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## MOLECULAR BARAMINOLOGY OF MARINE AND FRESHWATER FISH

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### ABSTRACT

A pertinent question posed to the Creation/Flood model is how different fish species could adapt to the drastic changes in water salinity that were inevitable due to the upheavals during the Flood that affected land animals as well as aquatic ones. Uniformitarianism sees difficulties in this since it projects the stenohaline status of many fish species today into the past. However, according to the Creation model, fish genomes may have been more robust and varied in the past, allowing for the euryhalinity of many fish kinds during the Flood. Euryhaline fish species could have had a more varied genetic machinery that allowed them to survive in saltwater or freshwater environments. Due to gene loss, this genetic machinery was then constricted, forcing fish to adapt to one or another narrower level of water salinity.

Several factors influence adaptation to differing water salinities, the most important being the presence or absence of various ion channels, including the sodium-potassium ATPase (NKA),  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  cotransporter 1 (NKCC1), cystic fibrosis transmembrane conductance regulator (CFTR), apical  $\text{Na}^+/\text{H}^+$  exchanger 3 (NHE3), and  $\text{Na}^+/\text{Cl}^-$  cotransporter (NCC). Other factors include the presence of different predators, parasites, and pathogens, water temperature, pH, and oxygen content, lighting, and even sexual factors. All these factors play a role, for example during the landlocking process, whereby fish species transition from facultative migration patterns involving adaptation to varying salinities to obligatory adaptation to freshwater environments.

The mitogenomes of 655 fish species belonging to nine orders (Acipenseriformes, Anguilliformes, Beloniformes, Characiformes, Clupeiformes, Cypriniformes, Elasmobranchii, Pleuronectiformes, and Salmoniformes) were analyzed. Overall mtDNA sequence similarity was determined to cluster these species into putative holobaramins. A total of 47 putative holobaramins were discovered. The distribution of saltwater, brackish water, and freshwater species was noted in all groups. A total of 22 (46.9%) of all groups were found to be euryhaline, where a group was determined to be euryhaline if at least one of its species was known to live in all three water environments. This indicates that some fish baramins are still euryhaline, in the process of adapting to narrower levels of water salinity (either salt or freshwater).

### KEYWORDS

freshwater, brackish water, saltwater, adaptation, molecular baraminology, Genesis Flood, mitochondrial DNA

### INTRODUCTION

After the Flood, the Earth had become a very different place for organisms to inhabit. Entire biomes and ecosystems had changed during the massive upheaval, and aquatic environments were definitely not an exception. The Flood was a violent geological process that drastically changed the surface of the Earth, including its water sources, such as oceans, lakes, and rivers. The erosion of the different landmasses and volcanic activity would have drastically increased the salt level of the post-Flood waters compared to the pre-Flood waters. This means that fish would have had to rapidly adapt to changing water salinities.

Skeptics raise the question, how could aquatic organisms, such as fish, survive such drastic changes in salinity? Elevated salt levels disrupt the osmotic balance within cells and draw water out of them.

On the other hand, hypoosmotic conditions tend to bloat cells, which is also undesirable. How did freshwater and saltwater fish survive the Flood? Is it even possible for fish to adapt rapidly between saltwater and freshwater? What kind of biological mechanism makes this process possible? What is the distribution of freshwater and saltwater fish species in different fish baramins?

Several initial explanations present themselves. First, changes in salinity may not have been that rapid for fish and other aquatic animals to be able to survive. Experiments performed by Smith and Hagberg (1983) on the blue damselfish (*Abudefduf unioellatus*), a species of marine reef fish, tested the organisms' capability of surviving at different levels of salinity. Fast rates of dilution (15 0/00 salinity/hour) resulted in the loss of the fish's locomotion at 0.88 0/00 salinity, where freshwater is defined as less than 0.5 0/00 of dissolved salts.

At slower rates of dilution (0.031 0/00 salinity/hour), however, the fish stopped swimming at 20.3 0/00 salinity. McCairns and Bernatchez (2010) studied freshwater and marine sticklebacks and found that freshwater populations can survive in saltwater conditions, albeit at lower survival rates. The reverse was also true with marine populations in freshwater habitats.

Second, it is a well-known fact that saltwater and freshwater can form layers on top of one another. The mixing of freshwater and saltwater in places was not complete. Pockets of water of varying salinity can exist next to one another. For example, MacGinitie (1939) reported layers of freshwater persisting atop layers of saltwater for several days. Fish with different salinity tolerance levels could have survived the Flood in these layers of varying salinity (Oard 1984).

Lastly, aquatic organisms can tolerate varying levels of salinity, even within the same species, genus, or family. Similarly, it could also be possible that different species within the same kind (baramin) of fish exhibit differing levels of tolerance towards adverse levels of salinity. In other words, both freshwater and saltwater fish species could be part of the same kind (Whitcomb and Morris 1961, p. 387).

Woodmorappe lists several examples of euryhaline organisms, namely animals that can not only tolerate both saltwater and freshwater but have been observed living in these environments. These include the roofed turtle (*Kachuga* sp.), the diamondback terrapin (*Malaclemys* sp.), the American crocodile (*Crocodylus acutus*), the cichlid fish *Tilapia grahami*, and the crab species *Telphusa* sp. (Woodmorappe 1996, p. 144). Adaptation to varying saltwater concentrations is a trait that is not exclusive to fish alone.

This also implies that certain changes in gene regulation allow for rapid physiological changes in response to a challenge in salinity in fish. These changes can involve hundreds or even thousands of genes. Similar regulatory changes in genes exist in microorganisms that allow them to adapt to adverse environmental factors. For example, the green alga *Chlamydomonas reinhardtii* can accumulate carbon in response to low CO<sub>2</sub> levels (Brueggeman et al. 2012) as well as form multicellular clusters in the presence of predators (Cserhati 2019). Although this is an example only from a species of algae, this could possibly be true in the case of vertebrate animals, as seen in various cases of sexual dimorphism that are due to genetic differences.

These pre-existing gene repertoires appear to constitute a divinely engineered regulatory circuitry that may be activated during adaptation to a water environment differing in salinity, such as a landlocked environment (an environment that is permanently closed to influx of external saltwater). These gene repertoires may have allowed for the post-Flood adaptation of fish to new environments due to the receding Flood waters (Genesis 8:13–14).

These changes in the regulatory machinery would have allowed fish not only to survive during the Flood but also to adapt to new environments that arose after the Flood, such as lakes, ponds, rivers, inland seas, and estuaries. Landlocking is a process whereby diadromous fish (species that migrate between saltwater and freshwater environments during their lifetime) lose their capability of adapting to saltwater and permanently end up in a landlocked lacustrine environment, and therefore cannot migrate back to a marine environment. Species that migrate as adults from saltwater to freshwater to spawn,

after which juveniles swim back to the ocean are called anadromous species. Conversely, species that migrate from freshwater to saltwater to spawn, after which juveniles migrate back to freshwater are called catadromous species. Amphidromous fish migrate between freshwater and saltwater for purposes other than spawning. Such species are born in freshwater, then float out into the sea then return to freshwater as adults to spawn (McDowall 2007). See Figure 1 for the differences between these migratory lifestyles, along with several examples of species that belong to these groups.

Actinopterygian (ray-finned) fish make up 96% of all fish species. In this clade, there are 15,150 freshwater and 14,740 (roughly 50%–50%) marine species, even though oceans make up 90–99% of the Earth's surface volume. When we look at non-fish species, Dawson (2012) estimates that there are around 4,000 marine gastropods versus 30,000 freshwater ones and 40 marine hydrozoans versus 3,500 found in freshwater habitats. Clearly, due to greater species richness, freshwater habitats are the scenes for rapid speciation, although freshwater fish can also acclimatize to saltwater conditions.

Genesis 1:20 describes the waters as abounding with living creatures, which would naturally include fish. As such, we can be sure that fish had high numbers at Creation Week. As of February 2022, the FishBase database has described 34,800 species of fish (Froese and Pauly 2022). Fish are the most abundant and diverse group of vertebrates (Magurran et al. 2011). As to whether the created diversity in a particular fish kind was low or high would classify it as a type 2b or type 3b Carter baramin (Carter 2021).

Within the same family of fish that inhabit both freshwater and saltwater, the more closely related species differ in their level of salt tolerance in many cases (Huysse et al. 2004). Carrete Vega and Wiens (2012) claim that species from the clade Percomorpha repeatedly invaded freshwater habitats, along with cichlids, percids, and pecciliids in several minor and major radiations. Although only 4% of extant actinopterygians are found in both freshwater and saltwater, many freshwater species have direct sister species in marine habitats (Seehausen and Wagner 2014), such as in the fish faunas of Iceland, New Zealand, Madagascar and Australia (Lévêque et al. 2008). Furthermore, freshwater sub-species may arise from the same marine species several times, as in the case of the threespine stickleback (*Gasterosteus aculeatus*) species complex (Jones et al. 2012).

In this paper, I shall examine the scientific literature to see what kinds of biological factors influence the adaptation of fish to new environments, such as the landlocking process. These could include things such as changes in water salinity, presence of pathogen or predator species (Choi et al. 2013; Perry et al. 2022), changes in lifestyle, geographical location (mainland or island populations), epigenetic factors, and sex-based differences (for example the disproportionate dispersal of one sex due to mating) (Hutchings and Gerber 2002). Furthermore, molecular baraminological analyses will be performed to verify whether freshwater and saltwater species exist within the same kind. This will be done by calculating the sequence similarity between the mitochondrial genome sequences of various fish species within a selected group. Then, clusters will be formed based on these sequence similarities. Species membership of these putative baramins will be checked against ecological annotation from FishBase to see the distribution of species that live in freshwater, brackish water, and saltwater.

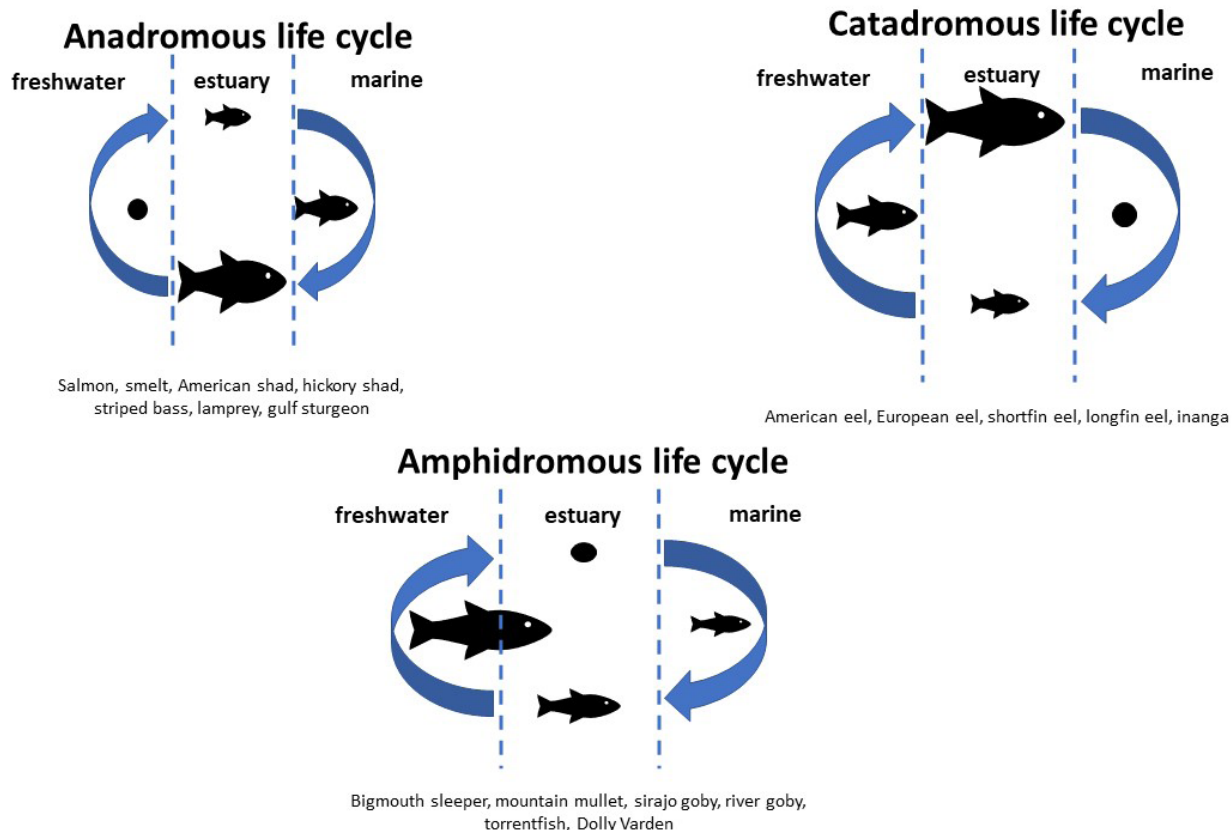


Figure 1. Depiction of differences between anadromous, catadromous, and amphidromous lifestyles.

## MATERIALS AND METHODS

### A. Mitochondrial sequence data (MitoFish)

The mitogenomes of 3,118 fish species were downloaded on September 8, 2022, from the MitoFish database, version 3.75 (Sato et al. 2018) at <http://mitofish.aori.u-tokyo.ac.jp/download.html>. The mitogenomes of several sets of species were selected from this dataset, and aligned with one another using the ClustalW software (Thompson et al. 1994). Table 1 shows a list of the selected taxonomic units as well as the number of species that have mitogenomes from the MitoFish dataset. The selected taxa (containing 29–167 species each) represent groups that are between the level of order and subclass, meaning that they are above the level generally accepted as the boundary of the kind. Three lamprey species were used as the outlier species in each analysis, *Lampetra aepyptera*, *Lampetra appendix*, and *Lampetra fluviatilis*.

