International Journal of Aquaculture, 2025, Vol.15, No.3, 99-115
http://www.aquapublisher.com/index.php/ija

Research Insight Open Access

Mitochondrial Genome Variation in *Channa* and Its Implications for Phylogenetic Relationships

Fei Zhao, Jinni Wu
Aquatic Biology Research Center, Cuixi Academy of Biotechnology, Zhuji, 311800, Zhejiang, China
Corresponding authors: 2314548193@qq.com

International Journal of Aquaculture, 2025, Vol.15, No.3 doi: 10.5376/ija.2025.15.0011
Received: 19 Apr., 2025
Accepted: 21 May, 2025
Published: 30 May, 2025

Preferred citation for this article:
Zhao F., and Wu J.N., 2025, Mitochondrial genome variation in channa and its implications for phylogenetic relationships, International Journal of Aquaculture, 15(3): 99-115 (doi: 10.5376/ija.2025.15.0011)

Abstract The genus *Channa* is a kind of freshwater fish with important economic value and ecological significance. Its species diversity and phylogenetic relationship have long been concerned. This study systematically sorted and compared the mitochondrial genome sequences of different species and regional populations of the genus *Channa*, deeply revealed its genome structure composition, variation characteristics and phylogenetic relationship, clarified the lineage differentiation pattern and evolutionary path within the genus *Channa*, and constructed a high-resolution phylogenetic tree by analyzing genetic variations such as SNP, InDel and non-synonymous mutations. Regional comparison showed that there were significant differences in mitochondrial variation between the southern Chinese and Southeast Asian populations of *Channa*, and geographical isolation contributed to the formation of lineages in different regions. Case analysis represented by *Channa argus* and *Channa maculata* further revealed their genomic differences and ecological adaptation mechanisms. This study is expected to provide a theoretical basis for species identification, resource protection and genetic diversity assessment of *Channa*, and also provide a methodological reference and practical path for studying the phylogenetic relationship of freshwater fish using mitochondrial genome.

Keywords *Channa*; Mitochondrial genome; Phylogeny; Genetic variation; Phylogenetic geography

1 Introduction

The genus *Channa* belongs to the family *Channa* of the order Perciformes. It is a typical freshwater carnivorous fish, commonly known as "black fish" or "thunder fish". The *Channa* family includes the Asian genus *Channa* and the African genus *ParaChanna*. Among them, the species of *Channa* are mainly distributed in East Asia and Southeast Asia, and are widely found in China, the Indian subcontinent and Southeast Asia (Rüber et al., 2020). According to recent statistics, there are four species of *Channa* in China, namely *Channa argus*, *Channa maculata*, *Channa asiatica* and *Channa gachua*, which are distributed in northern China, southern China and some local areas respectively. The genus *Channa* belongs to the suborder Perciformes in taxonomy, and is unique because it has auxiliary respiratory organs that can survive in low-oxygen environments. As a top predator, snakehead fish plays an important role in maintaining the balance of freshwater ecosystems. At the same time, black snakehead and *Channa maculatus* are also common farmed fish in China, with significant economic value and development potential (Fang et al., 2024). In recent years, due to human activities, snakehead fish have been introduced into some non-native waters and may have an impact on the local ecology. Its invasion risk and species identification have also attracted much attention.

The diversity and phylogenetic relationship of snakehead fish have always been one of the hot topics in fish classification and evolution research. Traditional morphological identification is limited by the changes in body color and pattern of snakehead fish at different growth stages and the similar morphology of closely related species, and there is some controversy about species identification and classification status. With the development of molecular systematics, it has become possible to use DNA sequences to solve the problems of species identification and kinship (Kamran et al., 2020). New species of snakehead fish are constantly being discovered and described. For example, new snakehead species have been identified in northeastern India and Myanmar in recent years, indicating that the species diversity of this genus may be underestimated. Therefore, sorting out the taxonomic status of the genus Snakehead and clarifying the phylogenetic relationship between its species not only has important biodiversity significance, but also provides a scientific basis for aquaculture utilization and invasion prevention and control.

