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Mitochondrial Genome-Based Phylogenetic Reconstruction and Taxonomic Classification of Siluriformes

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Abstract Siluriformes (commonly known as catfishes) are one of the most diverse groups of freshwater fishes in the world. Their evolutionary history and taxonomic relationships have always been a hot topic in systematic taxonomy and biogeography. In recent years, mitochondrial whole genome data have been widely used in fish phylogenetic analysis and taxonomic reconstruction studies because of their rich genetic information. This study takes Siluriformes as the object, reviews the application progress of mitochondrial genome in phylogenetic research, compares the characteristic differences of mitochondrial genomes of different major groups of Siluriformes, reconstructs the phylogenetic relationship of Siluriformes, and combines molecular clock methods to explore the origin and differentiation time and historical biogeographic pattern of Siluriformes. Specific groups (such as Silurus) are selected as cases to analyze their phylogenetic patterns and species diversity. The results show that mitochondrial genome sequences can effectively analyze the evolutionary relationships between and within Siluriformes families, discover hidden species diversity, and promote the revision of the classification system. This study looks forward to the development direction of phylogenetic research on Siluriformes fish, emphasizing that nuclear genomes and more extensive species sampling should be combined to further improve the systematic classification system of Siluriformes fish and provide a scientific basis for species protection and aquaculture resource utilization.

Keywords Siluriformes; Complete mitochondrial genome; Phylogeny; Molecular clock; Taxonomic reconstruction; Species diversity

1 Introduction

Siluriformes fish, commonly known as catfishes, are a highly diverse and ancient group of teleost ray-finned fishes, widely distributed in freshwater ecosystems around the world (only a few groups are distributed in coastal waters). According to the latest statistics, Siluriformes includes about 40 families, hundreds of genera, and more than 3 000 species, making it the third largest freshwater fish group after Cypriniformes and Perciformes. Siluriformes fish are diverse in morphology, ranging from small catfish of a few centimeters to giant catfish of up to several meters (such as the Amazon giant catfish) (Silvano et al., 2020; Chen et al., 2024). They play an important role in ecological function, economic value, and evolutionary research. For example, some catfish are important food and aquaculture species, while others are key species in watershed ecosystems.

Catfish have a long history in evolution and are considered a typical model system for studying the biogeography of freshwater fishes and the impact of continental drift. In the past, taxonomic studies have proposed many hypotheses on the family level and inter-family relationships of the Siluriformes based on morphological characteristics, but due to the convergence and conservation of morphological evolution, traditional classification is controversial in some aspects. The rise of molecular systematics has provided a new way to solve these problems. In particular, mitochondrial DNA sequences have become a commonly used molecular marker for fish phylogenetic and taxonomic studies due to their rapid evolutionary rate, maternal monophyletic inheritance, and stable genome structure. Since the end of the 20th century, mitochondrial gene fragments (such as COI, Cyt b, 16S, etc.) have been widely used in phylogenetic analysis of taxa at all levels of the Siluridae family (Carvalho et al., 2016; Wu et al., 2021). However, due to the limited amount of single gene information, early molecular studies were still insufficient in distinguishing some deeper branch relationships. In the past five years, with the development of sequencing technology and the accumulation of data, the complete mitochondrial genome (about 16 Kb in size, containing 13 protein-coding genes and 37 RNA genes in total) has become an important data source for phylogenetic studies. Large-scale phylogenetic analyses based on the whole mitochondrial genome continue to emerge, greatly advancing our understanding of the evolutionary relationships of Siluriformes fish. For example, a study assembled almost all the publicly available mitochondrial genome data of Siluriformes species, reconstructed a time-corrected Siluriformes phylogenetic tree, and provided many new insights (Prosdocimi et al., 2017). Therefore, it is of great significance to summarize the progress of mitochondrial genomes in the phylogenetic and taxonomic research of catfishes, which not only helps to clarify the evolutionary history and biogeographic pattern of this group, but also provides a scientific basis for the classification and protection of related species.

This study took fishes of the order Siluriformes as the research object. Based on the large-scale data of mitochondrial genome, it systematically explored the phylogenetic relationships and classification system within the order Siluriformes, including comparative analysis of the mitochondrial genome characteristics of the Catfish family and related groups, reconstruction of the phylogenetic tree using complete mitochondrial genome data, analysis of the kinship structure and topological characteristics among the main families and genera, identification of deep phylogenetic divergence and estimation of differentiation time, and inference of the origin and global diffusion history of the order Siluriformes using molecular clock methods. Taking typical groups such as the genus Silurus as cases, it explored the processes of species formation, diversification and geographical diffusion, in order to provide a scientific basis for practical issues such as protecting genetic diversity, optimizing fishery management, improving aquaculture varieties and preventing the invasion of alien species, and promoting the sustainable utilization and ecological protection of Catfish species resources in the future.