### B. Ecological data (FishBase)

The FishBase database (Froese and Pauly 2022) was used to help in determining the ecological status of a given fish species (freshwater, brackish water, or saltwater). Data analysis on the ecological status of a given fish species was done using the R package, ‘rfishbase’, version 2.0, using the `fb_tbl(“species”)` command. R version 4.1.0 was used. Heatmaps were created using the ‘heatmap’ function, and Silhouette plots with the ‘fviz\_nbclust’ function. The sequence similarity matrix was calculated using the ‘seqinr’ package.

Table 1. Number of genera and species with mitochondrial genomes from the Mitofish database.

Taxonomic unit	No. of genera	No. of species
Acipenseriformes	6	29
Anguilliformes	38	61
Beloniformes	12	32
Characiformes	38	54
Clupeiformes	47	96
Cyprinodontiformes	20	69
Elasmobranchii	84	167
Pleuronectiformes	45	72
Salmoniformes	11	75

### C. Entropy calculation

Shannon entropy was used to calculate the variety of freshwater, brackish water, and saltwater species in a given cluster. Entropy was calculated using the following equation:

$$S = - \sum_{i=F,B,S} p_i \cdot \log_2 p_i$$

where F stands for freshwater, B for brackish water, and S for saltwater. The variable  $p_i$  stands for the proportion of fish that fall into each of the three categories. In order to get a normalized entropy value, the entropy was divided by the maximum entropy value for three categories, which is approximately 1.585, where  $p_i = 1/3$ .

Supplementary files containing the results of the analysis of the nine fish groups are available on Zenodo at <https://zenodo.org/record/7227028#.Y1C9zEzMLrc>.

## RESULTS AND DISCUSSION

### A. Literature review

Adaptation to differing salinities is a process that entails large-scale genetic and morphological changes. One example is landlocking whereby diadromous fish lose their capability of migrating back to the ocean and permanently end up in a lacustrine environment. This may be accompanied by the loss of genes that allow the fish to adapt to marine environments. During this process, the differential expression of a large repertoire of genes may take place. Due to the changes in selective pressures and possible ensuing differential genetic regulation and gene loss, the newly landlocked species are rendered less capable of adapting to future changes in its new environment (Hunt et al. 2011). The landlocking process is similar to how eyeless fish lose genetic information associated with sight in caves. In the dark recesses of aquatic caves, fish lose the need for organs involving sight, and thereby lose the genes that are necessary for eyes.

Evolutionists assert that landlocking is evidence of natural selection producing new species, but this claim is rather problematic. The mere fact that the number of freshwater fish species is the same as marine species, despite the greater volume of inhabitable marine water implies a much faster diversification rate in freshwater fishes (Rabosky 2020). This also implies selection factors that lead to the diversification of fish species during their adaptation to lacustrine environments.

The landlocking process happens at a much faster rate than expected according to the evolutionary timescale. Palkovacs et al. (2008) found, based on mtDNA and microsatellite analysis, that the alewife (*Alosa pseudoharengus*) adapted to saltwater multiple times within as little as 300, but up to 5,000 years (both within the biblical timescale). Hendry et al. (2000) found, based on microsatellite data and phenotypic variation, that two populations of sockeye salmon (*Oncorhynchus nerka*) became reproductively isolated from one another and inhabited a lacustrine and a river environment after only 13 generations. Besides fish, Lee and Bell (1999) list 18 marine species that have adapted to freshwater environments over only the past 200 years.

This leaves little time and chance for many new genes to arise during adaptation to lacustrine environments, as per the evolutionary models. This is all the more significant, since the ion composition of saltwater is largely in the range of that of body fluids, and maintaining this ion composition in freshwater has high energy costs (Lee and Bell 1999). Adapting to freshwater demands quite a bit of adaptation on the part of fish, since the cell would have to expel superfluous

water entering it. It would make more sense if the expression of a wide range of already existing genes is either differentially expressed or these genes undergo differential epigenetic regulation. These processes have been found to occur fairly rapidly. The discovery of such differentially expressed gene (DEG) repertoires is greatly facilitated by RNA-seq technology, whereby dozens, or even hundreds of genes can be identified that take part in the transition between saltwater and freshwater.

Several factors can play a role during landlocking, and the adaptation to different water salinities in general. These include salt concentration, temperature, dissolved oxygen levels, new pathogens, parasites and predators, lighting, sex-associated differences, anthropogenic factors, geographical location, food sources, and also epigenetic factors.

The most important factor that plays a role in the landlocking process is water salinity. As anadromous fish species invade lacustrine environments, their bodies must get accustomed to hypo-osmolarity, whereas catadromous species must contend with hyper-osmolarity. The most important cellular components that regulate osmotic relationships within the body are ion channels, and transmembrane transport systems. Ions that are the focus of establishing osmotic relationships within the cell are  $K^+$ ,  $Na^+$ , and  $Cl^-$ , and to a lesser extent  $Ca^{2+}$  and  $NH_4^+$ .

Teleost fish adjust to varying salinity levels by either secreting or absorbing ions via special mitochondrion-rich cells called 'ionocytes' that line the gill epithelium and the intestines, the main sites of water uptake (Velselvi et al. 2021). Since the gills also play a major role in fish immunity, parasites are often found in this area of the fish's body. These ionocytes contain several ion-transport proteins, including sodium-potassium ATPase (NKA),  $Na^+/K^+/2Cl^-$  cotransporter 1 (NKCC1), cystic fibrosis transmembrane conductance regulator (CFTR), apical  $Na^+/H^+$  exchanger 3 (NHE3), and  $Na^+/Cl^-$  cotransporter (NCC) (Hiroi and McCormick 2012), the first three being the most important in the regulation of osmolarity in fish.

The function of NKA is to maintain a  $Na^+$  gradient across the membrane by exuding  $Na^+$  ions to facilitate ATP production. NKCC1 admits  $Cl^-$  ions into the cell, whereas the CFTR channel allows  $Cl^-$  out of the cell in saltwater conditions (Evans 2008). Hypotonic conditions inhibit  $Cl^-$  secretion by NKCC1. The efflux of  $Cl^-$  from the cell in turn enables the secretion of  $Na^+$  from the blood vessels towards the external seawater (Marshall 2010). The regulation and the coordination of these ion channels all work together to maintain cell volume, which is critical when transitioning between freshwater and saltwater (Whitehead et al. 2012). Saltwater fish can also adapt to freshwater if calcium is present in significant amounts since intracellular  $Ca^{2+}$  is important for activating these osmolyte channels (Whitehead et al. 2011).

Euryhaline fish are capable of differentially regulating the expression of these three ion channels when adjusting between freshwater and saltwater environments. Euryhalinity may have been the ancestral state, allowing fish to adapt to various salt concentrations, whereas stenohalinity is more derived due to the loss of ion channel regulation. This was found to be the case in killifish (*Fundulus*), which adapted to a freshwater environment from a saltwater environment

on several occasions, while losing the genetic factors responsible for hyperosmotic plasticity (Whitehead 2010). This goes according to the creation model which involves the devolutionary loss of genetic elements. It may be that while adapting to a new environment, concomitant gene loss barred species from reverse adaptation to their previous environment.

Osmoconformers are fish species that do not expend energy to maintain the osmolarity of their internal medium, by minimizing the osmotic gradient. These include some species of stenohaline fish. Osmoregulators use energy-dependent mechanisms to maintain a higher external osmolarity. These include several estuarine and freshwater species of fish (Rivera-Ingraham and Lignot 2017).

Ionocytes vary according to the contents of the first three types of ion channels. Type-I ionocytes occur in both freshwater and saltwater fish and contain only a basolateral NKA channel. Type-II and type-III ionocytes occur in freshwater species, where the type-II ionocyte has a basolateral NKA channel and an apical NCC channel, and the type-III ionocyte has a basolateral NKA and NKCC1a channel, sometimes with an apical NH3 channel. This configuration of ion channels allows for ion absorption. Type-IV ionocytes are the same as type-III ionocytes, except that they have an apical CFTR channel instead of an NHE3 channel, and they also occur in saltwater fish species. This configuration allows for ion secretion (Hiroi and McCormick 2012), and indicates that the CFTR channel is mainly responsible for salt excretion. Figure 2 shows the location of the three main types of ion channels in a typical fish ionocyte.

A second way of classifying ionocytes is whether their apical mem-

brane binds a protein called peanut agglutinin (PNA), which was originally used to identify cells that secrete  $\text{HCO}_3^-$ . In certain fish, such as freshwater rainbow trout (*Oncorhynchus mykiss*),  $\text{PNA}^-$  and  $\text{PNA}^+$  ionocytes both have a basolateral NKA channel, whereas only  $\text{PNA}^+$  ionocytes have an apical NHE3 channel, which is restricted to the gills (Ivanis et al. 2008; Dymowska et al. 2012).

In other fish species, such as zebrafish (*Danio rerio*), ionocytes can be classified in yet another manner. Zebrafish have an ionocyte repertoire that includes an NCC ( $\text{Na}^+/\text{Cl}^-$ ) cotransporter cell, which corresponds to the type-II ionocyte described earlier, an NaR (NKA-rich) cell, that absorbs  $\text{K}^+$  into the cell, and secretes  $\text{Na}^+$ . The third type of ionocyte in this ion channel repertoire is the H-ATPase (HA) ionocyte, which secretes  $\text{H}^+$  outwards to the lumen (Chang et al. 2009; Hwang et al. 2011).

In the transition between freshwater and saltwater environments, euryhaline fish species, such as tilapia can switch between ion-absorbing type-III and ion-secreting type-IV ionocytes. In contrast, the stenohaline zebrafish has only type-III ionocytes. The NKA ion channel can also be differentially expressed in freshwater and saltwater. The NKA channel has an  $\alpha$  and a  $\beta$  subunit, where the  $\alpha$  subunit binds the ATP,  $\text{Na}^+$ , and  $\text{K}^+$  substrates, and the  $\beta$  subunit is a structural element. The  $\alpha$  subunit has two isoforms, of which  $\text{NKA}\alpha 1a$  is more abundant in freshwater, whereas  $\text{NKA}\alpha 1b$  is expressed in higher levels in saltwater (Pfeiler and Kirschner 1972). The NHE3 channel allows saltwater fish to excrete metabolic acids, by exploiting the  $\text{Na}^+$  gradient across the ionocyte membrane (Claiborne et al. 2002).

Fluctuating salinity levels represent a form of oxidative stress, lead-

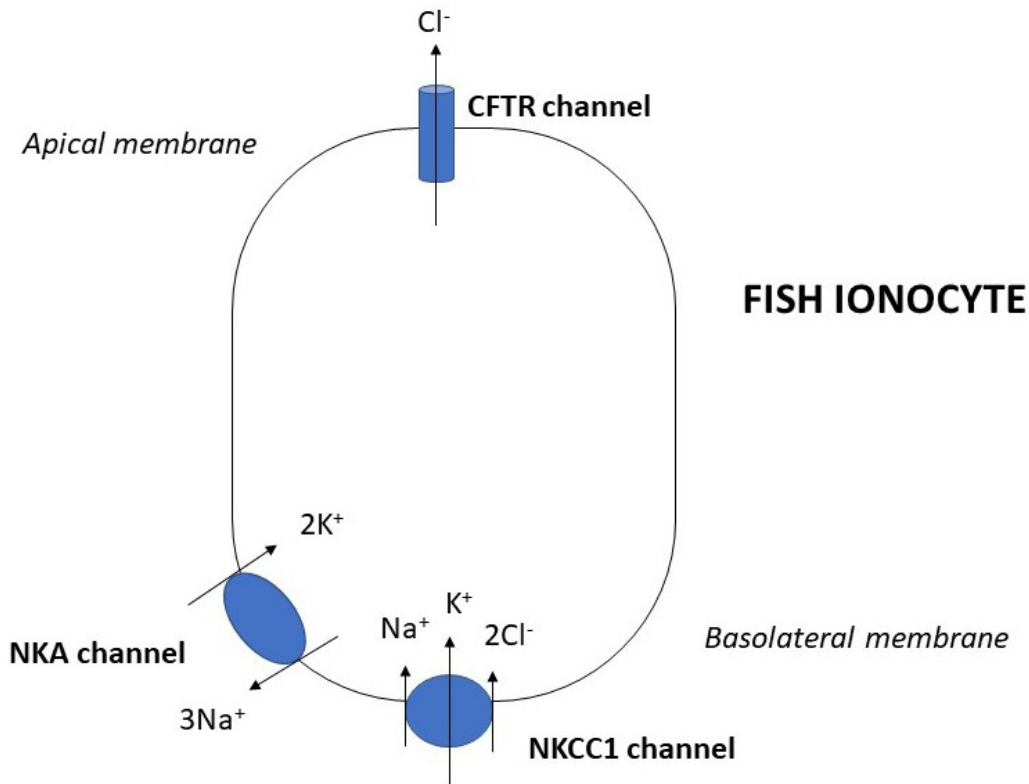


Figure 2. Schematic depiction of a fish ionocyte, showing the regular placement of the CFTR, NKA, and NKCC1 ion channels.

ing to the production of reactive oxygen species (ROS) (Bal et al. 2021). Taurine acts as a potent antioxidant that protects fish from this form of stress (Zeng et al. 2009). The levels of antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GPx) are good measures of salinity-induced oxidative stress. Certain types of aquaporin (AQP) also play a role in regulating the response to water salinity in fish (Hirose et al. 2003). As water salinity increases, the number of AQP channels decreases to mitigate the amount of water lost due to hyperosmotic conditions. In general, salinity tends to have an osmo-protective role against heavy metal ions, such as nickel, cadmium, zinc, and mercury (Gioda et al. 2007; Saglam et al. 2013; Blewett et al. 2016), which are mainly found inland, thus representing a challenge to fish during the landlocking process. Increased salinity induces AQP channels that facilitate salt ion transport, as well as the NKA ion channel to produce energy via ATP.