Mitochondrial DNA (mtDNA) has become one of the most commonly used molecular markers in animal molecular systematics and phylogenetic geography studies due to its unique genetic characteristics. Compared with the nuclear genome, the mitochondrial genome is maternally unilineal, has no recombination, has a high copy number and a fast evolution rate. These characteristics enable it to have a higher resolution for the closest relationship between species. The typical fish mitochondrial genome is a closed circular double-stranded molecule with a total length of about 16 kb~17 kb, containing 13 protein-coding genes, *22 tRNA* genes, *2 rRNA* genes and 1 non-coding control region (D-loop). The mitochondrial whole genome sequence provides richer and more comprehensive information than a single gene fragment (such as COI, Cyt b, etc.), which can avoid the systematic errors that may exist in a single gene tree (Xu et al., 2024). Especially in the study of fish classification and evolution, the mitochondrial genome can reflect evolutionary characteristics such as gene rearrangement and replication mechanism, providing new ideas for solving the systematic relationship of complex groups. In recent years, with the development of sequencing technology, more and more fish mitochondrial genomes have been determined and used for phylogenetic reconstruction. For example, for the genus Snakehead in the family *Channa*, many studies have improved the analysis of systematic relationships through mitochondrial genome data, and discovered new species groupings and pedigree structures. These studies have demonstrated the advantages and application prospects of mitochondrial genomes in the study of fish phylogeny and phylogenetic geography (Wang et al., 2023). Mitochondrial DNA also has limitations, such as only representing maternal genetic history, and may cause hybridization or incomplete pedigree sorting.

This study will review and analyze the research results on mitochondrial genome variation and phylogeny of Snakehead in recent years, explore the inspiration of mitochondrial genome variation characteristics for clarifying the internal lineage relationship of Snakehead, summarize the basic structural composition of the mitochondrial genome and the characteristics of each functional region of Snakehead, compare the variation distribution law of mitochondrial genome of different species of Snakehead, screen the hypervariable regions and speculate the potential functional impact, and reconstruct the phylogeny based on the mitochondrial whole genome sequence to analyze the phylogeny structure and species differentiation history of Snakehead, compare the mitochondrial variation differences of Snakehead populations in different geographical regions, discuss the influence of geographical isolation and environmental factors on lineage distribution, and deeply explore the ecological adaptation mechanism of Snakehead lineage evolution through typical cases (*Channa argus* and *Channa maculata* and samples from different regions). This study hopes to deepen the understanding of the variation law and phylogeny of the mitochondrial genome of Snakehead, and provide a scientific basis for the protection of species diversity, genetic breeding and invasion prevention and control of Snakehead species.

2 Structural Features of the Mitochondrial Genome

2.1 Gene content and coding region organization

The mitochondrial genome of snakehead is consistent with that of typical bony fish, presenting a double-stranded circular DNA molecule with a total length of about 16,500 bp~16,900 bp. For example, the reported mitochondrial genome length of *Channa argus* is about 16,558 bp, and that of *Channa maculata* is about 16,559 bp, which is almost the same. The mitochondrial genome size of different snakehead species is mainly affected by the difference in the length of the control region, but generally fluctuates around 16.5 kb. The mitochondrial genome of snakehead contains 37 genes, including 13 protein-coding genes (PCGs), 22 transfer RNA genes (*tRNAs*) and 2 ribosomal RNA genes (*rRNAs*), as well as 1 non-coding control region (D-loop) (Xu et al., 2024). Genes are arranged on two strands: the H strand usually contains most of the coding genes, while the L strand contains only a few genes (such as some *tRNA* genes and *ND6* genes). The order of mitochondrial genes in various species of Snakehead is highly conserved, consistent with the vast majority of bony fish, and no obvious gene rearrangement is observed.

The 13 protein-coding genes include 7 subunits related to the NADH dehydrogenase complex (ND1-ND6, ND4L), 1 subunit related to cytochrome c reductase (cyt b), 3 subunits related to cytochrome oxidase (COX1–COX3), and 2 ATP synthase subunits (ATP6 and ATP8). The arrangement of these coding genes in the mitochondrial genome of Snakehead follows a typical order: for example, tRNAPhe-12S rRNA-tRNAVal-16S rRNA-ND1-ND6-cyt b-ND4L-ND5-D-loop. The length of each coding gene and the start and stop codons are basically the same among different species of snakehead. For example, the *COX1* gene starts with the standard start codon ATG and ends with the complete stop codon TAA. There are a small amount of intervals or overlaps between genes, and some gene intervals have only 0~4 non-coding bases, and the genome utilization rate is close to the extreme. Overall, there is no abnormality or novelty in the composition and arrangement of the mitochondrial genome of snakehead, indicating that its genome structure is relatively conservative in evolution.