2 Application of Mitochondrial Genomes in Phylogenetic Studies

2.1 Basic structure and features of mitochondrial genomes

Due to its compact structure, moderate evolution rate and no recombination, the mitochondrial genome has unique advantages in animal systematic research. The whole mitochondrial genome is usually about 16 kb~17 kb, containing 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes and a control region. This structure is highly conserved, and the base composition shows a certain degree of A+T enrichment, providing stable and rich genetic information for phylogenetic research.

2.2 Advantages of mtDNA in fish phylogenetics

For fish, mitochondrial whole genome data has been widely used in species identification, phylogenetic relationship reconstruction, and evolutionary history inference. Compared with a single or a few mitochondrial genes, the complete mitochondrial genome provides an exponentially increased amount of base information, greatly improving the resolution and credibility of the phylogenetic tree (Lv et al., 2020). For example, in the past, when using COI barcodes to classify catfishes, the monophyly of some genera could not be strongly supported, while the use of mitochondrial whole genome data can clearly distinguish these evolutionary lineages. For another example, in terms of species identification, mitochondrial genome sequences can improve the accuracy of identification and help discover cryptic species or misclassified populations (Wen et al., 2017; Wang and Zhao, 2024). Another important application of mitochondrial genomes in fish phylogeny is to infer the branch lengths of phylogenetic trees and then perform molecular clock analysis. Since mitochondrial DNA evolution is relatively uniform, it can be used as an approximate molecular clock and performs well in estimating divergence time after combining fossil calibration. Mitochondrial genomes can also be used in phylogeographic studies to infer the historical diffusion paths of species by analyzing the genetic structure between populations.

2.3 Molecular markers suitable for Siluriformes analysis

Of course, mitochondrial genomes also have limitations, such as only representing maternal genetic history and being easily affected by hybridization and incomplete pedigree sorting. Therefore, many studies have begun to combine mitochondrial data with nuclear gene data to obtain more comprehensive and reliable conclusions (Santos et al., 2019). In the study of catfish phylogeny, in addition to the commonly used genes such as Cyt b, COI, and 16S, in recent years, more emphasis has been placed on using the complete mitochondrial genome sequence or its extracted hypervariable regions (such as control regions and ND2 genes) as reconstruction tools to improve the resolution and support rate of the phylogenetic tree. The mitochondrial genome has played a huge role as molecular evidence in the study of fish phylogeny and classification, and has become one of the core tools for the study of the evolution of Siluriformes fish.

3 Mitochondrial Genome Characteristics in Siluriformes

3.1 Gene content, rearrangements, and gene loss

The size and gene composition of the mitochondrial genome of Siluriformes fish are generally similar to those of most bony fish, generally about 16.5 Kb, encoding 13 proteins, 22 tRNAs and 2 rRNAs, a total of 37 genes, and a control region (D-loop). For example, the mitochondrial genome of yellow catfish (*Pelteobagrus fulvidraco*) is about 16,540 bp long, containing a typical 37 genes and a control region (Liu et al., 2019). Another example is the mitochondrial genome of the marine catfish Arius maculatus, which is 16,710 bp long, and its gene composition and arrangement are similar to those of other reported Siluriformes fish. The gene arrangement of the mitochondrial genome of most Siluriformes fish follows the standard order of bony fish, and no major rearrangement events have occurred. In terms of protein-coding genes, the promoters of each gene are usually ATG, and only the COI gene often uses GTG as the start codon. Some stop codons are incomplete (such as "T-" or "TA-"), and complete stop signals are formed by tailing after transcription. For example, in the mitochondrial genomes of some Siluriformes fish, up to 7 protein genes end with incomplete terminators (Yang et al., 2022). All 22 mitochondrial tRNA genes can fold into a typical cloverleaf secondary structure, except that tRNA-Ser (AGY) lacks the DHU arm, which is a common feature of vertebrate mitochondrial genomes.