Differences in salinity may be associated with a massive number of genes to be differentially expressed, numbering in the thousands. Gibbons et al. (2017) found that 2,515 genes were differentially expressed in threespine stickleback due to changes in salinity. These genes are involved in the migration of epithelial cells during gill remodeling, transmembrane ion transport, epithelial Ca<sup>2+</sup> channels (ECaCs), the NKA and NHE3 channels. Claudins and occludins, genes that code for proteins that form tight junctions and reduce ion permeability also change their gene expression levels in freshwater conditions. AQP3 is also differentially regulated in varying salinity conditions, with its expression level changing up to thirteen-fold in freshwater conditions (Whitehead et al. 2011).

Several genes that are differentially regulated between freshwater and saltwater conditions include the NF- $\kappa$ B family of transcription factors that respond to infection, stress, and injury (Xiao and Ghosh 2005). V-type H<sup>+</sup> ATPase was also found to play an important role in osmoregulation in freshwater fish species, such as sticklebacks, but also in non-fish species, such as copepods (Lee et al. 2011; Kozak et al. 2014), by facilitating the uptake of sodium into the cell (Katoh et al. 2003). Slowing down the progression of the cell cycle is a way in which fish can repair DNA damage before cell duplication, as has been observed in fish kidney cells (Kammerer and Kültz 2009; Dowd et al. 2010). Several genes involved in energy production are also differentially expressed, such as the subunits of the NADH dehydrogenase, ATP synthase, and cytochrome B and C (Newmeyer and Ferguson-Miller 2003).

Water salinity is such an important factor that it also affects other adaptational factors as well, such as food abundance, immunity, and exposure to parasites and predators (Saboret and Ingram 2019; Blasco-Costa et al. 2013), and can even lead to reproductive differences (Kozak et al. 2014). For example, Blonar et al. (2011) found that salinity played a role in the structure and composition of pathogen species infecting mummichog (*Fundulus heteroclitus*) in two polluted estuaries in New Brunswick, Canada.

## B. Mitochondrial DNA analysis of different fish groups

In the following, the mtDNA of the nine fish groups around the level of the order listed in Table 1 will be analyzed to discover what kinds of putative baraminic relationships exist within them.

## 1. Acipenseriformes

Acipenseriformes is an order of ray-finned fishes including sturgeons and paddlefishes. The mtDNA of 29 species was examined from this group according to the Materials and Methods section. The results can be seen in Figure 3, and are also available in Supplementary File 1. According to the heatmap (Figure 3A), there appear to be two groups within Acipenseriformes, four species from the genus *Scaphirhynchus* and the remaining 24 species from various genera, *Acipenser*, *Huso*, *Polydon*, *Psephurus*, and *Pseudoscaphyrhynchus*. The three *Lampetra* species formed a distinct outgroup compared to the two other clusters. The Hopkins clustering statistic was very good at 0.878. The Silhouette plot in Figure 3B shows a maximum silhouette value at two clusters, but there may be distortion in the data. All three groups had a statistically significant p-value.

The smaller group (*Scaphirhynchus*) had a lower normalized entropy value (0.512), with information from FishBase for four out of five species, and among these three from freshwater. In contrast, the larger group of 24 species had a normalized entropy value of 0.999, amongst which an almost even number of species inhabit freshwater, brackish water, and saltwater. These results clearly show that several species from this group can inhabit habitats of varying salinities, and that transitioning between habitats is not difficult. Indeed, 15 of the 22 species which had migratory annotation in FishBase were anadromous, with the remaining seven being potamodromous (completing their entire life cycle in freshwater).

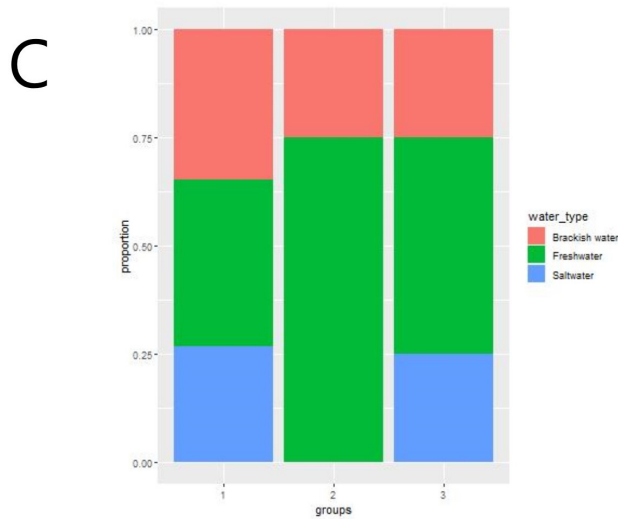
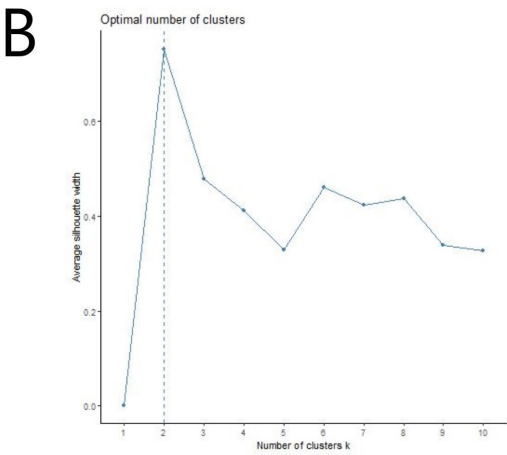
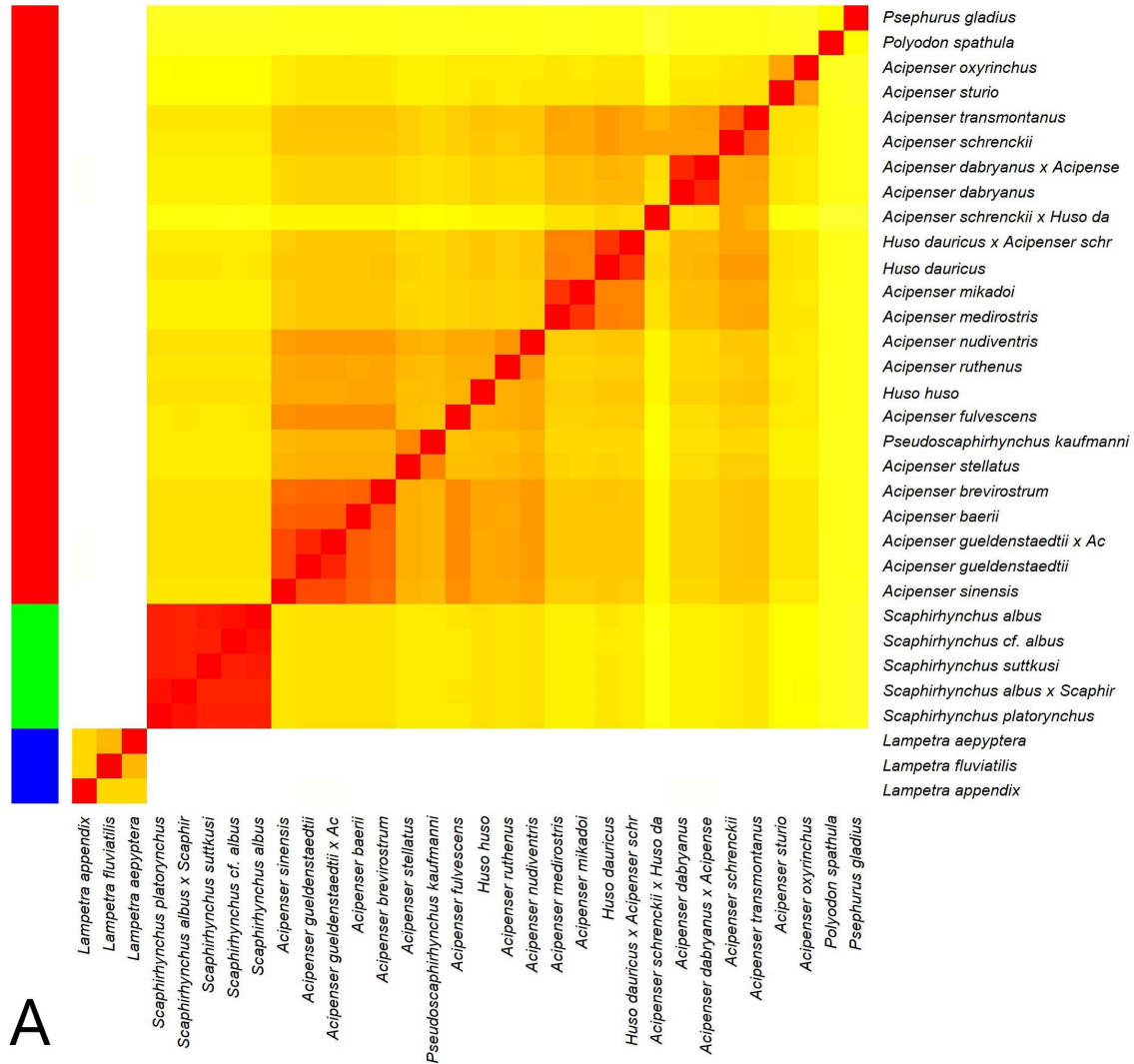
## 2. Anguilliformes

Anguilliformes (eels) are long-bodied fish that use peristaltic movement to swim, undulatory waves that are propagated posteriorly through the animal's body. These characteristics make these animals a distinct apobaramin compared to all other fish groups.

A total of 61 species of Anguilliformes were studied. For the heatmap, the 'average' clustering method was used. Besides the three outlier lamprey species, there are two larger groups, with 19 and 41 species, respectively, as can be seen in Figure 4A. The Silhouette plot in Figure 4B shows a maximum silhouette value at three clusters. The Hopkins clustering statistic is 0.804, indicating good clustering.

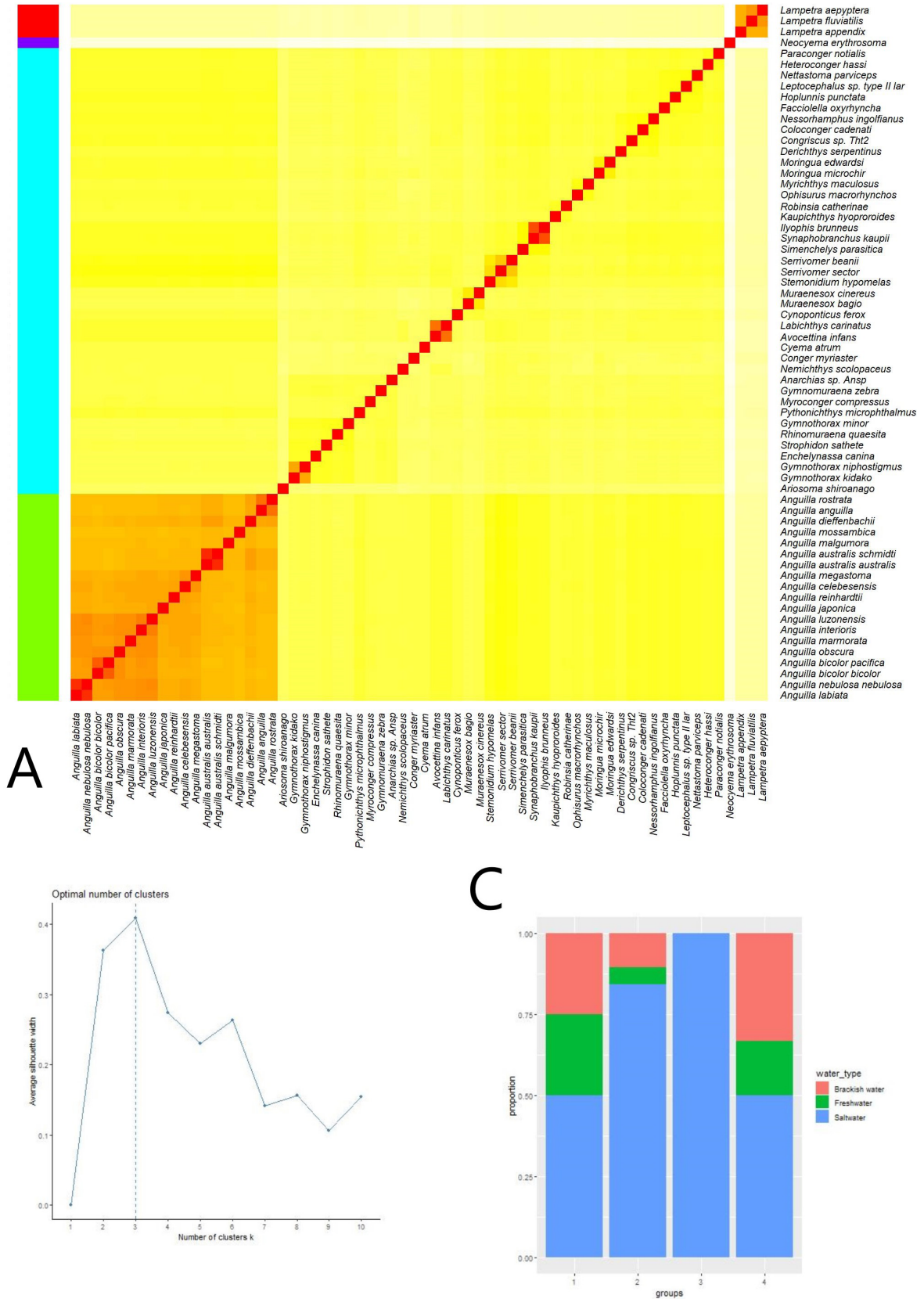
However, the larger group of 41 species is not statistically significant, with a p-value of 0.201. Furthermore, the species *Neocyema erythrosoma* does not fit in with either cluster. *N. erythrosoma* is also a deep-sea eel, found in depths between 6,600 and 7,200 ft. As such, it inhabits a different habitat than most eels. This is more evidence that this species belongs to its own holobaramin (Wise 1992). Poulsen et al. (2018) place members of the genus *Neocyema* into their own family, called Neocyematidae, based on divergent mtDNA sequences and mitochondrial gene order. In contrast with members of the family Cyematidae, the mitochondrial gene order of *N. erythrosoma* resembles that of *Eurypharynx* and *Sacchopharynx* but is significantly shorter in length (17,765 bp as opposed to 18,978 bp of *Eurypharynx pelecanaoides*).