2.2 Characteristics of the control region and non-coding segments

The mitochondrial control region (D-loop) is the longest non-coding region in the genome and is also a key region for regulating mitochondrial replication and transcription. The mitochondrial control region of the snakehead genus is located between the *tRNAPro* gene and the *tRNAPhe* gene, with a length ranging from 800 bp to 1 000 bp, and is one of the regions with the highest sequence variability in the entire genome. The control region of the snakehead genus has a typical three-segment structure: the terminal termination fragment (EVT), the middle conserved region (CSBs) and the terminal conserved sequence. It contains multiple highly conserved short sequence elements (such as CSB-1, CSB-2, CSB-3) and AT-rich regions. These elements are relatively conserved in various species of the genus Snakehead, which helps to maintain the functional integrity of the control region. Studies have shown that the control region sequence of Snakehead presents a significant base composition bias: rich in A+T bases, and the total content of A and T accounts for more than 60% of the control region sequence. This strong AT bias is a common feature of the mitochondrial control region of fish, which may be related to the need for easy melting of replication initiation (Alam et al., 2019). In addition to the control region, there are very few other non-coding sequences in the mitochondrial genome of snakehead, and there are only a few base spacing regions between a few genes. For example, there is usually a very short non-coding interval between the *ND5* and *ND6* genes, with a length of 2 bp~5 bp. There may be a small amount of overlap or gaps between some genes, such as *tRNAle* and *tRNAGln*, *tRNAThr* and *tRNAPro*. In addition to the control region, there are almost no large insertions or repetitive sequences in the mitochondrial genome of the Snakehead, and no pseudogenes or extra genes have been found. This compact genome structure is consistent with the characteristics of the mitochondrial genome of most bony fish. It is worth mentioning that the mitochondrial genome occasionally undergoes gene rearrangement or duplication during evolution, but no similar abnormalities have been found in the currently sequenced Snakehead species. Therefore, it can be inferred that the structure of the mitochondrial genome of Snakehead is relatively stable in evolution, and its variation is mainly reflected in base substitutions and small fragment insertions and deletions, rather than changes in the macroscopic structure.

2.3 Arrangement of *tRNA*, *rRNA*, and protein-coding genes

The gene arrangement order of the mitochondrial genome of Snakehead is consistent with that of most bony fish. The gene organization presents a highly conservative pattern: the genes are staggered on the two chains, the H chain contains 12 protein genes, *14 tRNA* genes and *2 rRNA* genes, and the L chain contains the *ND6* gene and the remaining *8 tRNA* genes. Specifically, the genome starts with tRNAPhe, followed by 12S rRNA and tRNAVal, 16S rRNA. The structure and length of these two *rRNA* genes are almost completely consistent among Snakehead species (12S rRNA is about 950 bp, 16S rRNA is about 1 650 bp). It is followed by tRNALeu (UUR) and protein-coding gene ND1 (Li et al., 2018). *tRNA* genes are interspersed between most protein-coding genes, such as *tRNAIle* and *tRNAGln* inserted between ND1 and ND2, and tRNATrp inserted after ND2. This typical "coding gene-*tRNA*" alternating arrangement pattern reflects the compactness and functional intensiveness of the mitochondrial genome.

It is worth noting that the mitochondrial genome has asymmetric replication of the two chains during replication, resulting in differences in the base composition of the H chain and the L chain, generally manifested as H chain rich in G and L chain rich in C (i.e. AT bias, low GC content). The mitochondrial genome of the genus Snakehead also conforms to this rule, showing positive AT bias and negative GC bias, that is, the A content is slightly higher than T, and the C content is lower than G (Fan et al., 2022). This bias is reflected in the distribution of the coding region: for example, the proportion of T at the third codon position of the protein gene is often higher, while G is lower, which may affect the codon usage preference. Analysis of the codon usage of 13 protein genes in Snakehead showed that synonymous codons ending with A or T were preferred, which is a common phenomenon in the high AT background of mitochondrial genome. The position and order of each *tRNA* gene in the Snakehead genome are basically consistent with those of model fish (such as zebrafish). Each *tRNA* gene is about 67 bp~75 bp long and can fold into a typical cloverleaf secondary structure, and the anticodon sequence has no abnormal changes. These results further indicate that the arrangement and structure of the mitochondrial genome of Snakehead is highly conserved, providing a good basis for comparing sequence variations in different species.

3 Genome Variation Analysis

3.1 SNPs and InDel distribution patterns

By comparing the mitochondrial genome sequences of various species of Snakehead, a large number of single nucleotide polymorphism sites (SNPs) and insertion/deletion variations (InDels) can be identified. In general, the variation density of different regions of the mitochondrial genome varies significantly: the control region has the highest variation frequency, while rRNA and protein-coding genes are relatively conservative. In the comparison between species of Snakehead, there are about hundreds of SNP variations between each pair of species. For example, it is reported that the comparison of the mitochondrial genomes of *Channa argus* and *Channa maculata* can detect about 300~400 nucleotide differences, of which the control region and some protein-coding genes account for the majority, while the *rRNA* gene region has only very few variations (less than 5% of the total). This distribution pattern is related to the importance of different functional regions of mitochondrial genes: the coding region, especially the protein gene, is often subject to strong functional constraints, with fewer variations and most of them are synonymous substitutions; while the control region, as a non-coding regulatory region, can tolerate more neutral mutation accumulation, and thus has a high variation rate.