3.2 Control region variation and replication origin features

The length of the control region varies slightly among different species, such as the control region of the Amazon giant catfish, which is as long as 911 bp. The base composition of the mitochondrial genome of Siluriformes fish usually shows a high A+T content, with a GC content ranging from about 37% to 45%. Taking the Piraíba giant catfish (*B. filamentosum*) as an example, the GC content of its mitochondrial genome is 42.21% (Formiga et al., 2021).

3.3 Selective pressures and adaptive changes in coding regions

In terms of functional sequence evolution, each protein-coding gene is subject to strong purifying selection, and its nonsynonymous/synonymous substitution ratio (K_a/K_s) is far below 1. This indicates that mitochondrial protein genes are highly conserved in function, and the accumulated amino acid changes in these genes in different catfish species are limited by selection pressure. It is worth noting that there are also some differences in the details of the mitochondrial genomes of catfish from different families. For example, a study compared the mitochondrial codon usage frequency of spotted catfish and closely related species and found that the preference was basically the same, but there were lineage-specific differences in the relative usage frequency of a few codons. The mitochondrial genome structure of Siluriformes fish is conservative and the functional genes evolve slowly, which provides a reliable basis for reconstructing their phylogenetic relationships using mitochondrial genome data. However, small differences such as control region length variation and codon usage patterns can still be used as molecular features between different evolutionary lineages, providing additional information for classification and evolutionary studies (Yang et al., 2022).

4 Phylogenetic Relationships within Siluriformes

4.1 Branching structure and topology of major families and genera

Phylogenetic analysis based on a large amount of mitochondrial genome data has made the relationship between fish families in the order Siluriformes increasingly clear. In general, molecular phylogenetic evidence supports that the order Siluriformes originated in the Southern Hemisphere and differentiated into two main branches in the early stage: the Neotropical catfish suborders (Loricarioidei and Diplomystoidei) and the remaining Siluroidei suborder. In other words, the South American armored catfish suborder (Loricarioidei, including benthic adsorbed groups such as the family of Siluroidei) was restored as the most basal evolutionary branch in the order Siluriformes, followed by the Diplomystidae, which is also distributed in South America, together forming the basal group of the order Siluroidei. This result is consistent with the hypothesis that "Siluriformes originated in the Gondwana ancient land and spread outward from South America." On this basis, the vast majority of the remaining catfish families (collectively known as the Siluroidei) further differentiated into several large branches, among which the geographical distribution can be summarized as the two major evolutionary lines of "Asia + America branch" and "Africa branch" (Kappas et al., 2016; Brito et al., 2024). Long before the emergence of molecular evidence, studies based on fossils and limited molecular markers have proposed the concepts of "Big Asia" and "Big Africa". The latest mitochondrial genome phylogenetic tree well supports this view and makes a more detailed description of the family-level relationship.

4.2 Identification of deep divergence nodes and time estimation

Specifically, in the “Asia+America clade”, several families living in East Asia and North America were found to be closely related, such as the Cranoglaniidae (only found in China) and the Ictaluridae (only found in North America) forming a sister group, which together with the marine Ariidae (mainly distributed in subtropical and tropical coastal waters) and Pangasiidae (Southeast Asia) form a highly supported monophyletic clade on the phylogenetic tree (Schedel et al., 2022). Interestingly, the African-specific Austroglanididae (endemic to South Africa) was not classified as a branch with other African catfishes in the molecular tree, but was clearly embedded in the above-mentioned Asian clade as a sister group of Pangasiidae (Duong et al., 2023). This discovery updates our understanding of the evolutionary status of African catfishes: the family Clariidae was traditionally believed to be a member of the African lineage, but new evidence shows that it shares a closer common ancestor with the Asian giant catfish family. In terms of the "African lineage", it mainly includes several families such as the Mochokidae (commonly known as the upside-down catfish) and the Claroteidae, which live on the African continent. They form a monophyletic group with each other and are sister groups to the above-mentioned "Asian + American lineage".