When the gene order for the mtDNA of the four putative Anguilliformes groups was analyzed, it was found that some species in group #3 had a slightly different gene order than the rest of the species. Thus, group 3 was reassigned to groups 3 and 4, and group 4 was



**Figure 3.** Results of the analysis of Acipenseriformes. A. Heatmap showing tentative baraminic relationships between several groups of species based on mtDNA sequence similarity. Darker, redder colors denote higher sequence similarity, thus common baraminic status, whereas lighter, yellower colors denote lower sequence similarities, and different baraminic status. B. Silhouette plot showing the optimum number of clusters in the mtDNA sequence similarity heatmap. C. Barplot showing the different proportion of species living in freshwater (green), brackish water (red), and saltwater (blue) in the groups in the heatmap.





**Figure 4.** Results of the analysis of Anguilliformes. A. Heatmap showing tentative baraminic relationships between several groups of species based on mtDNA sequence similarity. Darker, redder colors denote higher sequence similarity, thus common baraminic status, whereas lighter, yellower colors denote lower sequence similarities, and different baraminic status. B. Silhouette plot showing the optimum number of clusters in the mtDNA sequence similarity matrix. C. Barplot showing the different proportion of species living in freshwater (green), brackish water (red), and saltwater (blue) in the groups in the heatmap.

reassigned to group 5. Figure 5 displays the gene order of the mtDNA from the five revised groups. The difference in the gene order between these two groups is an inversion of two pairs of genes. In group 3, the fifth to the second last genes are NAD6, tRNA-Glu, cytochrome B, and tRNA-Thr. In group 4, the order of these genes is cytochrome B, rTNA-Thr, NAD6, and tRNA-Glu. Furthermore, the mean mtDNA length of the species displayed in Figure 5 is 16,686 bp, whereas the mean mtDNA length in group 4 is 17,713 bp.

Cytochrome b is also missing from *Myrichthys maculosus* in this group. *Cyema atrum* is another anomalous species, as the length of its mitogenome places it in group 4, whereas its gene order is similar to that of group 3. As to whether there are four or five holobaramins

within the Anguilliformes apobaramin, more study is needed.

The larger cluster of 41 species also has a relatively low normalized entropy value of 0.485, with 84.1% of the species living in saltwater. This cluster is made up of 36 genera, listed in Supplementary File 2. The smaller cluster consists of species from the genus *Anguilla*. It is more balanced, with an equal number of species (14) inhabiting all three aquatic environments. Here the normalized Shannon entropy is the highest (0.946). This gives more evidence that the species in this cluster have a better potential to adapt to different water salinities (see Figure 4C). All 14 *Anguilla* species studied here are catadromous (meaning that they migrate to the sea to spawn).

### 3. Beloniformes

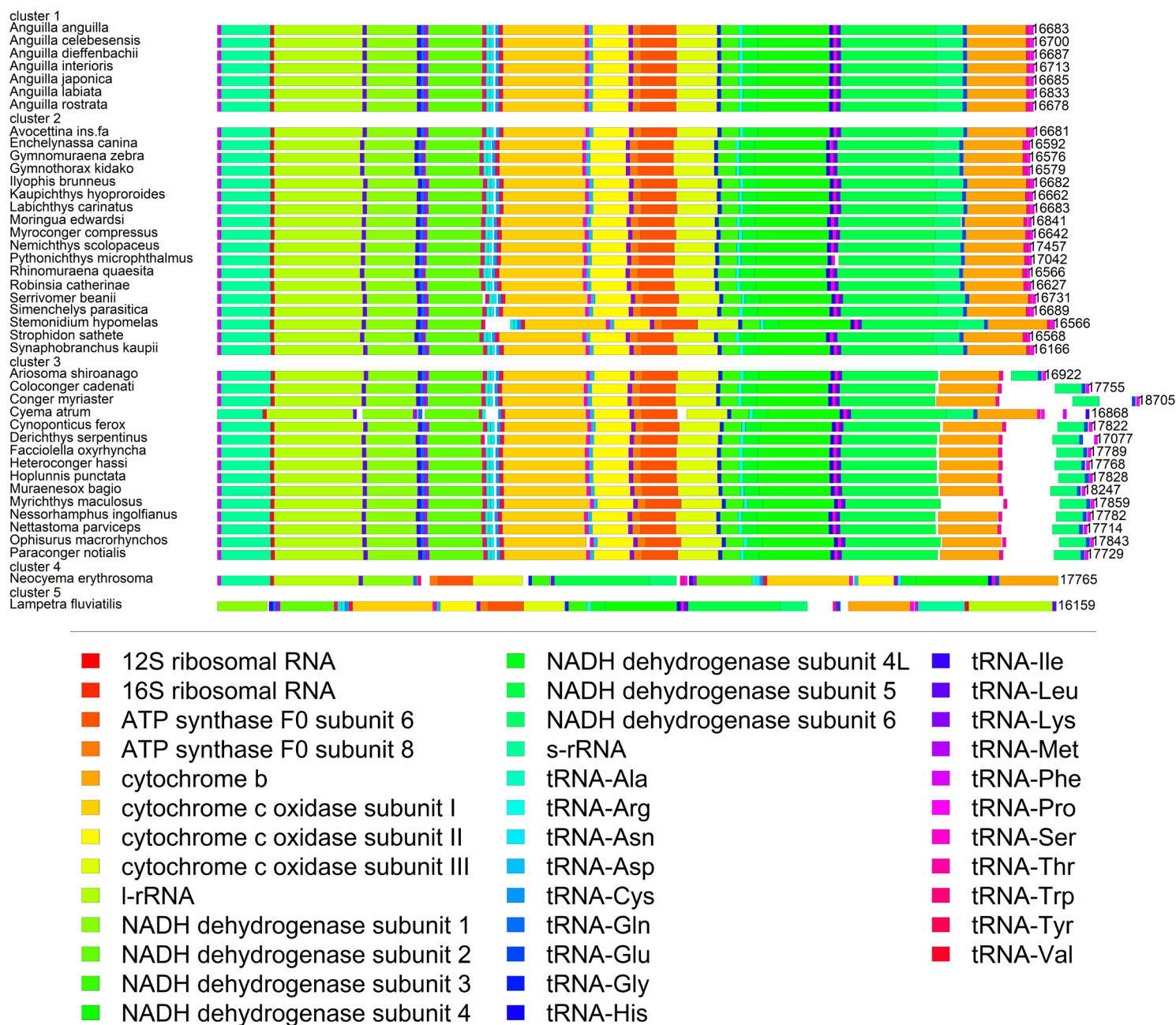


Figure 5. Gene order map of species from several groups from the mtDNA analysis of Anguilliformes.

Beloniformes is an order composed of six families of marine and freshwater fish with elongated bodies. They are commonly known as needlefishes or long toms. In this study, the mtDNA of 32 species was examined. The four putative clusters can be seen in Figure 6A. The Silhouette plot shows an optimum of four clusters, but this may be due to distortion in the data. The Hopkins clustering value is 0.777, which represents good clustering. All four clusters are statistically significant. The results of the analysis of these 32 species are available in Supplementary File 3.

Besides the three control species, three putative baramins were found. These include twelve species of exclusively marine fish, mainly from the genus *Cheilopogon*, but also *Cypselurus hiraii*, *Exocoetus volitans*, *Hirundichthys rondeletii*, and *Prognichthys sealei*. These are all species from the family Exocoetidae or flying fishes. This group has a normalized water type entropy value of 0, with all species being oceanodromous; fully adapted to saltwater.

Besides this, ten species from various genera (*Ablennes*, *Cololabis*, *Strongylura*) mainly from Belonidae also formed a cluster. However, several species from other families also are part of this cluster, such as *Dermogenys pusilla*, five species from *Hyporhamphus*, and *Par-exocoetus brachypterus*. These fish come from a mix of freshwater, brackish water, and saltwater environments, and have a normalized water-type entropy value of 0.958 (see Figure 6C). Four species were annotated as oceanodromous in FishBase, two as anadromous, and one as potamodromous.

Also, ten species of *Oryzias* (ricefish) were found that populate mainly freshwater and brackish water. The normalized water-type entropy value is 0.579. Of the six *Oryzias* species that had migratory annotation from FishBase, five were non-migratory, and only one was amphidromous. This indicates that these fish species have fairly well adapted to environments with lower salinity levels.

#### 4. Characiformes

This order contains around 2,000 species classified into 18 families. Fish such as characins, piranhas, and tetras belong to this group. A total of 54 species from Characiformes were studied. According to Figure 7A, nine putative holobaramins were found, with a Hopkins clustering value of 0.778, indicating good clustering. The Silhouette plot in Figure 7B also shows an optimum of nine clusters. A list of species clusters and statistics is available in Supplementary File 4. However, cluster #8, made up of three species, has a statistically insignificant p-value of 0.148.

A characteristic of these Characiformes clusters is that almost all species live in freshwater, meaning that they have almost completely adapted to this type of environment. With the exception of cluster #1, all other groups had a normalized entropy value of 0. All ten characids with migratory FishBase annotation were potamodromous.

#### 5. Clupeiformes

This diverse group includes species such as anchovies and herrings and other fish that are caught for human consumption. Its 400 species populate marine, euryhaline and freshwater environments in tropical, subtropical, and temperate climate zones (Lavoué et al. 2014).

Mitochondrial genomes from 96 species from Clupeiformes were analyzed. The results can be seen in Figure 8, and are available in

Supplementary File 5. The Silhouette plot in Figure 8B shows eight putative clusters (the outlier group and seven clupeiform baramins). The Hopkins clustering statistic is 0.832, which indicates good clustering. However, cluster #4, containing 17 species, has a statistically insignificant p-value of 0.342.

Lavoué et al. (2013) also analyzed the mitogenomes of Clupeiformes and uncovered nine main lineages. In the present analysis, of the 93 species that were also studied by Lavoué et al., 75 (80.6%) fell into the same lineage as defined by Lavoué et al. Cluster #5 of the present analysis of Clupeiformes corresponds to lineage 3+Pristigasteridae, and clusters #6 and #7 of the present study both correspond to the family Engraulidae as defined by Lavoué et al. However, Engraulidae is made up of the subfamilies Engraulinae and Coiliinae, thus reflecting the clustering discovered in this analysis. Of the five Coiliinae species, all five can live in freshwater and brackish water, with only three species inhabiting saltwater. The species from the subfamily Engraulinae, on the other hand, inhabit mainly brackish water and saltwater, with 15 and 17 species, respectively. Only seven engraulids inhabit freshwater. Thus, since adaptation to freshwater and saltwater are occurring in opposite trajectories for these two groups, it may be that these two groups are separate holobaramins.

The normalized water type entropy values are all above 0.85 indicating that all the clusters were euryhaline (adaptable to different water salinities). Since all these clusters are euryhaline, this seems to indicate that not much time has elapsed since the Flood, after which fish species would have had the chance to adapt to environments with narrower salinities.

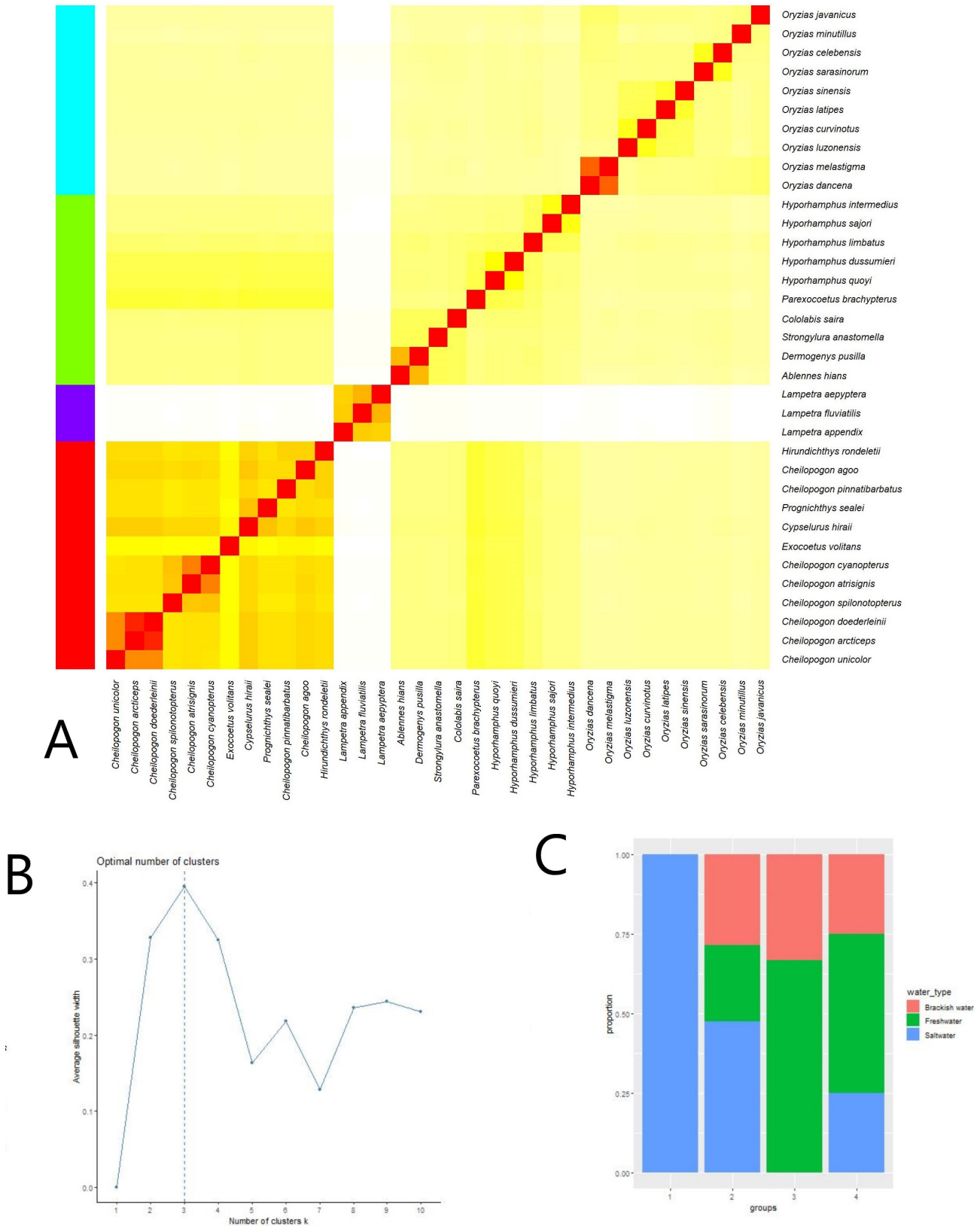
For example, Wilson et al. (2008) found that the freshwater herring (*Clupea*) species of Lake Tanganyika could be the result of a recent invasion by their marine relatives, which have not diverged much from their freshwater counterparts in their morphology. When the waters of the Flood receded, some portions could have formed inland lakes in Africa, whereas the rest drained off the continent. These specific *Clupea* species could have been localized to that portion of the receding waters that ended up inland, and hence adapted to freshwater circumstances, whereas their baraminic relatives adapted to a saltwater environment in the ocean, being localized to the portion of the receding Flood waters that drained into the ocean.

