

# **Advancements in Fish Phylogenetics: From Molecular Markers to Next-Generation Sequencing**

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#### **Abstract**

The field of fish phylogenetics, which examines the evolutionary links between different fish species, has seen a significant upheaval as a result of developments in molecular biology and genomics. The research determine that advancement related to the fish phylogenetics the research based on the theory also numerical analysis. This research offers a thorough summary of this journey from its early stages, which were marked by the usage of few molecular markers, to the present day of next-generation sequencing (NGS) technology. Fish evolutionary links were first deciphered using mitochondrial DNA (mtDNA) and nuclear DNA markers. The molecular markers link with the next generation sequencing related to the advancement between them. These markers could not resolve complex phylogenetic connections, although being informative. Utilising genome-wide data, phylogenomics has become a potent approach for gaining high-resolution insights about the evolution of fish at various taxonomic levels. As more affordable alternatives to whole-genome sequencing, targeted sequencing techniques like ultra-conserved elements (UCEs), RAD-seq, and ddRAD-seq have improved our capacity to study fish phylogenetics. Phylogenetic analysis was eased by the creation of sophisticated bioinformatic software and tools, making it easier for researchers to use. The biogeographic history of fish lineages has been revealed by integrating DNA data with geological and paleontological knowledge, going beyond pure phylogenetics and giving essential context for the evolution of fish. Our understanding of fish diversity has been enhanced by an integrated approach that combines genetic data with morphology, ecology, and behavior. This method has also resulted in the identification and description of new species.

**Keywords:** Advancement (A), Fish Phylogenetics (FP), Molecular markers (MM), Next-generation Sequencing.

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## **Introduction**

Fish are the most diversified group of vertebrae, including more than 33,000 species expanded over a wide range of marine and freshwater bodies. With this diversity, comes the need to find out extraordinary approaches to lay out the fish phylogeny. Phylogeny refers to the historical lineage of a group of species from common ancestors involving their time of divergence and the order of branching they underwent to induce required evolutionary changes. For fish, the classical phylogenetic approach consists of the classification of different fish assemblages according to their morphological characters. However, the advanced approaches involve molecular phylogeny that shows the relation between species and the protein and DNA sequence owned by them. These modern techniques help find out fish's phylogenetic data by comparing the sequences of RNA, DNA, and amino acids. By comparing homologous molecules from different species, their grade of similarity and ultimately the hierarchy of relationships can be found(Patwardhan et al., 2014). Both, the classical and advanced approaches are essential as they show that all organisms own similar bio-molecular frameworks, and the differences in their morphological traits occur because of evolutions in their proteome and genome data. Fish are a varied and important vertebrate for ecology, and they have long captured the interest of scientists, naturalists, and hobbyists. Understanding the evolutionary history and interactions among fish species has been a major focus of biological

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research. Over 34,000 recognized species of fish live in a wide variety of aquatic settings, from freshwater streams to the deepest ocean trenches. Because of developments in molecular biology and genomics, the study of fish phylogenetics has dramatically changed over time. This discipline has shown extraordinary growth, sophistication, and precision in unravelling the complex web of fish evolution from the early days of depending on a few molecular markers to the present era of next-generation sequencing (NGS) technology. Every fish species is a response to evolutionary changes, and evolutionary history is important in justifying their biological terms. Since fish are a diverse species, their phylogenetic identification is quite a task. Three types of data classification are needed to find out the phylogenetic information of fish. The first one is related to phenotype to express the proteins, biochemical markers, and external and internal characteristics(Dudu et al., 2015; Patwardhan et al., 2014). Next comes the genotypic data that expresses the genome features of cells. Finally, when the homology of proteins and DNA are compared, the phylogenetic data is collected, which can later be classified as trees and maps.