4.3 Comparison of new lineages with existing taxonomy

In addition to the lineage division at the continental scale, mitochondrial genome data also clarified many systematic relationships between and within families. For example, molecular phylogenetic trees consistently support that the Clariidae (mainly distributed in Africa and South Asia) and the Heteropneustidae (South Asia) are the closest relatives to each other. Both families have auxiliary respiratory organs in anatomy, and molecular evidence confirms the kinship behind their convergent characteristics. For example, the American families Doradidae and Auchenipteridae are both thoracic catfishes, and their taxonomic status has long been unclear. The latest phylogenetic analysis shows that the two families constitute sister groups and evolved together in the freshwater system of South America. In terms of the relationship within the family, the affiliation of some genera that were controversial in the past has also been resolved with molecular evidence. For example, the classification of the subfamily *Pelteobagrus* in East Asia (formerly classified into the genus *Pseudobagrus* and the genus *Leiocassis*, etc.) has been confusing for a long time. Through mitochondrial genome analysis, it was found that these species with different genus names are actually clustered into one branch and have no obvious difference from the genus *Tachysurus* (whip catfish) (Zou et al., 2020). This result is consistent with the new classification scheme based on morphology, that is, *Leiocassis*, *Pseudobagrus*, *Pelteobagrus*, etc. are incorporated into the genus *Tachysurus* and unified into the broad genus *Pelteobagrus*. For example, the famous Bagridae family in the Siluriformes was found not to be a strictly monophyletic group in the past: the South Asian genus Rita is independent of other cichlids on the molecular phylogenetic tree. The latest study has also verified this again. Rita rita is not clustered on the main trunk of the cichlid family, but is closer to other groups, so that if it is excluded, the rest of the cichlid family can form a monophyletic group. It is suggested that the taxonomic status of the genus Rita should be re-evaluated, for example, it may be necessary to set up a separate family for it (Zhang et al., 2022).

5 Case Studies of Phylogenetic Patterns

5.1 Pangasiidae: divergence history and taxonomic revision

Fishes of the genus Silurus are mainly distributed in Eurasia, including several economically and ecologically important species, such as the common yellow catfish (*Silurus asotus*) in East Asia and one of the largest freshwater fish in Europe, *Silurus glanis*. The classification and evolutionary relationships of species in this genus have long been controversial. On the one hand, the morphological variation of populations in different regions makes the species definition complicated; on the other hand, Silurus is widely distributed in Eurasia, and its origin and diffusion history involve the influence of multiple geological and climatic events (Kishimoto et al., 2022). Recently, Chen et al. (2024) used mitochondrial whole genomes to conduct a comprehensive study of the phylogeny and biogeography of Silurus, providing new insights. In the study, 109 mitochondrial whole genome sequences of 13 morphological species of Silurus (89 of which were newly sequenced) were collected, and a phylogenetic tree was constructed using maximum likelihood and Bayesian methods. The results showed that Silurus can be divided into 8 highly supported main branches. These eight phylogenetic lineages do not completely correspond to species in traditional morphological classification. For example, *Silurus asotus* (catfish), which is widely distributed in East Asia, is not of a single origin, but contains four clearly differentiated branches (MOTUs), indicating that multiple species may be implied under the current nomenclature. For another example, the Chinese endemic species *Silurus microdorsalis* is also divided into two genetic branches, suggesting the existence of subspecific differentiation or hidden new species. These findings show that the species diversity of the genus Silurus has been underestimated in the past, and mitochondrial genome data help reveal hidden lineages.

5.2 Clariidae: evolution of hypoxia tolerance

Molecular clock analysis based on time calibration further reveals the evolutionary time frame of the genus Silurus: the last common ancestor of the genus Silurus lived in the early Oligocene about 37.6 million years ago. In the subsequent long evolution, the main branches successively differentiated between about 29.4~11.6 Ma; and within several recognized species, the differentiation time of different hidden branches is roughly between 11.6~3.7 Ma, roughly corresponding to the geological period from the middle and late Miocene to the Pliocene. This means that the species differentiation of the genus Silurus mainly occurred in the middle Cenozoic, when East Asia experienced significant geological and climatic changes (Chen et al., 2024).