#### 6. Cyprinodontiformes

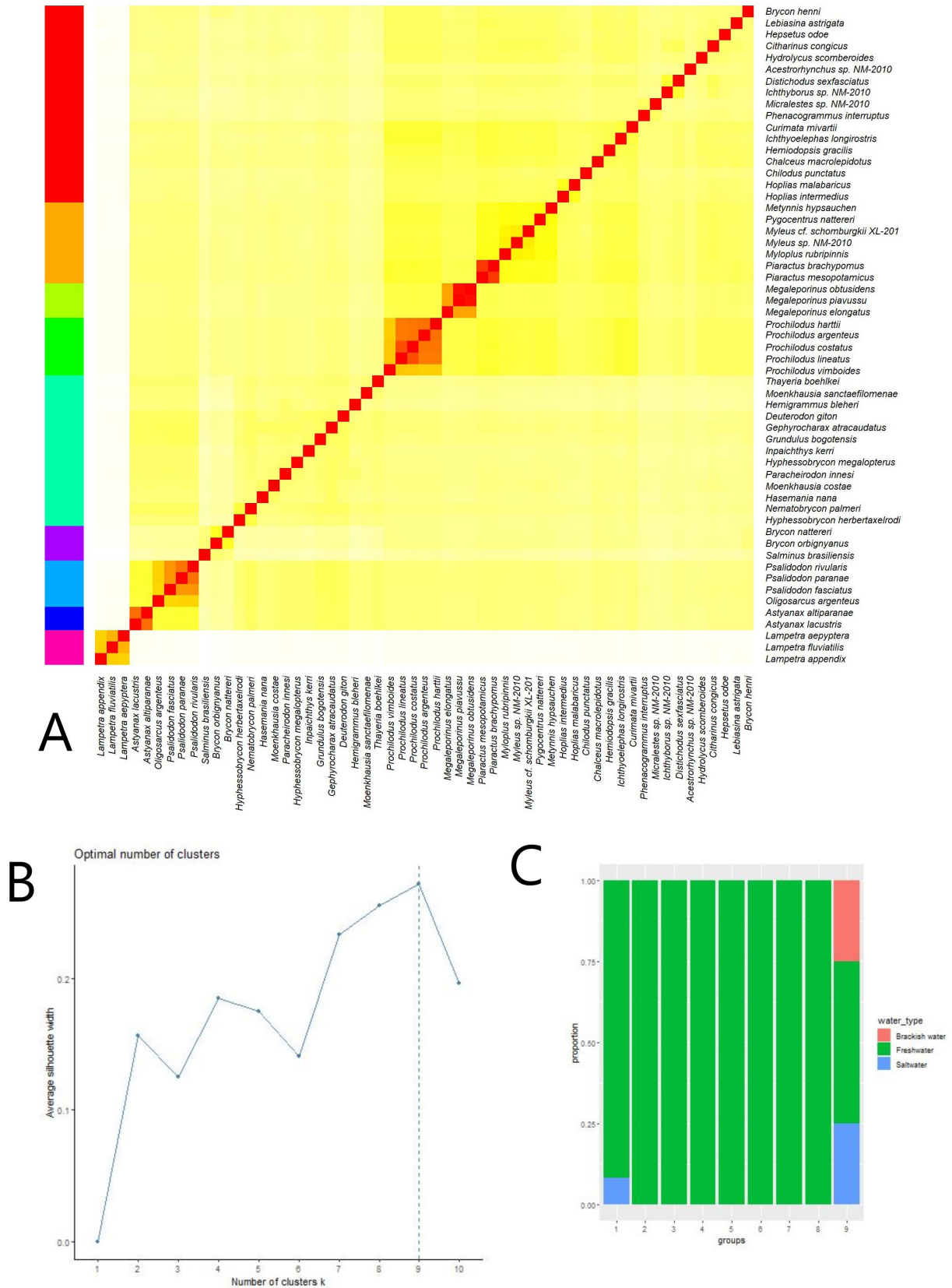
Cyprinodontiformes include small-sized fish, such as killifishes, minnows, pupfishes, and livebearers, which live mainly in freshwater and brackish water. They are represented by 1,400 species.

The mtDNA of 69 species was analyzed. The results can be seen in Figure 9, and are also available in Supplementary File 6. The Hopkins clustering statistic is 0.831, which means good clustering. Besides the outlier group, there are eight putative holobaramins as indicated by the Silhouette plot in Figure 9B. The first seven groups are statistically significant.

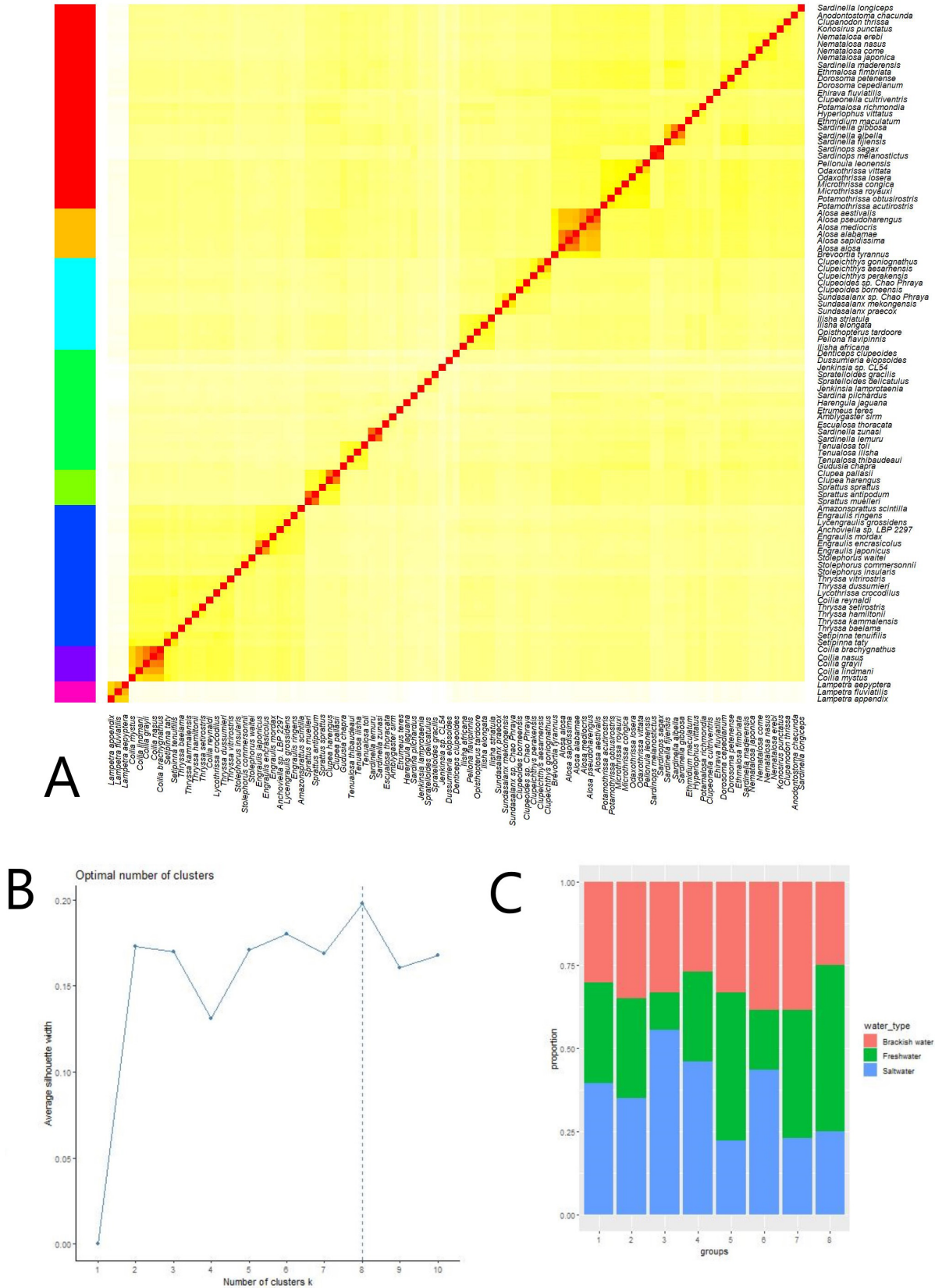
Five of the eight groups contain no saltwater species (see Figure 9C), and there are only three species, one from each of the remaining three groups, which can live in saltwater. The majority of the species (56 out of 69) are non-migratory, which means that these species have well adapted to freshwater environments (see Figure 9C). The three species that can live in saltwater, *Cyprinodon variegatus*, *Fun-*



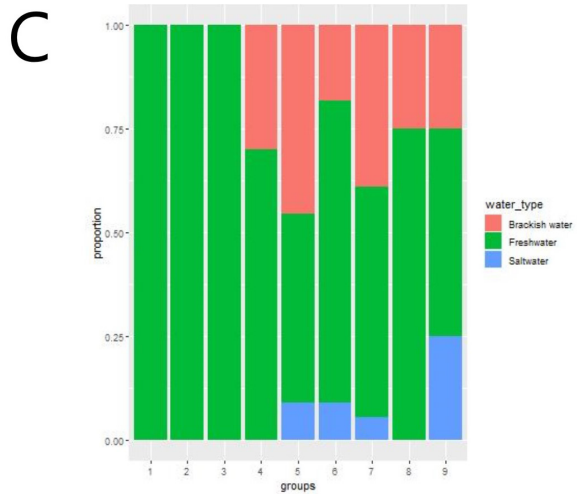
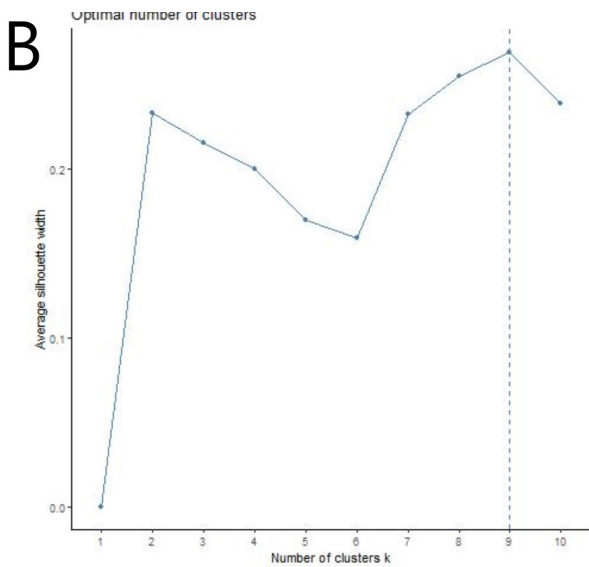
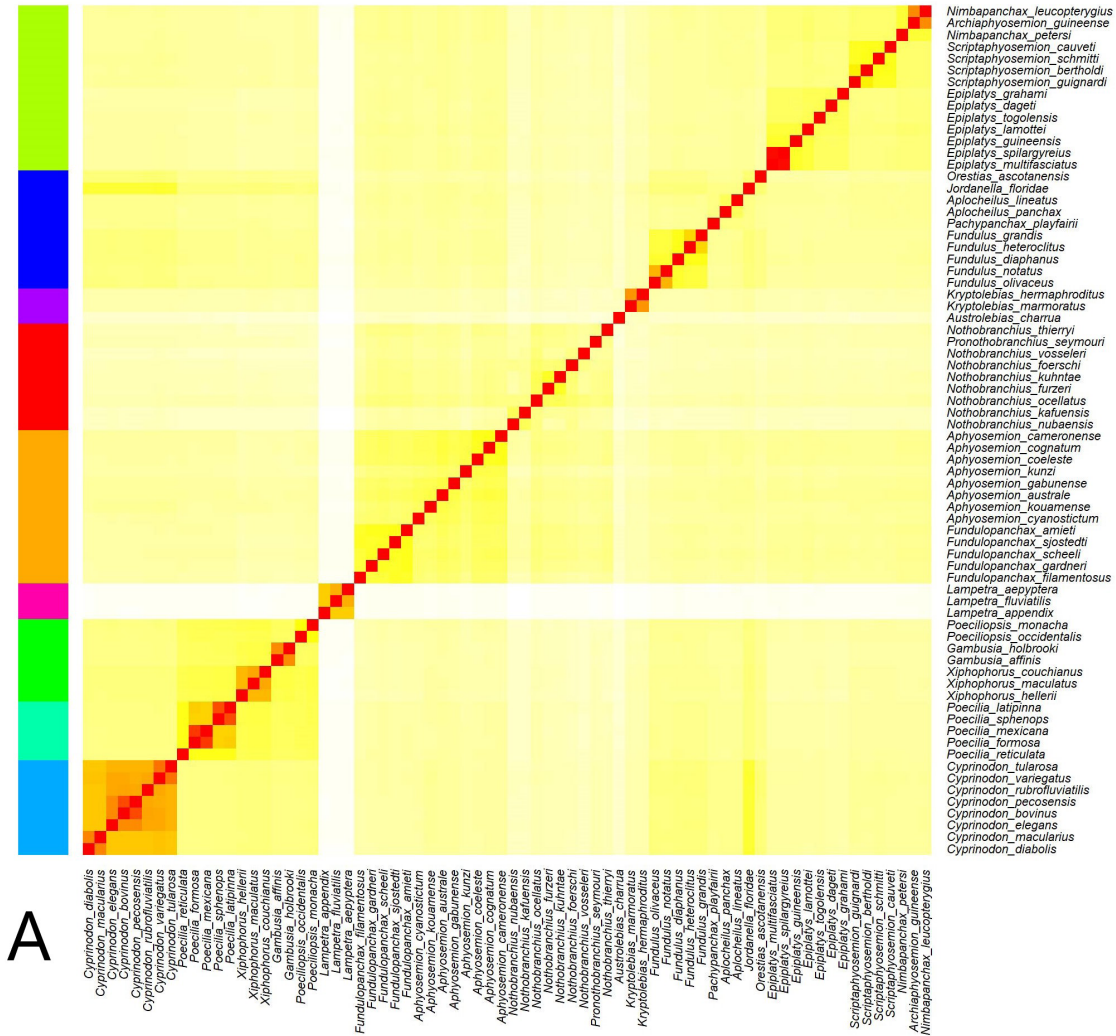
**Figure 6.** Results of the analysis of Beloniformes. A. Heatmap showing tentative baraminic relationships between several groups of species based on mtDNA sequence similarity. Darker, redder colors denote higher sequence similarity, thus common baraminic status, whereas lighter, yellower colors denote lower sequence similarities, and different baraminic status. B. Silhouette plot showing the optimum number of clusters in the mtDNA sequence similarity matrix. C. Barplot showing the different proportion of species living in freshwater (green), brackish water (red), and saltwater (blue) in the groups in the heatmap.