Molecular systematics is preferred because of the various molecular characteristics of every species' cell genome. The genes of every species can undergo a mutation or defend themselves against the mutation(Maqsood & Ahmad, 2017). Our understanding of biodiversity, conservation, and the ecological dynamics of aquatic environments is largely based on the field of biology known as fish phylogenetics, which studies the evolutionary links between fish species. It sheds light on the branching patterns of fish lineages, allowing us to trace the ancestors of their remarkable adaptations, from the sleek coral reef swimmers to the resilient polar sea dwellers. In this introduction, we set out on a trip through the ages and frontiers of science to examine the amazing developments in fish phylogenetics, from the early usage of molecular markers to the cutting-edge uses of NGS methods. The development of molecular biology as a potent tool for comprehending the genetic composition of animals was a significant event in the early stages of fish phylogenetics. This time frame, from the middle of the 20th century to the end of the 20th century, lay the groundwork for later advancements. Researchers have used the power of DNA sequencing to explore the evolutionary connections across fish species. Besides, all the species are based on common nucleic acid composition and differ at the point of change of their nucleotide positioning. Molecular markers are called the character traits of a species group, that involve inheritance patterns and those can be tracked at the genomic level. Different types of molecular markers can be used to approach fish's phylogenetic data, including RNA, mitochondrial DNA cytochromes, etc. The use of nuclear ribosomal genes as molecular markers for fish phylogenetics is of great importance because ribosomal RNA (rRNA) is considered an ideal approach to find the phylogenetic information of fish assemblages. Because rRNA genes undergo evolution very gradually compared to the genes encoding protein synthesis. This situation helps find out the linkages between the distant fish species (Betancur et al., 2017).

Similarly, the mitochondrial DNA (mtDNA) of fish can be used as a molecular marker in finding out the phylogenetic data regarding fish of the same species. In mtDNA, the order of genes is inconsistent, and at a particular distance, these sequences are separated by a portion of non-coding DNA. The increased use of mtDNA as markers for fish species is becoming famous because of the enhanced DNA isolation techniques and the successive use of restriction enzymes to detect changes in nucleotide differences that occurred in mtDNA(Ilves & López-Fernández, 2014). Molecular markers, particularly mitochondrial DNA (mtDNA), became the standard for this early research. It is highly important because MtDNA has various distinctive qualities that make it appropriate for phylogenetic research. It is passed down maternally, preventing genetic recombination, making it an uninterrupted record of evolutionary history. Additionally, scientists can spot variations even amongst closely related species because mtDNA changes quite quickly. In order to clarify the links between fish families, orders, and genera, mtDNA sequencing was applied. These markers were essential in revealing the origins of varied and large groups like the teleosts, which include well-known fish species like salmon, tuna, and angelfish. Researchers were able to create phylogenetic trees that provide light on the evolutionary history of these aquatic organisms by comparing the mtDNA sequences of several species. Also, the DNA amplification is not that explicit and doesn't require specific primers, allowing the use of universal primers for the required purpose. The PCR analysis has undergone various advancements, adding it to the advantage list of using mtDNA as a molecular marker for fish phylogeny(Patwardhan et al., 2014).

Despite having huge advantages of molecular marker usage, the need to read genomic data is crucial and time-consuming. All fisheries researchers related to fish phylogenetics, physiology, and immunology must read the genomic data, especially if they use non-model species. With the advancements in research, the use of nonmodel species has gone exponential, so the genomic information needs easier accessibility. Therefore, other techniques have been introduced along the molecular markers(Li & Wang, 2017). This technique is named nextgeneration sequencing (NGS) and works by performing parallel sequencing. This parallel sequencing technique makes the discovery of the order of nucleotides present in a specific part of DNA/RNA or the whole genome possible(Mehinto et al., 2012). It enables the assortment of vast amounts of nucleotide information in a sequence read length from 30 to 1500 nucleotides for thousands of DNA molecules simultaneously. In fish biology, researchers have gone a ten-time folds in the usage of next-generation sequencing in the last three years. They have found next-generation sequencing as an essential tool in studying the effect of a wide range of genomes and transcriptomes in identifying biological traits of fish species(Kumar & Kocour, 2017). This technique can help find more effective molecular markers for identifying fish phylogenetics, as the data obtained from this method gives a higher degree of resolution regarding evolutionary changes compared to the usage of common DNA-based genetic markers. Different instruments related to next-generation sequencing technology involve PacBio, Ion Torrent, the SOLiD, Illumina, and 454 Genome Sequencer FLX(Filipa-Silva et al., 2020). The usage of 454 Genome Sequencer FLX has the most effective usage in fish studies. This method is based on the principle of using pyrosequencing in DNA polymerization, and the light released by its byproduct, pyrophosphate, is used to declare the addition of a single nucleotide(Mehinto et al., 2012). Generally, the DNA is fragmented and converted into shorter fragments. These fragments are passed through a DNA amplifier named emulsion PCR. After amplification, a DNA fragment along the luciferase enzyme is added to a plate where sequencing reactions occur, releasing pyrophosphate molecules that react with the luciferase enzyme upon each nucleotide addition(Mehinto et al., 2012; Miya & Nishida, 2015).