5.3 Loricariidae: lineage radiation in south America

In order to reconstruct the origin and diffusion path of Silurus, the study further conducted an ancestral distribution area reconstruction analysis. The results support that the ancestors of the genus Silurus are likely to originate in East Asia, that is, the area centered on today's China and the Korean Peninsula. From the end of the Oligocene to the middle Miocene (about 26.7~21.8 Ma), some branches of the genus Silurus spread westward from East Asia many times, reaching Central Asia, West Asia and even Europe. This period coincided with the complete closure of the Tethys Sea and the uplift of the Pamir Plateau, and the inland rivers of East Asia were connected with the Eurasian water system, providing a corridor for the cross-regional diffusion of freshwater fish. Subsequently, from the late Miocene to the Pliocene (about 18.4~2.5 Ma), some branches of the ancestors of the genus Silurus migrated eastward to the Japanese Islands. It is worth noting that climate events in different periods had a profound impact on these diffusion and differentiation activities. The study pointed out that the Paleocene-Eocene extinction event at the turn of the Eocene-Oligocene, the establishment and strengthening of the East Asian monsoon in the Neogene, and the repeated glacial sea level rise and fall in the Pleistocene may all be key driving factors in shaping the evolutionary history of the genus Catfish. For example, the East China Sea shelf was exposed during the glacial retreat, forming a land bridge between the continent and Japan, which facilitated the spread of catfish to Japan; and the rise in sea levels during the interglacial period isolated its population on the island, leading to genetic differentiation (Figure 1) (Chen et al., 2024). For another example, the strengthening of the East Asian monsoon has brought about periodic climate fluctuations, which may lead to the fragmentation of catfish habitats and accelerate the geographical isolation of populations and the formation of lineages. Through the above-mentioned Catfish case, it can be seen that the mitochondrial genome combined with molecular clock and biogeographic analysis can finely depict the phylogenetic pattern and historical distribution dynamics at the species level. On the one hand, it clarifies the relationship and hidden diversity between species, providing a basis for taxonomic revision (for example, suggesting that the *Silurus asotus* complex should be split); on the other hand, it links species evolution with geological and climatic events, revealing the spatiotemporal background of species differentiation (such as the changes in the inland water system of East Asia in the Miocene that led to the differentiation of eastern and western populations). This comprehensive case fully demonstrates the ideas and value of conducting phylogenetic and taxonomic reconstruction research based on mitochondrial genome data (Yang et al., 2019; Li and You, 2024).