**Figure 7.** Results of the analysis of Characiformes. A. Heatmap showing tentative baraminic relationships between several groups of species based on mtDNA sequence similarity. Darker, redder colors denote higher sequence similarity, thus common baraminic status, whereas lighter, yellower colors denote lower sequence similarities, and different baraminic status. B. Silhouette plot showing the optimum number of clusters in the mtDNA sequence similarity matrix. C. Barplot showing the different proportion of species living in freshwater (green), brackish water (red), and saltwater (blue) in the groups in the heatmap.



**Figure 8.** Results of the analysis of Clupeiformes. A. Heatmap showing tentative baraminic relationships between several groups of species based on mtDNA sequence similarity. Darker, redder colors denote higher sequence similarity, thus common baraminic status, whereas lighter, yellow colors denote lower sequence similarities, and different baraminic status. B. Silhouette plot showing the optimum number of clusters in the mtDNA sequence similarity matrix. C. Barplot showing the different proportion of species living in freshwater (green), brackish water (red), and saltwater (blue) in the groups in the heatmap.



**Figure 9.** Results of the analysis of Cyprinodontiformes. A. Heatmap showing tentative baraminic relationships between several groups of species based on mtDNA sequence similarity. Darker, redder colors denote higher sequence similarity, thus common baraminic status, whereas lighter, yellower colors denote lower sequence similarities, and different baraminic status. B. Silhouette plot showing the optimum number of clusters in the mtDNA sequence similarity matrix. C. Barplot showing the different proportion of species living in freshwater (green), brackish water (red), and saltwater (blue) in the groups in the heatmap.

*dulus heteroclitus*, and *Poecilia latipinna* are euryhaline and can live in all three salinity habitats.

Recently, two new species of killifishes from the genus *Austrolebias* (*Austrolebias botocudo* and *Austrolebias nubium*) were discovered 1000 meters above sea level in the Araucaria Forest domain in the highlands of southern Brazil (Lanés et al. 2021). Species from the genus *Austrolebias* and *Kryptolebias* from group #8 are non-migratory. While it is possible that *A. botocudo* and *A. nubium* became landlocked species by migrating to their present location, it could also be possible that these fish were entrapped in their highland environments as the receding waters of the Flood flowed off the South American continent. The species from this cluster belong to the family Rivulidae (killifishes), the fourth most diverse clade of Neotropical fishes. They have a characteristic annual life cycle, diapausing eggs, and delayed embryonic development. Two species of the genus *Kryptolebias* are self-fertilizing (hermaphroditic), whereas two genera from Cynopoecilini fertilize internally (Loureiro et al. 2018; Costa et al. 2016). Costa (2004) and Hertwig (2008) list 22 synapomorphic traits, mainly of the cranium, that characterize the family Rivulidae, suggesting that this family is discontinuous with all other fish.

The genus *Austrolebias* is an interesting monobaramin within Rivulidae. This baramin was the only one with an insignificant p-value (0.472), and a mean mtDNA sequence similarity of 0.566. These differences could be due to the high rate of base substitutions in their mitochondrial genomes (García et al. 2002). Furthermore, the genomes of *Austrolebias* species underwent large-scale expansions, and are approximately twice the size of the genomes of most rivulid species. The DNA content of  $5.95 \pm 0.45$  pg, compares to the mean C-value of other rivulids of 2.98 pg (García et al. 2014). This results in larger species diversity (Mank and Avise 2006). Indeed, of the 36 genera within Rivulidae, *Austrolebias* has the largest number of species (53), according to the National Center for Biotechnology Information (NCBI) Taxonomy Database (see Table 2).

## 7. Elasmobranchii

This subclass of Chondrichthyes consists of sharks, rays, skates, and sawfishes, and numbers around 1,150 species, making up around 3.3% of all fish. These fish form an apobaramin in that their skeleton is made up of cartilage, as opposed to bony fish (Osteichthyes). They are characterized by five to seven gill slits behind their head, rigid dorsal fins, and multiple rows of teeth. Their skin is also covered by tough dermal scales called placoid scales. Sharks make up the superorder Selachii, whereas rays, skates, and sawfishes make up the superorder Batoidea.

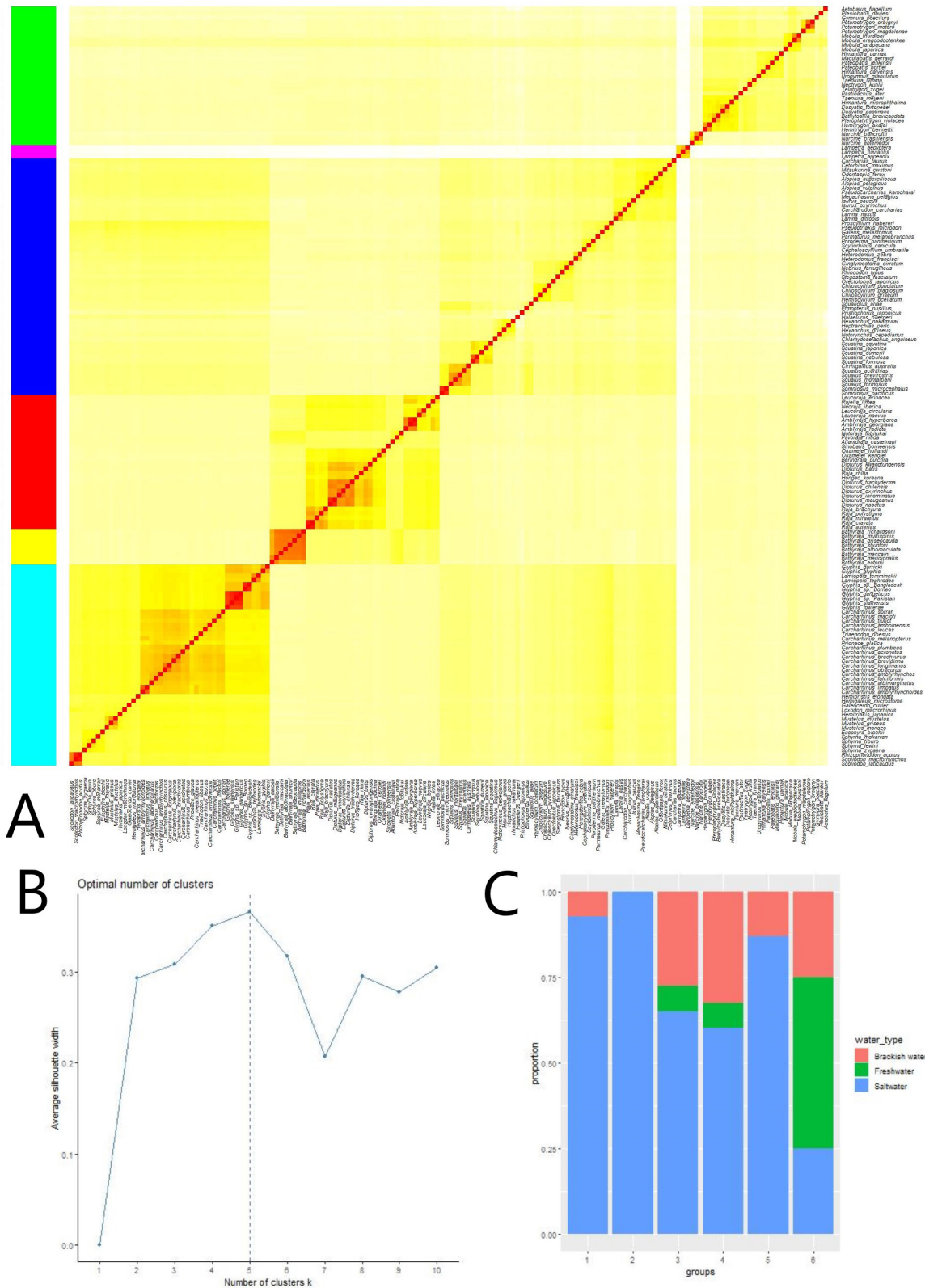
In this study, the mtDNA of 167 species was examined. The results can be seen in Figure 10 and are also available in Supplementary File 7. The heatmap in Figure 10A shows six putative baramins. However, the Silhouette plot shows five optimal clusters in Figure 10B, which indicates possible torsion in the data. The Hopkins clustering statistic is 0.890 which denotes very good clustering. Besides the outlier group, there are five putative holobaramins, each of which has statistically significant p-values.

While sharks and rays have different body plans, it might be the case that God created multiple shark and ray baramins, as suggested by

**Table 2.** Number of species in each of the 36 genera in the family Rivulidae.

Genus	No. species
<i>Austrolebias</i>	53
<i>Hypsolebias</i>	39
<i>Anablepsoides</i>	31
<i>Melanorivulus</i>	21
<i>Cynolebias</i>	18
<i>Cynodonichthys</i>	15
<i>Laimosemion</i>	14
<i>Kryptolebias</i>	9
<i>Austrofundulus</i>	8
<i>Cynopoecilus</i>	8
<i>Atlantirivulus</i>	6
<i>Moema</i>	5
<i>Ophthalmolebias</i>	5
<i>Spectrolebias</i>	5
<i>Rachovia</i>	4
<i>Rivulus</i>	4
<i>Simpsonichthys</i>	4
<i>Trigonectes</i>	4
<i>Xenurolebias</i>	4
<i>Campellolebias</i>	3
<i>Leptolebias</i>	3
<i>Nematolebias</i>	3
<i>Notholebias</i>	3
<i>Gnatholebias</i>	2
<i>Maratecoara</i>	2
<i>Neofundulus</i>	2
<i>Papiliolebias</i>	2
<i>Pituna</i>	2
<i>Plesiolebias</i>	2
<i>Pterolebias</i>	2
<i>Aphyolebias</i>	1
<i>Llanolebias</i>	1
<i>Micromoema</i>	1
<i>Millerichthys</i>	1
<i>Renova</i>	1
<i>Terranatos</i>	1





**Figure 10.** Results of the analysis of Elasmobranchii. A. Heatmap showing tentative baraminic relationships between several groups of species based on mtDNA sequence similarity. Darker, redder colors denote higher sequence similarity, thus common baraminic status, whereas lighter, yellower colors denote lower sequence similarities, and different baraminic status. B. Silhouette plot showing the optimum number of clusters in the mtDNA sequence similarity matrix. C. Barplot showing the different proportion of species living in freshwater (green), brackish water (red), and saltwater (blue) in the groups in the heatmap.

the evidence here. Molecular baraminological analysis of snakes and lizards showed that they are separate apobaramins, and that there are likely several snake and lizard baramins (Cserhati 2020). As such, putative elasmobranch holobaramins would be classified as type 3b Carter baramins.

The first three groups are rays, whereas the fourth and fifth are sharks. Two of the three ray baramins have a normalized entropy value of less than 0.25, indicating that the species in these baramins are adapted to either brackish or saltwater conditions. This is no surprise since sharks and rays are mainly marine animals. These animals are less adapted to freshwater, as they must constantly swim to keep afloat; the less dense freshwater makes this more difficult for them.