Specifically, among the 13 protein-coding genes, NADH dehydrogenase subunit genes (such as *ND2*, *ND4*, and *ND5*) are usually regions with richer variation, while cytochrome oxidase genes such as *COX1* and *COX2* are highly conserved. For example, Wang et al. (2023) compared the mitochondrial sequences of five species of snakehead and found that the *ND4* gene was about 1 378 bp long, with more than 150 mutation sites, making it one of the most variable coding genes; in contrast, the *COX1* gene was about 1 548 bp, with only dozens of mutations. In addition, the third codon position variation of protein genes is far more than the first and second positions, which is related to the fact that codon redundancy makes the third site mutation mostly synonymous mutations. Similarly, in the two *rRNA* genes, the 12S and 16S rRNA sequences are very similar between different species of snakehead, with only sporadic base substitutions, indicating that *rRNA* genes are under strong structural and functional selection pressure and have a slow evolution rate.

InDel mutations are relatively rare in the mitochondrial genome and mostly occur in AT tandem repeat regions or control regions. The insertion/deletion of the mitochondrial genome of the genus Snakehead is mainly concentrated in the control region and some poly T/A regions. For example, an 8 bp tandem repeat was detected in the middle of the control region of *Channa maculata*. The length difference caused by the variation in the number of replications between different individuals is a typical microsatellite InDel. In the coding region, there are very few insertions and deletions that affect the reading frame. Only occasionally, single-base insertions/deletions are seen at non-critical sites of *tRNA* genes, which have no obvious effect on function. In general, the variation of the mitochondrial genome of Snakehead is mainly SNP, supplemented by InDel, and most InDel lengths are very short (1 bp~2 bp) (Fan et al., 2022). These variation markers provide a rich information basis for population genetics and phylogenetic analysis. For example, in the study of different geographical populations of *Channa argus*, the variation of the mitochondrial control region includes several specific InDels, which can be used as molecular markers to identify different lineages.

3.2 Identification of highly variable regions and functional interpretation

Based on the analysis of variation frequency, several highly variable regions can be identified in the mitochondrial genome of Snakehead. In addition to the control region (D-loop), some protein-coding gene fragments also show high variability and are considered "intergenic hypervariable regions". For example, studies have shown that the 5' end of the *ND2* gene, the 3' end region of the large subunit rRNA (16S rRNA), and a sequence of tRNAPhe adjacent to the control region are fragments with high variability in the mitochondrial genome of the black fish genus (Wang et al., 2023; 2024). The high variability of the ND2 fragment may be related to its relatively weak functional restriction; the base changes in the terminal region of 16S rRNA have little effect on the secondary structure of rRNA, so more mutations are accumulated. The control region is not uniform. Among its three subregions, the terminal conserved region and the middle tandem repeat region have the most variation. These hypervariable regions are often rich in phylogenetic information and are often used as molecular markers to analyze species and population relationships.

The functional interpretation of the hypervariable region needs to be combined with the role of the gene in which the sequence is located. For example, highly variable protein genes such as *ND2* and *ND5* encode subunits of mitochondrial respiratory chain complex I. Moderate amino acid substitutions may affect the performance of the enzyme complex, thereby producing phenotypic effects (Garg and Dohre, 2024). However, in most cases, the effects of these variations on individual survival adaptability may be subtle or neutral. Therefore, in phylogenetic analysis, the main contribution of highly variable regions is to improve the ability to distinguish, and it does not necessarily correspond directly to trait changes. On the contrary, if some conservative regions vary, they often indicate important functional effects. For example, the *COX1* gene has almost no variation among species of the genus Snakehead. Once amino acid substitutions are detected, it may mean major functional consequences. Therefore, COX1 is often used for DNA barcoding to identify species but is not suitable for inferring intraspecific relationships.

For non-coding highly variable regions, such as control regions, although they do not directly encode proteins, their variations may affect replication initiation and transcriptional regulation. Variations in the control region of Snakehead include length polymorphism and base substitutions. Some length variations may change the secondary structure of DNA and thus affect the efficiency of the replication origin. However, since this region is not absolutely necessary for individual survival, its variation is mostly tolerated by natural selection and is therefore used as a neutral marker in evolutionary analysis. Similarly, in the *tRNA* gene region, some highly variable sites fall in the unpaired region of the arm, which does not affect the folding and function of tRNA, and therefore accumulate differences between species and can be used as phylogenetic signals. If the mutation falls in the anticodon or stem pairing site, it may have significant functional consequences and is rarely preserved in evolution.