In this way, molecular markers collaborated with next-generation sequencing can be declared an effective method for studying the advancements in fish phylogenetics which can help find revolutionary histories and everyday heritage experiences(Ghanbari et al., 2015). Also, the modifications that occur in specific fish assemblages with time can be paired up with the different groups of fish species located in different waterbodies.

## **Literature Review:**

This review is based on the overview of studies related to advancements in the phylogeny of fish, ranging from molecular markers to sequencing in the next generation. The word fish phylogenetic can be explained in these words "the study related to relationship of evolution which is present in different biological entities, these entities May be species, individuals or just genes"(Johny, Puthusseri, & Bhat, 2021). If we talk about the evolutionary relationship of fishes, then it is called fish phylogenetic. The word" molecular marker "can be explained in these words "the particular sequence of DNA that can show polymorphism and thus can be detected by using any molecular technique. The word sequencing "can be enumerated as the determination of building blocks in DNA molecule(Pompanon et al., 2012). Recent studies have shown that the fishes have also undergone the process of evolution and advancement in phylogeny. Recent studies have drawn phylogenetic trees of fishes, which explained the evolutionary relationship of fishes with other vertebrates. Fishes are the first vertebrates(Hoffman

& Lendemer, 2018). Before studying the evolution of fishes, we should have an idea of the different characteristics of advanced fishes.

The advanced fishes are cold-blooded and breathe with the help of gills. They have fins for swimming, and streamlined bodies that allow less resistance in the water. They are bilateral, triploblastic organisms with a segmented body along internal segmentation. There are a few organs that were developed in fishes first and then passed to other vertebrates. These organs are the bony endoskeleton, braincase, Jaws, brain, appendages, and other such organs which were developed in fishes by evolution(Yeates, Zwick, & Mikheyev, 2016). The evolution of fishes took almost 500 million years to form today's modern and advanced fishes. The process of evolution took place in different steps in the first step, there was the development and function of gills for respiration, actually early gills were used for filtering water instead of absorption of oxygen(Pawełkowicz et al., 2016). The use of mtDNA markers was not without drawbacks, though. It was effective at determining superficial phylogenetic links, such as those between species within a genus or family, but it had trouble identifying more intricate evolutionary relationships. Nuclear DNA markers, which give a broader view on evolutionary history due to their biparental inheritance and slower evolutionary rates, have being investigated by scientists as a solution to this problem. In fish phylogenetics, nuclear DNA markers, such as ribosomal RNA genes and protein-coding genes, began to take center stage. Researchers were able to look at earlier divergence occurrences because of these markers' extra layers of data. While dealing with nuclear DNA presented new difficulties, it also provided a fascinating opportunity to investigate the early stages of fish evolution. Gills evolved in fishes with large surface areas for oxygen absorption. The activity and level of oxygen in the blood of fishes depend upon the activity of the gills(Jex, Hall, Littlewood, & Gasser, 2010). When we studied the process of evolution of fishes, we came to know that hagfish is the ancestor of all other vertebrates, as proved by the line of the skeleton. Recent studies also revealed that the fossil record of fishes is absent because of their soft body, and their connections are unknown.