In contrast with the several putative baramins found within the Cyprinodontiformes apobaramin, it seems that the several Elasmobranchii groups have adapted to saltwater (see Figure 10C) after the initial euryhaline stage, as 74% of the species with FishBase annotation were oceanodromous.

Nevertheless, five shark species from group #4 and three ray species from group #3 can also inhabit freshwater. These species are *Carcharhinus leucas* (bull shark), *Glyphis fowlerae* (Borneo river shark), *Glyphis gangeticus* (Ganges shark), *Glyphis glyphis* (Bizant river shark), *Rhizoprionodon acutus* (milk shark), *Potamotrygon magdalenae* (Magdalena River stingray), *Potamotrygon motoro* (ocellate river stingray), and *Potamotrygon orbignyi* (smooth back river stingray). Of these, *C. leucas* is known to inhabit freshwater lakes in Mozambique, KwaZulu-Natal, Nicaragua, and Sydney Harbour, Australia, a large temperate estuary (Smoothey et al. 2019). This species leads a catadromous lifestyle, using natural rivers and estuaries as nursery grounds before migrating out to the sea (Werry et al. 2012). It is possible that as the Flood waters receded, separate groups of bull sharks could have been entrapped in inland lakes in these three locations. The capability of male great white sharks to undertake transoceanic migrations observed by Pardini et al. (2001). These animals could have been entrapped in these freshwater lakes recently since they have not had much time to diverge morphologically.

## 8. Pleuronectiformes

The order Pleuronectiformes consists of around 700 species such as flounders, turbot, and soles. These fish are characterized by the following synapomorphies: a flat body, with both eyes on one side of their head, with one of the eyes migrating to the other side of the head during development. Their dorsal fin is also positioned dorsal to their skull (Campbell et al. 2013). They also have a muscular sac in the eye called a recessus orbitalis, which can fill with fluid, thereby protruding the eyes above the plane of the fish's body (Chapleau 1993). No extant flatfish have been discovered that have intermediate skull morphology. These fish apparently form an apobaramin, as they are unrelated to all other fish. Evolutionists such as Lamarck (1809) hypothesized that the ancestors of flatfish lay flatly on the seabed in extremely shallow water. This is exceedingly hard to imagine, because, as such, flatfish ancestors would become prey animals that would be very easy to capture.

The mtDNA of 72 species of Pleuronectiformes was analyzed. The results can be seen in Figure 11 and are also summarized in Supple-

mentary File 8. The heatmap in Figure 11A shows six clusters. The Hopkins statistic is 0.809. The Silhouette plot (Figure 11B) shows a maximum value at 8 groups, although there may be distortion in the data. Just as with sharks and rays, flatfish also may form multiple baramins, despite their general morphological similarity with one another.

Besides the outlier group, there were four putative holobaramins with at least three species, each one of them with a statistically significant p-value. Groups #2 and #3, of 27 and 16 species respectively, both have a normalized water-type entropy value of just over 0.5. These fish live predominantly in saltwater.

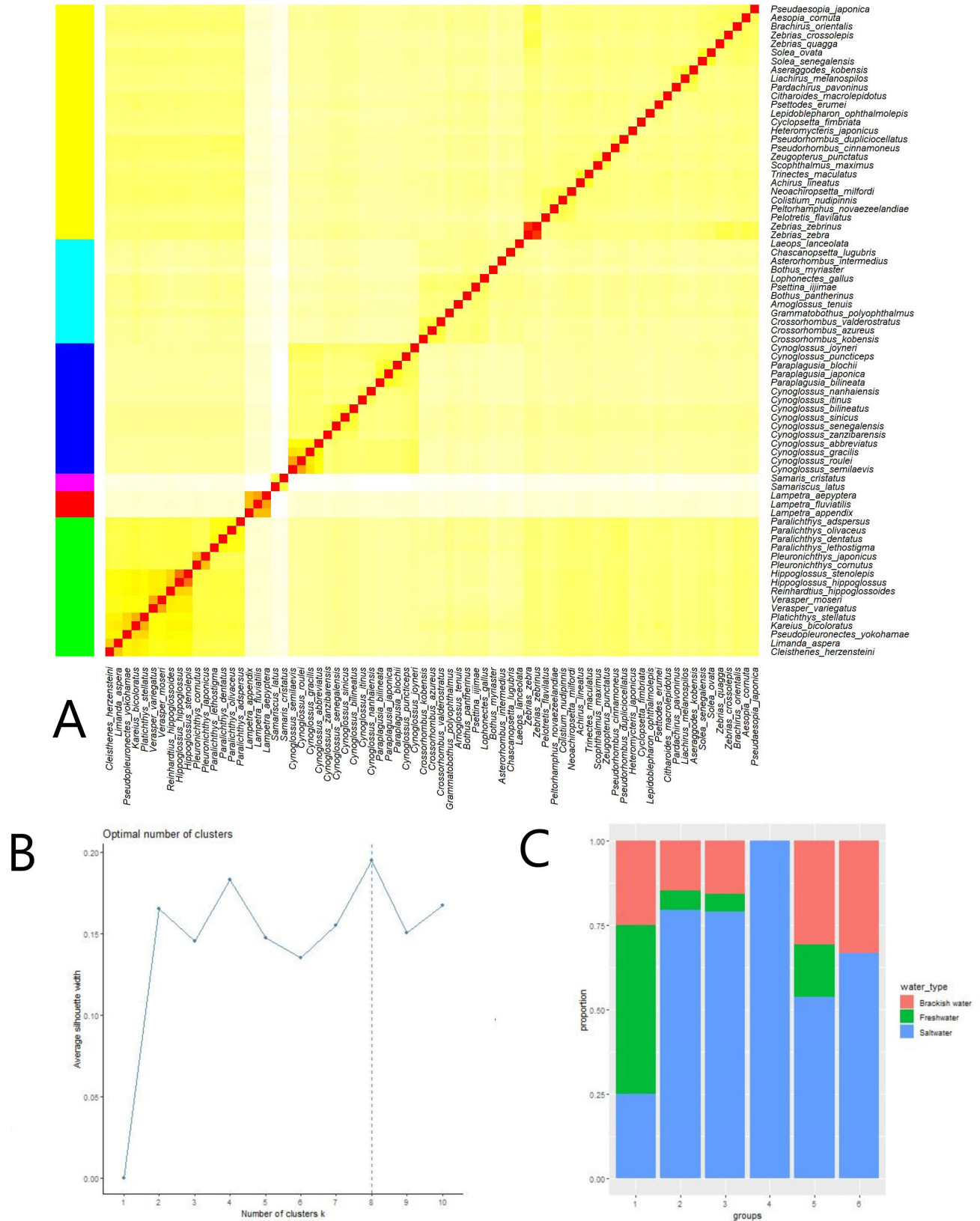
Group #4 with 12 species is comprised of species that exclusively inhabit saltwater environments (see Figure 11C); thus, their normalized entropy value is 0. The last group, with 15 species has a normalized water type entropy value of 0.896, with four, eight, and 14 species from the genera *Cynoglossus* and *Paraplagusia*, living in freshwater, brackish water, and saltwater respectively. This suggests that this putative baramin is still in the euryhaline stage.

It is noteworthy that here also the gene order of the mtDNA differs between groups #4 (*Arnoglossus*, *Asterorhombus*, *Bothus*, *Chascanopsetta*, *Crossorhombus*, *Grammatobothus*, *Laeops*, *Lophonectes*, and *Psettina*) compared to groups #2, #3, and #5 (see Figure 12). The individual mitogenomes of all Pleuronectiformes species analyzed in this study can be seen in Figure 12. The 3' end of the mtDNA in group #4 contains nine gene rearrangements compared to the mitogenomes of groups 2, 3, and 5. These genes are tRNA-Gln, tRNA-Ala, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Asp, NADH6, tRNA-Glu, and tRNA-Pro. *Asterorhombus intermedius* differs from the regular mtDNA gene order in that tRNA-Val has been inserted between the 16S rRNA and tRNA-Leu (Luo et al. 2019). Furthermore, it appears that the mtDNA of this archaeobaramin had two control regions, CR1 and CR2, one of which was differentially lost in some species and the other in other species (Li et al. 2015).

Group #6, made up of only two species (*Samaris cristatus* and *Samariscus latus*) also has a gene order configuration that is different from all the other groups. These fish live in deep-water benthic zones and inhabit only saltwater environments. Their mtDNA gene order signals discontinuity not only from other flatfishes but also from all other vertebrates, making them a truly unique group. For example, the mitogenome of *S. latus* has 39 genes (two rRNA genes, 24 tRNAs, 13 protein-coding genes), as well as a duplicated control region and a 376 bp non-coding region, inserted between tRNA-Phe and tRNA-Pro (Shi et al. 2014).

## 9. Salmoniformes

These fish include species such as salmon, trout, charrs, whitefishes, graylings, taimens, and lenoks. The mtDNA of 75 species was analyzed in this study. The results can be seen in Figure 13 and are also available in Supplementary File 9. The Hopkins clustering statistic is 0.885, which corresponds to very good clustering. The Silhouette plot in Figure 13B shows a maximum value at four clusters. There is a difference between the number of optimum clusters as shown in the elbow plot and the number of groups that seem to be present in the heatmap. The difference could be due to distortion in the data. However, besides the outlier group, six statistically significant putative



**Figure 11.** Results of the analysis of Pleuronectiformes. A. Heatmap showing tentative baraminic relationships between several groups of species based on mtDNA sequence similarity. Darker, redder colors denote higher sequence similarity, thus common baraminic status, whereas lighter, yellower colors denote lower sequence similarities, and different baraminic status. B. Silhouette plot showing the optimum number of clusters in the mtDNA sequence similarity matrix. C. Barplot showing the different proportion of species living in freshwater (green), brackish water (red), and saltwater (blue) in the groups in the heatmap.

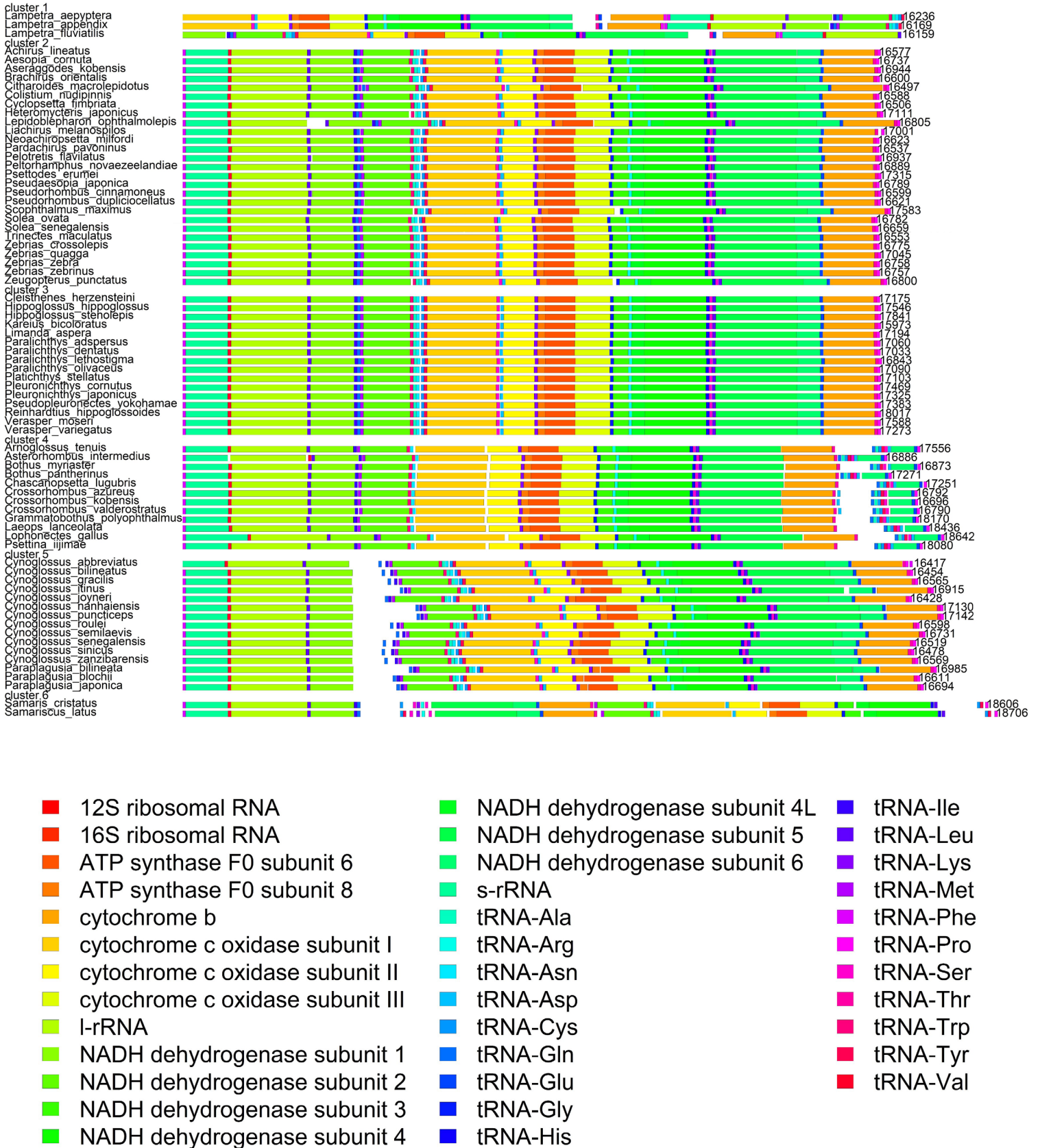
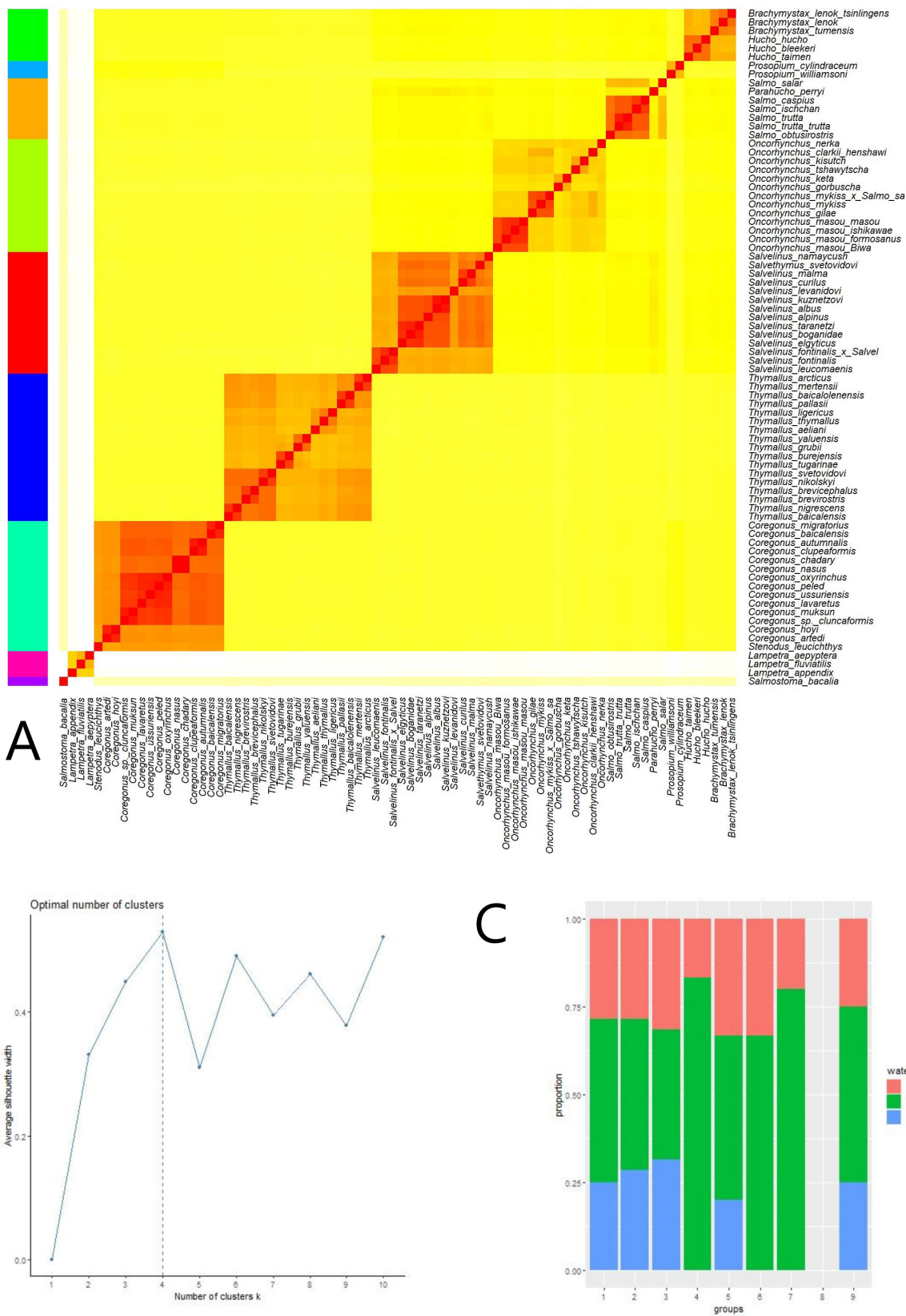