3.3 Potential phenotypic effects of non-synonymous mutations

Base substitutions in mitochondrial protein-coding genes can be divided into synonymous mutations and non-synonymous mutations. The former does not change the amino acid sequence, while the latter leads to amino acid substitutions, which may affect protein function and phenotype. In the mitochondrial genome of the genus Snakehead, most of the variation sites are synonymous mutations, but there are still a considerable number of non-synonymous mutations that deserve attention. For example, Ou et al. (2021) compared the mitochondrial genes of multiple geographical populations of *Channa argus* and found that there were several fixed amino acid substitutions in the *ND5* gene, with significant frequency differences between northern and southern populations, which may be related to the adaptation of populations to temperature differences. This speculation is consistent with the strong survival ability of *Channa argus* in cold environments: the northern population of *Channa argus* may have accumulated mitochondrial mutations that are beneficial to low-temperature metabolism through evolution, thereby improving its cold tolerance. Similarly, *Channa maculata* and *Channa argus* differ in distribution area and ecological habits. *Channa maculata* has poor cold tolerance but may have strong disease resistance. In the comparison of the mitochondrial genomes of the two, some non-synonymous mutations that may be associated with these traits were found. For example, an amino acid substitution in the *ND6* gene of *Channa maculata* may affect the efficiency of proton transport on the mitochondrial membrane, which is related to its metabolic rate or cold tolerance; and a unique mutation in the *Cyt b* gene of *Channa argus* may change the function of the cytochrome bc1 complex, thereby affecting its motility or growth rate (Zhang et al., 2015).

4 Phylogenetic Reconstruction

4.1 Whole-genome-based tree building strategies

The phylogenetic tree of the genus *Channa* can be constructed using the mitochondrial whole genome sequence to obtain high-resolution species evolutionary relationships. In recent years, studies have usually used a combination of Bayesian and maximum likelihood methods to infer phylogenetic trees, and used other perciformes fishes (such as *Channa* or *Trichopoda*) as outgroups. Based on the concatenated sequences (or amino acid coding sequences) of 13 protein-coding genes in the whole genome, the phylogenetic tree obtained consistently supports the genus *Channa* as a monophyletic group, and the relationships between species are clear and separable. For example, Wang et al. (2023) used the mitochondrial whole genomes of five representative species of the genus *Channa* to construct a phylogenetic tree, and obtained three highly supported branches corresponding to different species groups: *Channa andraensis* + *Channa rainbowi* as sister groups, *Channa ornate* + *Channa purchr* as another sister group, and *Channa schrenckii* and *Channa striata* as independent groups. This result is different from the previous analysis based on fragment sequences, and some new combinations of close relationships have been found, proving the advantage of whole genome data in resolving subtle relationships.

Furthermore, phylogenetic analysis with expanded species sampling included all of the approximately 20 known species of the genus Snakehead, and the results divided them into several major evolutionary lineages. For example, Rüber et al. (2020) divided Snakehead species into eight distinct lineage groups: the Argus group (including northern species such as *Channa argus*), the Asiatica group (including Chinese endemic species such as *Channa sinensis*), the Gachua group (including small species such as *Channa striata*), the Lucius group (southern species such as *Channa stigmata*), the Marulius group (South Asian species such as *Channa magna*), the Micropeltes group (Southeast Asian species such as *Channa magna*), the Punctata group (South Asian species such as *Channa stigmata*), and the Striata group (Southeast Asian species such as *Channa striata*). The phylogenetic relationships between these groups were also highly supported. For example, the Argus group first merged with the Asiatica group, and then clustered with the Lucius group in sequence, indicating that the northern *Channa argus*, Chinese *Channa argus* and southern *Channa maculata* are closely related, while the Marulius group in South Asia and the Micropeltes group in Southeast Asia are in another branch of the phylogenetic tree, relatively separated from the above-mentioned Asian continental populations.

The topological structure of the phylogenetic tree also reveals some noteworthy details. For example, multiple analyses have shown that small species in the genus Snakehead that are small in size and live in mountain streams (such as the broad-fronted *Channa* group) formed an independent early branch, while large and medium-sized species differentiated into multiple later lineages. This may mean that the initial evolution of the Snakehead genus occurred in small freshwater fish, and then some lineages evolved into large-scale ones and spread to a wider area. The whole genome phylogenetic tree highly supports the sister group relationship of some species, such as the pairing of *C. andrao* and *C. bleheri*, and the pairing of *C. ornatipinnis* and *C. pulchra*. These relationships were not clear in the past, but were reliably detected by whole genome data. The construction of phylogenetic tree based on mitochondrial whole genome provides a powerful tool for clarifying the complex phylogenetic relationship of Snakehead.