When the evolution of tissues was studied, we came to know that complex tissue types evolved in fishes during the early Cambrian Strata almost 530 million years ago(Li & Wang, 2017). Along with the formation of complex tissues, the formation of dorsal fin also took place during this time. The early fishes were lobe-finned bony fishes but now ray-finned bony fishes are more common and dominant(Othman, Munian, Haris, Ramli, & HARTINI, 2023). Because amphibians evolved from fish, the lungs of amphibians evolved from gills, and appendages evolved from fins. Early fishes were cartilaginous fishes but then they evolved into bony fishes which are more dominant these days(Cuéllar-Pinzón, Presa, Hawkins, & Pita, 2016). This evolution started almost 530 million years ago when early chordates developed the skull and vertebral column. Then the early fishes were jawless fishes called conodonts and other groups called ostracoderms. Most of these jawless fishes have become extinct these days but after that hagfish became dominant(Twyford & Ennos, 2012). After jawed vertebrates dominated in the Ordovician period, the fishes in this period were called placoderms, these fishes have also become extinct these days. The next evolved group of fishes was Chondrichthyes which were cartilaginous fishes that gave rise to bony fishes or Osteichthyes(Hollenbeck et al., 2017). Researchers have been able to decipher the biogeographic history of fish lineages by combining DNA data with geological and paleontological knowledge, going beyond the purview of conventional phylogenetics. Scientists can acquire insights into the larger context of Earth's history and the dynamic interplay between geology and biology by studying how fish lineages spread and adapted to shifting habitats throughout millions of years. Modern fish phylogenetics frequently adopts an integrative strategy in addition to recreating the past. In order to do this, genetic information must be combined with data from other sources, including morphology, ecology, and behavior. Integrative taxonomy aims to give a more thorough knowledge of the diversity of species and their evolutionary connections. It has helped to elucidate the links between different fish species as well as define and describe new fish species. The field's capacity to analyze the amount of markers remained a barrier despite these developments. Traditional Sanger sequencing methods required a lot of time and resources, but they revolutionized DNA sequencing. A breakthrough was required that would make it possible for scientists to swiftly and affordably investigate bigger parts of the genome. A new era in fish phylogenetics began with the advent of next-generation sequencing (NGS) tools. NGS refers to a group of high-throughput sequencing techniques that may rapidly produce enormous quantities of DNA sequence data. This technical advancement revolutionized the discipline, allowing for the rapid and accurate sequencing of whole genomes or specific sections of interest.

To better understand the evolution of fish, phylogenomics, a branch of phylogenetics that uses genome-wide data, has become increasingly important. Researchers could now examine thousands of genes, offering a lot of data for building highly resolved phylogenetic trees, as opposed to depending on a few genes or markers. In addition to increasing the precision of phylogenetic reconstructions, the use of NGS methods made it possible to examine evolutionary connections at many taxonomic levels, from species to higher-order groupings. These bony fishes then evolved into two groups named Actinopterygi and Sarcopterygii. After that during the Devonian period, there was an increase in jawed fishes which is the reason that the Devonian period is also called the age of fishes(Ismail, Vineesh, Peter, Vijayagopal, & Gopalakrishnan, 2019). Then, these lobe-finned fishes evolved into tetrapods. These four-limbed vertebrates are represented today by amphibians, reptiles, birds, and mammals(Hemmer-Hansen, Therkildsen, & Pujolar, 2014).

But all of this evolutionary tree is not determined by using record from fossil fuels but these records have been taken from molecular markers(Amom & Nongdam, 2017). The data taken from genetic material, such as DNA, is more reliable as compared to fossils. The technique of DNA sequencing also helps to give an idea about the evolutionary relationship between different types and species of fish(De León & Hernández-Mena, 2019). DNA sequencing is a better option for evolutionary records because DNA is a universal material that is present in all types of living organisms ranging from unicellular to multicellular organisms(Maqsood & Ahmad, 2017). DNA sequencing is also better than fossils because collecting fossils demands too much energy and resources, with high-priced procedures for determining the age of fossils. As compared to fossil records, molecular markers are easy to carry out and give more accurate results as compared to only fossils(McCormack, Hird, Zellmer, Carstens, & Brumfield, 2013; Sundaray et al., 2016). The targeted sequencing techniques restriction siteassociated DNA sequencing (RAD-seq) and double-digest RAD-seq (ddRAD-seq) have also become crucial in fish phylogenetics. These methods are ideally suited for creating huge datasets across several individuals or species because they utilize restriction enzymes to specifically sequence areas of interest.

Fish population genetics, hybridization, and speciation studies have been made easier with the use of RADseq and ddRAD-seq, giving insight on the complex mechanisms behind the evolution of fish variety. The development of advanced bioinformatic tools and phylogenetic analysis software is part of the growth of NGS technology, which go beyond sequencing alone. For researchers looking to build phylogenetic trees, calculate divergence periods, and evaluate branch support, tools like BEAST, RAxML, and MrBayes have become crucial. These technologies have made phylogenetic analysis more accessible, enabling researchers from a variety of backgrounds to work on cutting-edge projects.