Figure 12. Gene order map of species from several putative groups found in the mtDNA analysis of Pleuronectiformes. The gene order may suggest a possible division of these species into groups.



**Figure 13.** Results of the analysis of Salmoniformes. A. Heatmap showing tentative baraminic relationships between several groups of species based on mtDNA sequence similarity. Darker, redder colors denote higher sequence similarity, thus common baraminic status, whereas lighter, yellower colors denote lower sequence similarities and different baraminic status. B. Silhouette plot showing the optimum number of clusters in the mtDNA sequence similarity matrix. C. Barplot showing the different proportions of species living in freshwater (green), brackish water (red), and saltwater (blue) in the groups in the heatmap.

holobaramins were discovered (see Figure 13A). These include the following groups: group #1: the genus *Salvelinus* (charrs or trouts), group #2: *Salmo* + *Parahucho* (salmon and taimens), group #3: the genus *Oncorhynchus* (Pacific salmon and Pacific trout), group #4: *Brachymystax* + *Hucho* (lenoks and taimens), group #5: *Coregonus* + *Stenodus* (whitefishes), group #7: the genus *Thymallus* (graylings).

Four of the six statistically significant groups have a normalized entropy value greater than 0.9. This means that the species in these four putative baramins are in the process of transitioning from euryhalinity towards stenohalinity. 27 of the 28 species that are anadromous according to the FishBase migration annotation come from these four groups. Groups #4 and #7, with normalized entropy values less than 0.5 have no species that live in saltwater. Only one of the eight species from these two groups with migratory annotation in FishBase lives in saltwater and one is potamodromous and three are non-migratory (see Figure 13C).

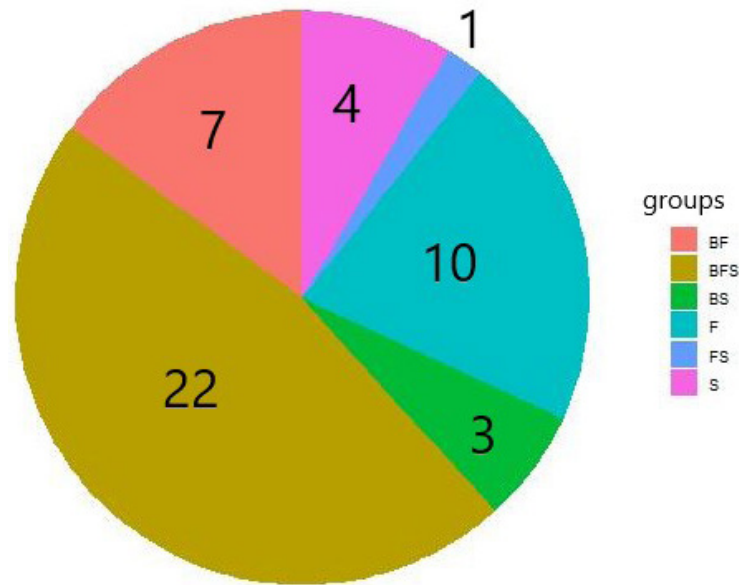
## CONCLUSIONS

The original question posed in this study was whether fish would have been able to survive different salinities during the Flood. The evidence presented here gives strong support to the idea that indeed this is possible. Even some evolutionary researchers think that euryhalinity was the ancestral state of many fish groups. According to some researchers, euryhaline species that inhabit estuaries are the most capable of invading new environments with different salinity levels (Lee and Bell 1999; Schultz and McCormick 2012). The great majority of fish before the Flood could have been euryhaline, allowing them to adapt to varying water salinity as they migrated into more specialized ecological niches. Different fish groups would then become obligatory saltwater or freshwater species after a period of adaptation, such as landlocking.

Some researchers suggest that the majority of extant fish are stenohaline (Gibbons et al. 2017). In contrast, as we have seen here, 46.8% of all putative groups found in this study (22/47) are euryhaline, where a euryhaline group is defined as having at least one species from saltwater, brackish water, and freshwater (see Figure 14). This also indicates that not much time has elapsed since the Flood since most kinds are still in transition from euryhalinity to stenohalinity. If longer time had elapsed, then we would expect to see the great majority of species having adapted to either freshwater or saltwater environments.

In Figure 14 we can also see that seven groups have species adapted to brackish water and freshwater, three adapted to both brackish water and saltwater, and one adapted to both freshwater and saltwater. In total, 33 out of 47 groups (70.2%) have lost the ability to adapt to one of the environments. In comparison, 22 of the 47 groups (46.8%) are 'still' adapted to all three water environments. This indicates that some of the species in many groups are still transitioning towards either freshwater or marine environments, whereas almost half are still undifferentiated. The species from ten groups live exclusively in freshwater, and four groups inhabit marine environments only. This means that only 29.8% of all groups have fully adapted to only one environment. Variation of freshwater and saltwater species within a group (a baramin) may also imply rapid adaptation by these species.

The Flood also serves as a good explanation as to why there are



**Figure 14.** The number of putative holobaramins discovered in this study that contain species that live in one or more water salinity categories. F = freshwater, B = brackish water, S = saltwater.

freshwater species within putative baramins with a majority of saltwater species. For example, shark species such as *C. leucas* can live in freshwater environments, such as lakes in Nicaragua. It also explains why certain freshwater *Clupea* species live in Lake Tanganyika, whereas their marine counterparts have not yet diverged from them morphologically. What this may mean is that during and after the Flood, different fish kinds were able to differentiate into either freshwater or saltwater specialists. It is possible that the euryhaline ancestor of species within a kind had a more robust (diverse) genome, which allowed it to survive in environments of varying salinities. Those extant stenohaline species that occupy only freshwater and saltwater niches most likely underwent gene depletion and lost part of their genetic machinery that allowed them to survive in saltwater or freshwater environments.

The holobaramins defined here are tentative and need further verification. The mtDNA is only a small fraction of the genome, and thus inferences based on sequence similarity are limited. As we have seen in the case of the three rivulid species, mtDNA substitution rates can vary.

It is also worth noting that in some cases, the gene order (or gene configuration) of the mtDNA might be useful to help delineate between kinds, as we have seen in the case of Anguilliformes and Pleuronectiformes. Based on sequence similarity as well as gene order, *N. erythrosoma* may be classified in its own holobaramin, even into its own family. Since there was little time since the Flood for mutations to accumulate in the mtDNA, the configuration of genes in the mtDNA, mtDNA sequence similarity, the length of the mitochondrial genome, and GC% (the percent of G's and C's in the genome) are very similar among species within the same holobaramin. We must note here that mutations themselves are only indicative of divergence from an ancestral mtDNA state. It has been shown that a very large proportion of vertebrates have a highly similar mtDNA gene order

(Liu et al. 2005). As such, differences compared to the general vertebrate mitochondrial gene configuration might possibly be used to discern between holobaramins in some cases.

This phenomenon is somewhat rare, as we have seen that in only three of 49 cases the gene order differs significantly in one baramin compared to other baramins. Besides *N. erythrosoma*, the gene order of two species of deep-sea gulper eels (Saccopharyngiformes), namely *Eurypharynx pelecanoioides* (the pelican eel), and *Saccopharynx lavenbergi* (the whiptail gulper) also diverge from the typical vertebrate mtDNA gene order (Inoue et al. 2003). In these two species, two gene groups, namely NAD5 and NAD6 as well as ATP8, ATP6, COIII, and NAD3 are positioned to the left of the gene group NAD2, COI, and COII.

Lastly, in several of these groups (eels, sharks, rays, flatfish), the use of mtDNA sequence similarity may be useful in delineating multiple putative holobaramins within these apobaramins. However, it may also be the case that mtDNA shows the existence of different sub-lineages within the same holobaramin. This highlights the utility of using molecular data in baraminology studies to discern possible molecular discontinuity beyond the available morphological discontinuity. Since we do not know the exact identity of the fish kinds created during Creation Week, it may be possible that multiple eel, shark, ray, or flatfish kinds were created. These kinds may look similar to one another externally, but their mtDNA sequences are different. However, molecular data must be augmented by other lines of evidence, such as morphological data.

## NOMENCLATURE

**Allogenic parasite:** a species of parasite that lives in both freshwater and saltwater.

**Amphidromous:** fish that migrate between saltwater and freshwater without the intent of spawning. This may involve laying eggs in freshwater estuaries, which then float out to sea.

**Anadromous:** fish that migrate from freshwater to saltwater in order to spawn.

**Apobaramin:** a group of one or more baramins, which is outwardly discontinuous with all other species.

**AQP:** Aquaporin, a cell membrane channel that allows water to cross the cell membrane.

**Baramin:** a created kind, a reproductive community, with inward continuity and outward discontinuity between species. This is true of the baramin created during Creation Week, however, by today these kinds may have differentiated so that there are reproductive barriers between groups within the same kind.

**Brackish water:** water that has a salinity level between that of freshwater and saltwater, from 0.5–29 ppt (parts per thousand) of dissolved salts.

**CAT:** catalase enzyme.

**Catadromous:** fish that migrate from saltwater to freshwater in order to spawn.

**CFTR:** cystic fibrosis transmembrane conductance regulator.

**DEG:** differentially expressed gene.

**Diadromous:** fish that migrate between saltwater and freshwater in order to spawn.

**Euryhaline:** an aquatic organism that can live in both freshwater and saltwater.

**Freshwater:** water that contains less than 0.5 ppt of dissolved salts.

**GPx:** glutathione peroxidase enzyme.

**GR:** glutathione reductase enzyme.

**Holobaramin:** the complete set of known organisms that make up a baramin.

**Hypertonic:** high salt concentration.

**Hypotonic:** low salt concentration.

**Monobaramin:** a group of species that are continuous with one another without regard to continuity with all other species.

**mtDNA:** mitochondrial DNA.

**NCC:** Na<sup>+</sup>/Cl<sup>-</sup> cotransporter.

**NHE3:** apical Na<sup>+</sup>/H<sup>+</sup> exchanger 3.

**NKA:** sodium-potassium ATPase (NKA) channel.

**NKCC1:** Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> cotransporter 1.

**Oceanodromous:** a fish species that complete its entire life cycle in saltwater.

**Potamodromous:** a fish species that complete its entire life cycle in freshwater.

**ROS:** reactive oxygen species.

**Stenohaline:** an aquatic organism that can tolerate only a narrow range of salinity.

**Saltwater:** water that contains >29 ppt of dissolved salts.

**SOD:** superoxide dismutase enzyme.

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