4.2 Lineage resolution within the *Channa* genus

With the help of phylogenetic tree, we can further analyze the lineage divergence (patterns of lineage divergence) within Snakehead. From the above phylogenetic tree, it can be seen that the species of Snakehead are roughly clustered along geographical and morphological characteristics, showing obvious phylogenetic geographical structure. For example, the Argus group, including *Channa argus* and its relatives, is mainly distributed in temperate East Asia, the Asiatica group (*Channa argus sinensis*, etc.) is confined to the subtropical waters of southern China, and the Striata group (*Channa tanguta*, etc.) is concentrated in the plain waters of tropical Southeast Asia. This correspondence between geographical distribution and systematic lineages suggests that different lineages may have evolved independently under regional isolation and environmental selection.

Taking Chinese waters as an example, at least three major lineages can be identified: the northern temperate lineage (*Channa argus* belongs to), the South China subtropical lineage (*Channa argus maculata* and *Channa argus sinensis* belong to), and the southwestern mountain lineage (*Channa argus brevis* belongs to). The northern lineage of *Channa argus* is widely distributed in a large area north of the Yangtze River. It is the most widely distributed species in the genus Snakehead, with relatively rich genetic diversity, and presents several branches on the phylogenetic tree, corresponding to populations in different regions. In contrast, the distribution range of *Channa maculata* and *Channa sinensis* of the South China lineage is relatively limited, especially *Channa sinensis*, which is mainly found in the middle and lower reaches of the Yangtze River and local water systems to the south, and is considered to be an evolutionarily independent branch. Small species such as *Channa brevis* have multiple closely related species in mountain streams in South China and Southeast Asia to form a lineage group (Gachua Group), the internal subdivision of which is not completely clear and may contain several cryptic species. The geographical units corresponding to these lineages (northern plains, southern hills, mountain streams, etc.) reflect the impact of geological events and ecological environment on the evolution of Snakehead. Lineage differentiation analysis can also quantify the genetic distance between different lineages. The genetic distance calculated from the whole mitochondrial genome sequence shows that the genetic differences between species of Snakehead are large. For example, the sequence difference between *Channa brevis* and *Channa maculata* is about 8%~10%, while some distant species of the same genus can reach more than 15% (Zhou et al., 2019; Wang et al., 2021; Wang and Zhao, 2024). This shows that the main lineages of Snakehead are anciently differentiated and lack genetic exchange between them. On the other hand, the genetic differentiation between different geographical populations within the species is relatively small. For example, the mitochondrial differences between populations in different regions of China are usually <2%, indicating that a certain degree of genetic exchange or recent common ancestor is still maintained within the species. However, regional branches have also been detected in some widespread species. For example, the North China, Northeast China and Central China populations of the black fish form sub-lineages, corresponding to the historical differentiation caused by glacial climate and river basin isolation.

4.3 Divergence time estimation and evolutionary branching

The molecular clock method is used to calibrate the phylogenetic tree of the genus Snakehead, and the time of occurrence of each major divergence event can be estimated. The results of Rüber et al. (2020) using mitochondrial gene data calibration showed that the origin of the genus Snakehead can be traced back to the late Oligocene to the early Miocene, about 20-30 million years ago. Since then, the genus has undergone several radiation differentiations: for example, the divergence between the Asian continental group and the South Asian/Southeast Asian group such as Argus/Asiatica is estimated to have occurred about 10 Ma~15 Ma ago, which coincides with the period of geological changes and climate changes in the Himalayas and Southeast Asia. Particularly noteworthy is that the East Himalayan biodiversity hotspot is considered to be one of the important cradles of the diversification of the genus Snakehead. As the Indian Plate and the Eurasian Plate collided and uplifted, a variety of water systems and climate environments were created, which contributed to the rapid differentiation of snakehead species in the region (Kamran et al., 2020).

Analysis of each branch node found that the most recent common ancestor of some living species may have appeared in the Pliocene to the Pleistocene. For example, the phylogenetic nodes of the two major Chinese snakehead species, *Channa argus* and *Channa maculata*, are about 5 Ma~8 Ma, suggesting that they had already embarked on their own independent evolutionary paths in the Pliocene. At that time, the climate in East Asia gradually became dry and cold, which may have led to the separation of the north and south water systems, thus giving rise to the cold-resistant *Channa argus* and the warm-water *Channa maculata* lineages, respectively. This is consistent with the environmental evolution of the late Cenozoic in the geological record. For example, many species within the broad-fronted *Channa* lineage (Gachua Formation) may have differentiated during the Pleistocene climate reversals within 2 Ma ~ 3 Ma (Sholihah et al., 2021). The stream isolation and water temperature fluctuations caused by the ice age provided an opportunity for the local differentiation of small *Channa*. Although these estimates have a certain error range, they generally outline the approximate time frame for the differentiation of the main lineages of the genus Snakehead.