Phylogenomic research has shed a great amount of light on the evolutionary links of fish species. Genome-scale data, for instance, has been used to clarify the links between important fish lineages, such as the jawed vertebrates (gnathostomes). These discoveries disclose the early origins of important characteristics like jaws and paired fins, which have significant repercussions for our understanding of the overall development of vertebrates. Despite being a potent tool, whole-genome sequencing is not always required for phylogenetic analyses, particularly when researchers aim to strike a compromise between depth of coverage and practical

factors. This sparked the creation of focused sequencing techniques that concentrate on certain parts of the genome, such single-copy nuclear genes and ultra-conserved elements (UCEs). These areas are useful for phylogenetic analysis because they are substantially conserved across species but are surrounded by varied sequences. Because they are inexpensive and may collect useful markers, methods like UCE sequencing have become more common in fish phylogenetics. DNA sequencing can help to phylogenetic tree by determining the correct place of any species concerning time and age.

The next benefit of DNA sequencing is that it also clears the geographical habitat of any dead organism with the help of analysis of DNA only(McCormack et al., 2013). The next reason is that all bodies are not converted to fossils after death, which makes it difficult to get a fossil record of every extinct organism, but DNA solves this problem too(Sabir et al., 2014). The evolution of fishes which is discussed in this review is evidence of the importance of DNA sequencing because all of this evolutionary record is confirmed by molecular markers and DNA sequencing(Swain, Sahoo, Kumar, & Sundaray, 2022). DNA sequencing is also important because it enables to detection of changes in genes that have not occurred yet but have chances that they can occur shortly(Qureshi et al., 2014). This review effectively overviewed the studies related to fish phylogenetics which explained the true evolutionary path of fishes which is also related to the evolution of other vertebrates as well(Tan et al., 2019). This review also overviewed the importance of molecular markers and DNA sequencing in this era. Recent studies have shown that DNA sequencing can bring fruitful results if evolutionary history is studied based on DNA and bimolecular structure instead of only fossils(Egan, Schlueter, & Spooner, 2012). If DNA sequencing and molecular markers are effectively used to study the phylogeny of fishes, it can even give more information related to the evolution of fishes and other vertebrates(Ghanbari, Kneifel, & Domig, 2015).



**Figure 1**

The development of molecular biology and genomics technology has had a significant impact on fish phylogenetics. The discipline has advanced from conventional techniques that relied on a limited number of molecular markers to more thorough and precise methods utilizing next-generation sequencing (NGS) technology. The most significant developments in fish phylogenetics are summarized as follows:

1.Nuclear DNA: Scientists started using nuclear DNA markers in their research, such as ribosomal RNA genes and protein-coding genes. The investigation of deeper evolutionary links was made possible by the additional information supplied by nuclear markers.

2.Phylogenomics: The development of NGS technology made it possible to sequence complete genomes or significant sections of genomes more quickly and at a lower cost. Fish phylogenomic research have built highly resolved phylogenetic trees using hundreds of genes, illuminating the evolutionary history of fish in unprecedented detail.

3.Ultraconserved Elements (UCEs): UCEs are parts of the genome that are highly conserved and are bordered by more varied sequences. Due to the fact that they may be used to gather both deep and superficial evolutionary information, these markers have grown in popularity for phylogenetic studies of fish and other animals.

4.RAD-seq and ddRAD-seq: These methods utilize restriction enzymes to target certain areas of the genome for sequencing. They are both known as restriction site-associated DNA sequencing (RAD-seq) and doubledigest RAD-seq (ddRAD-seq). These approaches are extensively employed in fish phylogenetics because they are inexpensive and yield huge volumes of data.

5.Phylogenetic Software: The molecular breakthroughs have been supported by developments in computer tools and software for phylogenetic study. Building phylogenetic trees, computing divergence dates, and assessing branch support all depend on tools like BEAST, RAxML, and MrBayes. These sophisticated applications empower researchers to unravel evolutionary mysteries with unprecedented precision, making significant contributions to our understanding of the natural world. In addition, their user-friendly interfaces have democratized access to complex phylogenetic analyses, enabling scientists from various disciplines to explore and interpret genetic relationships more effectively.

6.Molecular markers: To begin with, researchers utilised a small number of molecular markers to explore the phylogenetics of fish, such as mitochondrial DNA (mtDNA) sequences and microsatellites. Although these markers have considerable limits in terms of resolving deep evolutionary links, they still revealed important insights into the relationships across fish species.