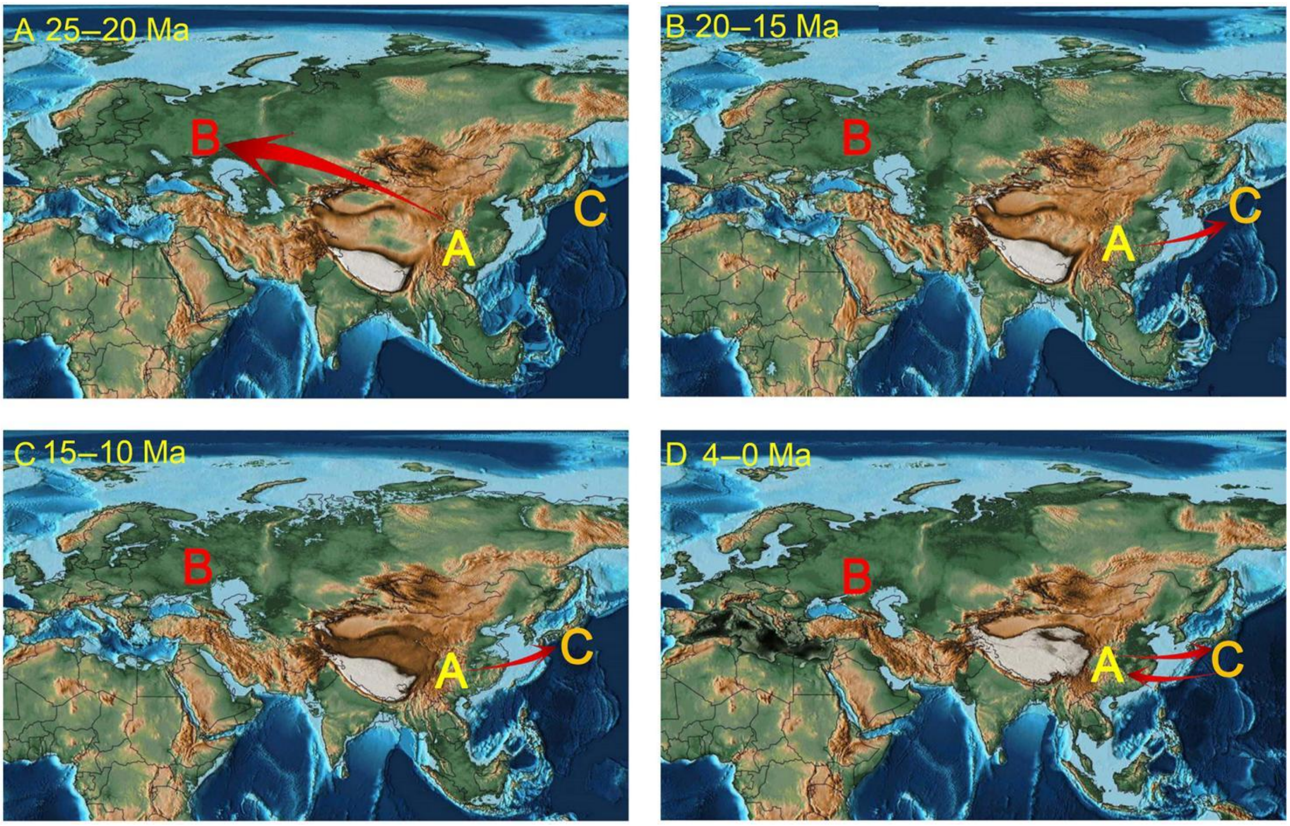

Figure 1 Dispersal routes of Silurus inferred from biogeographic analyses at different time scales (Adopted from Chen et al., 2024)
Image caption: A: 25-20 Ma. B: 20-15 Ma. C: 15-10 Ma. D: 4-0 Ma. A, B, and C on each map represent three recognized biogeographical regions, i.e., China and Korean Peninsula, Europe and Central and Western Asia, and Japan, respectively (Adopted from Chen et al., 2024)

6 Molecular Clock Analysis and Historical Biogeography

6.1 Methods for divergence time estimation using mtDNA

The phylogenetic tree itself can only reflect kinship, but the molecular clock model combined with the node time estimation can add time scale information to the system evolution, thereby inferring the age and rate of species differentiation. In the study of Siluriformes fish, the use of mitochondrial genomes for molecular clock analysis has achieved many important results. For example, the study by Kappas et al. (2016) provided the first quantitative estimate of the origin and divergence time of Siluriformes: the results showed that Siluriformes can be traced back to the Early Cretaceous (about 120 million years ago), and its origin and early evolution are consistent with the time frame of the breakup of the supercontinent Gondwana. This time is slightly earlier than the origin time of other freshwater fish such as cold-water fish, suggesting that catfish may be a fairly ancient branch of freshwater fish (Yang et al., 2019). Recent further analysis also supports similar conclusions. Brito et al. (2024) used the mitochondrial genomes of 138 Siluriformes species to construct a time correction tree and estimated that Siluriformes originated in the middle Cretaceous about 118 million years ago. The study also found that the oldest branch, the armored catfish (South America), first differentiated about 100 million years ago, followed by the South American catfish Diplomystidae slightly later, and the Siluriformes, which accounted for the vast majority of species, began to diversify rapidly about 94 million years ago. The timing of this series of differentiation events is very close to the final separation of South America and Africa (about 100 million years ago). Therefore, it can be inferred that the global radiation of Siluriformes fish is closely related to the isolation of continental plates in the middle and late Cretaceous period. After South America split from Africa, catfish populations on different continents evolved independently, gradually forming two major branch patterns of "African system" and "South American system".

6.2 Origin and dispersal routes of major Siluriformes lineages

In addition to clarifying the macroscopic evolutionary time of the entire Siluriformes, molecular clock analysis can also be used to compare the evolutionary rates of specific families or genera and study the rate of speciation. For example, some scholars have focused on the rate of body size evolution of Siluriformes fish. By plotting body length traits on a time-corrected tree, they found that in the past 100 million years of evolution, the body size changes of different lineages were not in a single direction, but showed 16 significant large-scale evolutionary events and 11 small-scale evolutionary events, and there was no overall trend of continuous increase or decrease. This result negates simple hypotheses such as the "large-scale trend", indicating that the body size evolution of catfish is driven by niche diversification, and different branches may evolve their body size in different directions according to environmental selection pressure. For example, the body mass index of the Piraíba giant catfish increased by 5.65 times in about 40.8 million years, while the cave catfish of another lineage evolved a dwarfed body shape. These trait evolution analyses based on time trees demonstrate the power of the molecular clock combined with phylogeny, that is, the rate and period of trait change can be quantitatively characterized, thereby inferring possible factors driving evolution (Kishimoto et al., 2020).