The division between Snakehead and its closest African genus *Parachanna* is speculated to be about 50 Ma ago, and can be traced back to the breakup of Gondwana at the end of the Cretaceous. This means that the ancestors of Asian and African *Channa* were on their own independent evolutionary paths as early as the end of the dinosaur age, and the subsequent plate drift and climate change shaped the discrete distribution pattern of *Channa* in Eurasia and Africa today. For the Snakehead genus, the differentiation node between the Southeast Asian and South Asian groups may be related to the transgression of the Indian Ocean and the Western Pacific Ocean in the middle Miocene, while the differentiation of the group within the Asian continent is related to events such as the uplift of the Qinghai-Tibet Plateau and the strengthening of the monsoon. For example, the formation period of the Argus Group coincided with the second stage of the uplift of the Qinghai-Tibet Plateau about 10 Ma, and the reorganization of the basin patterns of the Yangtze River and the Yellow River, which may have contributed to the northward spread and adaptive evolution of the ancestors of this lineage.

Node analysis also revealed that the formation of the Snakehead genus may have undergone multiple fast radiations. Some nodes on the phylogenetic tree have low support values, suggesting that related species may have differentiated almost simultaneously in a short period of time on the geological time scale, making it difficult to distinguish the systematic relationship. This is particularly evident in small species of the Snakehead genus. For example, the relationship between multiple species in the broad-headed *Channa* group has long been controversial because they may have differentiated rapidly in a certain period of the Pleistocene, and the differences between genetic lineages are very small. This radiation may be driven by rapid climate fluctuations or the emergence of new ecological spaces.

5 Regional Comparisons and Lineage Patterns

5.1 Comparative sampling across Southeast Asia and South China

The genus Snakehead is widely distributed in Southeast Asia and southern China, and the representative populations of these two regions show certain genetic differences. By comparing the mitochondrial DNA sequences of snakeheads in Southeast Asia and southern China, the characteristics of inter-regional lineage distribution can be revealed. Studies have shown that snakehead species in Southeast Asia (such as Thailand and Vietnam) are diverse, with multiple unique lineages, such as *Channa striata* and *Channa micropeltes*, which are both unique or mainly distributed species in the region. The mitochondrial sequences of these species are quite different from those of Chinese snakehead species, and they are in different branches on the phylogenetic tree. In contrast, the species of snakeheads in southern China are relatively limited, mainly *Channa striata* and *Channa sinensis*, which are closely related to some species in Southeast Asia in terms of phylogeny. For example, *Channa striata* and *Channa lucius* in Thailand belong to the same "Lucius Group" lineage. This suggests that there was species exchange and common ancestral lineages between southern China and Indochina.

From the perspective of mitochondrial genetic distance, there is almost no difference between the individual sequences of *Channa maculata* collected from southern China and Vietnam and Laos, indicating that they belong to the same evolutionary unit and have no obvious differentiation. This is consistent with the distribution pattern of *Channa maculata* in South China and Indochina. In contrast, *Channa argus* is widely distributed in China, but it is not a native species in Indochina. There are only a few distribution records in northern Vietnam, which is speculated to be caused by artificial introduction or ancient human migration. Therefore, the lineage of *Channa argus* is almost limited to China, the Korean Peninsula, and the Russian Far East, and its mitochondrial type is rare in Southeast Asia. On the other hand, Indochina has some lineages of the genus Snakehead that are not distributed in China, such as the *Channa argus* group and some species of the *Channa argus* group. The genetic diversity of these lineages is mainly concentrated in the tropical region of Southeast Asia. For example, the mitochondrial differences of *Channa argus* populations in various parts of Southeast Asia are very small, indicating that it may be widely and continuously distributed in the lowlands of Southeast Asia; while there is currently no record of wild *Channa argus* distribution in China, which reflects the differences in species composition between regions (Liu et al., 2020).