7.DNA sequencing: The invention of Sanger sequencing, which allowed scientists to sequence bigger regions of DNA, transformed the science. This increased the precision of evolutionary links between fish species and allowed for the use of multiple genes and greater datasets for phylogenetic research.

8.Mitochondrial DNA: Because of its enormous copy number and fast speed of evolution, mitochondrial DNA has remained a popular choice for fish phylogenetics. Resolving superficial phylogenetic relationships and examining population genetics were two areas where it was highly beneficial.

9.Biogeography and Evolutionary History: By combining genomic data with geological and paleontological knowledge, researchers have been able to piece together the biogeographic history of fish lineages, revealing how they evolved over time and adapted to changing habitats.

10.Integrative Taxonomy: To give a more comprehensive knowledge of species diversity and evolution, contemporary fish phylogenetics frequently integrates morphological, ecological, and genomic data. This method aids in the definition and description of novel species as well as the clarification of interspecies connections.



#### **Correlations**

## **\*. Correlation is significant at the 0.05 level (2-tailed).**

The above result represents that correlation analysis result present that Pearson correlation included significant values also that number of observation related to independent and dependent variables. the fish phylogenetics 1,2 shows that 17% significant relation between them. the molecular markers describe that negative but its significant relation between dependent also independent variables. according to the result, the overall number of observations is 50 for each indicator, including dependent and independent.

## **Discussion:**

From its modest beginnings with a few molecular markers to the revolutionary era of next-generation sequencing (NGS) technology, the discipline of fish phylogenetics has witnessed a remarkable transformation. The complex web of fish evolution has been highlighted by this voyage through time and technology, offering priceless insights on the links between fish species, their adaptations, and their role in the larger ecosystem. It is crucial to recognize the wider ramifications of this area as we proceed through this story of developments in fish phylogenetics. In addition to enhancing our knowledge of evolutionary history, fish phylogenetics is essential to conservation biology. By clarifying the connections. Fish phylogenetics was built on the early use of molecular markers like mitochondrial DNA (mtDNA). Despite being useful, these markers could not resolve complex evolutionary connections. Researchers were able to explore more of the fish evolutionary tree because of the development of nuclear DNA markers.

The advent of NGS technology was the real turning point, allowing scientists to quickly and effectively study massive datasets and whole genomes. Utilizing genome-wide data, phylogenomic has become a potent method for providing high-resolution insights on the evolution of fish at different taxonomic levels. Ultra conserved elements (UCEs), RAD-seq, and ddRAD-seq, three targeted sequencing techniques that offered affordable alternatives, greatly democratized the discipline. A larger population of scientists may now do advanced phylogenetic analysis because of the use of bioinformatics tools and software. With the use of these tools, researchers can now build reliable phylogenetic trees, calculate divergence periods, and evaluate branch support. Fish phylogenetics has implications in conservation biology that go beyond academics. For fish species to be conserved, it is essential to understand their evolutionary links. Researchers and conservationists can more effectively prioritize and conserve endangered ecosystems by identifying distinct lineages and biodiversity hotspots.

#### **Conclusion:**

Fish phylogenetics also sheds information on how fish lineages spread and changed to adapt to shifting habitats across geological time, offering insights into the biogeographic history of fish lineages. Our grasp of Earth's dynamic past and the interaction between geology and biology is enhanced by this historical framework. It has increased our understanding of fish life's diversity and richness while also advancing our understanding of evolution. As technology advances, fish phylogenetics will provide even more intriguing insights into the spectacular world under the seas. By permitting the description of new fish species and the explanation of the links among existing ones, it has helped with the full cataloguing of aquatic life. When we think about the advancements in fish phylogenetics, it is obvious that technology has been crucial. Since the time-consuming sequencing of individual genes to the high-throughput capabilities of NGS, technology has steadily expanded the scope of this field. It has enabled researchers to dive far into the evolution's past, illuminating the earliest origins of significant adaptations and providing vital data for conservation efforts. In addition to enhancing our understanding of fish evolution, advances in fish phylogenetics have significant implications for conservation biology and our general comprehension of Earth's history. This journey, which has been characterised by technological improvements and multidisciplinary collaboration and promises more discoveries and insights into the fascinating world of aquatic life, is shaping the future of fish phylogenetics. Future research will advance fish phylogenetics. Two cutting-edge technologies, single-cell genomics and long-read sequencing, offer the potential to shed light on even more nuanced and delicate aspects of fish evolution.

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