6.3 Influence of geological and climatic events on lineage formation

Historical biogeographic analysis is another important component of phylogenetic research. It attempts to link the branching of phylogenetic trees with geographical distribution, reconstruct the distribution range of the ancestors of species or groups, and infer events such as migration and isolation. In Siluriformes fish, historical biogeographic analysis has revealed many interesting patterns. For example, the aforementioned study of the genus Silurus showed that its ancestors probably originated from the East Asian continent and spread to other parts of Eurasia many times. This conclusion provides a basis for explaining the east-west distribution pattern of the genus Silurus. Similarly, at the family level, the North American Siluridae and the Chinese Siluridae form sister groups. The analysis of the ancestral distribution area speculates that their most recent common ancestor may have lived in the northern part of the ancient Laurasia in the late Cretaceous, and then separated into the east and west due to the breakup of the continent (Zhang et al., 2024). For example, the Clariidae from Africa and the Heteropneustidae from South Asia form sister groups. Their most recent common ancestor is presumed to be on the African-South Asian plate, which was still connected at the end of the Cretaceous. Later, as the plates separated, they evolved into today's distribution. These inferences need to be combined with paleogeographic evidence and fossil records for comprehensive judgment, but mitochondrial phylogeny provides key evolutionary sequence information. Historical biogeographic analysis can also test regional diversification hypotheses. For example, for small catfishes (such as *Glyptothorax*) in the rapids environment of the Asian Plateau, a study combined phylogenetic and geographic information and found that their diversification is highly consistent with the uplift event of the Qinghai-Tibet Plateau, that is, the geologically new river network provided a large number of new habitats for these mountain stream fish, driving the rapid differentiation of species (He et al., 2020). This "large-scale radiation" pattern is also reflected in other groups of Siluriformes, such as small electric catfish and hook catfish in the Congo River basin in Africa (Day et al., 2023).

7 Broader Implications for Evolution and Biodiversity

7.1 Origin and dispersal pathways of Siluriformes diversity

The results of phylogenetic and taxonomic reconstruction studies of Siluriformes fish have far-reaching significance for understanding the evolutionary mechanism of fish and biodiversity conservation. At the level of evolutionary biology, a complete phylogenetic tree is the basis for analyzing the evolutionary pattern of traits. By mapping morphological, physiological and ecological traits onto molecular phylogenetic trees, researchers can find out which characteristics are inherited from common ancestors (conservative evolution) and which are independently generated by different lineages (parallel or convergent evolution). Siluriformes fish show rich diversity in habitats (such as caves, rapids, seawater, etc.) and morphological characteristics (such as tentacles, blind, bony plates). With the help of a reliable phylogenetic framework, adaptive features that have evolved independently multiple times can be identified. For example, cave adaptation has appeared in unrelated lineages (such as the genus Cave Catfish in East Asia and the genus Blind Catfish in Africa), indicating that different catfish have evolved similar strategies under similar ecological pressures. For example, marine invasions occurred in at least two major lineages: the marine scorpion family and the catfish family, respectively. The phylogenetic tree shows that they come from two independent freshwater ancestors, reflecting the biogeographic response of convergent evolution (Vega and Wiens, 2012). These understandings all rely on accurate phylogenetic tree scaffolds, otherwise they are easily misled by surface similarities.

7.2 Speciation mechanisms and adaptive evolution

In terms of biodiversity research and species conservation, the latest phylogenetic studies continue to reveal underestimated species diversity and phylogenetic diversity in Siluriformes fish. For example, the multiple evolutionary branches hidden in the genus Silurus mentioned above, as well as some populations neglected by traditional classification under the genus Whipcatus, represent genetic diversity units that actually exist but have not been named or valued. This is crucial for biodiversity conservation. Many catfish live in specific aquatic ecosystems. If cryptic species are not identified, they may face the risk of extinction without being noticed. Therefore, phylogenetic studies can guide us to re-evaluate the conservation value of species and populations (Shao et al., 2021). Identifying lineages with high evolutionary uniqueness (such as single-genus, single-species families or endangered genetically isolated species) through molecular systematics methods can help to formulate "evolutionarily significant" conservation priorities. For example, the discovery that the family of Forked Tongue Catfish is a phylogenetic sister of the American Catfish family and only one species remains suggests that this family and its species should be given higher conservation attention because their extinction will lead to the loss of an entire unique lineage. For example, for widespread species, independent management units should be determined based on genetic differentiation. For example, different cryptic lineages of the genus Silurus should be treated as independent units in fishery management and conservation actions to maintain overall genetic diversity.

7.3 Applications of phylogenetic studies in endangered species conservation

Phylogeny and taxonomic reconstruction can also directly serve the fishery and aquaculture industries. Accurate classification is the basis for aquaculture variety improvement and disease prevention and control. Some aquaculture objects that were misclassified in the past (such as the alien species "channel catfish" that was once farmed as native catfish) have been corrected through molecular identification, thus avoiding the ecological risks caused by species confusion (Yen et al., 2023). Understanding the relationship between closely related species can also help to utilize hybrid advantages for variety breeding and predict the possible impact of invasive alien species. From a taxonomic perspective, phylogenetic studies provide an objective basis for the revision of the classification system of Siluriformes fish. For example, based on the molecular phylogenetic tree, taxonomists have processed some paraphyletic groups and split or merged non-monophyletic taxa to make the classification reflect natural evolutionary relationships. This series of reconstruction work has made the classification of Siluriformes more stable and predictable, and also facilitated the communication of subsequent research. In this process, molecular evidence and traditional morphological evidence corroborate and complement each other: for most families and genera, the two types of evidence are consistent (increasing confidence); for the few cases of conflict, in-depth research is prompted to find out the cause, either morphological misjudgment or molecular tree misleading, thereby advancing cognition. It can be said that the process of classification reconstruction is also a process of deepening the understanding of the evolution of the catfish family.

8 Concluding Remarks

The large-scale mitochondrial genome analysis not only verifies many traditional classification hypotheses (such as the evolutionary division of African catfish and Asian catfish), but also corrects several long-standing classification errors (such as the division of the genus of East Asian yellow catfish). The phylogenetic tree constructed by the mitochondrial genome is highly consistent with morphological evidence at the inter-family level, and reveals rich details and new discoveries at the intra-family level, including hidden species and complex phylogenetic structures. These advances have gradually improved the classification system of Siluriformes fish and laid a reliable systematic framework for evolutionary biology and ecological research. At the same time, the application of molecular clock and biogeographic analysis puts the diversification history of Siluriformes fish in the context of time and space, so that we have a preliminary answer to when and where this group appeared, how it spread and differentiated. For example, existing evidence supports that Siluriformes originated in South America in the Cretaceous, and then spread to the world through continental connection and drift events. The diversity of catfish on each continent was formed under the shaping of the geological history of the Cenozoic Era (such as the separation of Africa and South America, the evolution of the inland water system in Asia, etc.). This understanding is not only of academic value, but also provides a typical case for understanding the historical evolution of freshwater biota.

Despite this, there are still some limitations and gaps in the current research, which is also the direction of future efforts. First, at the data level, although the currently public mitochondrial genome data of Siluriformes covers most families, some rare or restricted distribution groups are still undersampled. For example, some small families in Africa and cave catfishes in the Andes region of South America lack genome data, making some branches of the phylogenetic tree still incomplete. In the future, it is necessary to strengthen the genome sequencing of these unsampled groups through international cooperation to achieve comprehensive coverage of the main evolutionary lineages of Siluriformes. For the cryptic lineages that have been discovered, their taxonomic status should be further confirmed through multi-gene data and morphological evidence. For example, whether those MOTUs of the genus Siluriformes correspond to new species needs to be comprehensively described and named. Second, at the methodological level, although a single mitochondrial genome has a large amount of information, it only represents the maternal lineage after all. With the reduction of the cost of sequencing technology, it is a general trend to introduce nuclear genome data (such as transcriptomes, ultra-conserved elements, UCEs, etc.) into the phylogeny of Siluriformes. Nuclear genes have a genetic history independent of mitochondria, which can provide independent verification for phylogenetic trees and solve the possible inconsistencies between mitochondria and species trees (such as hybridization, maternal bias, etc.). Future taxonomic reconstruction work should integrate evidence from mitochondria and nuclear genes to build a more robust comprehensive phylogenetic framework. Third, in terms of analytical methods, in addition to traditional sequence alignment and tree construction, new models and computational methods are worth trying. For example, the application of Bayesian models of biogeography (such as BioGeoBEARS software) can quantify the possibility of different distribution models and accurately reconstruct the ancestral geographical range of each node of the catfish family. For another example, the use of molecular clocks at the whole genome level (such as substitution rate drift models) can better characterize the differences in evolutionary rates between lineages and provide a basis for understanding the "slow clock" or "fast clock" phenomenon of certain lineages.

The study of phylogenetic and taxonomic reconstruction of the catfish family (Siluriformes) is entering a new stage of integrating multi-source data and analyzing deep problems. The fruitful results achieved in mitochondrial genome research in the past few years have laid a solid foundation for this field and inspired new scientific problems. Looking into the future, we look forward to further revealing the unsolved mysteries in the catfish evolutionary tree through more comprehensive genomic data, more advanced analytical methods and closer interdisciplinary collaboration.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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