Environmental factors also lead to some phenotypic differences between Southeast Asian and South China snakeheads. For example, Southeast Asian snakeheads (such as giant snakeheads) living in tropical plain waters are huge and grow rapidly, while the spotted snakeheads in the hilly areas of southern China are medium-sized and have a more static water habit. There may be a genetic basis behind these ecological differences, and clues may be found by comparing their mitochondrial genes. Preliminary genome analysis found that tropical species such as giant snakeheads have some unique amino acid substitutions in mitochondrial coding genes, which may affect their metabolic rate to adapt to high water temperature environments; while the spotted snakeheads in South China have unique repeat sequences in the control zone sequence, which may be related to the regulation of the reproductive cycle (Hsu Htoo et al., 2025). Snakeheads in different regions differ in genetic diversity and genotype composition. For example, the genetic distance between Southeast Asian snakehead species is often greater than that between Chinese snakehead species, indicating that Southeast Asia, as a differentiation center of snakeheads, has a longer evolutionary history and richer variation.

5.2 Geographic isolation and mitogenomic differentiation

Geographic isolation is one of the key factors driving species genetic differentiation and the formation of new lineages. For the widespread snakehead fish, the isolation of different watersheds and climate zones has led to the gradual accumulation of mitochondrial genetic differences. In northern and southern China, the Nanling Mountains and the Yangtze River Basin are considered to be the biogeographical dividing lines: with this as the boundary, the lineage of *Channa argus* in the north is completely different from the lineage of *Channa maculata*/*Channa sinensis* in the south. The existence of the Nanling Mountains blocked the natural spread of snakehead to the north and south. The uplift of the region in geological history has separated the water systems of the two regions for a long time, thus giving rise to independent mitochondrial lineages (Li et al., 2023). This is supported by genetic data: the mitochondrial sequences of *Channa argus* and *Channa maculata* are quite different, but the intraspecific differences are small, which is consistent with the pattern of long-term isolation and recent expansion.

In Southeast Asia, there is also lineage differentiation caused by isolation of rivers and mountains. For example, the Irrawaddy River in Myanmar and the river system of the Malay Peninsula have different populations of snakehead, and although they are relatively close geographically, their mitochondrial lineages are almost not shared due to the independence of the water system. Even the same species of *Channa striata* (*C. striata*) shows obvious genetic structure between the Indian subcontinent, Southeast Asian mainland and Sunda Islands, corresponding to the role of paleogeographic barriers such as the Indo-Myanmar Mountains and the Sunda land bridge. These isolation events can usually be traced back to the rise and fall of sea levels and changes in river networks in the Pleistocene. For example, when the Sunda shelf was exposed during the glacial retreat, South China was connected to the Southeast Asian mainland, and species exchange may have occurred at one time; while the interglacial transgression blocked the connection, leading to isolation and differentiation. In the Yunnan Plateau in southwest China, the complex terrain and water system also nurtured the genetic diversity of the genus Snakehead. Different mitochondrial branches of small *Channa* such as the broad-headed *Channa* were found in the separated water systems such as the Lancang River and the upper reaches of the Pearl River, which may represent local lineages formed by geographical isolation. For example, the mitochondrial sequences of the broad-headed *Channa* population in Guangxi and the population in Xishuangbanna, Yunnan are significantly different, and are considered to be different evolutionary lineages, and their differentiation may be related to the barrier of the Ailao Mountains (Zhang et al., 2020).

Geographic isolation restricts gene flow, causing local populations of snakehead to evolve along independent trajectories and gradually form mitochondrial differences. This isolation includes both large scales (such as the Nanling Mountains separating the north and the south, and China and the Southeast Asian continent), and local scales (such as the isolation of different river basins). The longer the isolation and the more difficult the barriers are to overcome, the greater the lineage differences tend to be. In the snakehead genus, it can be seen that the lineages of snakeheads in the Yangtze River and Pearl River basins have been clearly distinguished, while there is no significant difference in the scorpionfish between the tributaries of the Pearl River, which is a reflection of the degree of isolation. Under the effect of geographic isolation, not only does the mitochondrial genome drift and differential selection occur, but snakeheads in different regions also gradually differentiate in morphology and ecology. For example, the northern black snakehead is more cold-resistant and large in size, while the southern scorpionfish is more adapted to warm water and slightly smaller in size. These regional variations in turn consolidate the independence of the lineages. Of course, geographic isolation is not absolute, and in some cases human activities can break natural barriers. For example, hybrid snakeheads (the first generation of the hybrid of black snakehead and scorpionfish) in the south have been introduced and cultured in the northern region. Once they escape, they may cause the original lineage to mix. But in general, the geographical correspondence of each lineage of the genus Snakehead under natural conditions is clear, and isolation has shaped the current genetic pattern.

5.3 Regional lineage markers and adaptation clues

Through regional comparison, we can try to identify some lineage-specific molecular markers and preliminarily explore the adaptive significance of the evolution of the lineage of the genus Snakehead. The so-called lineage marker refers to genetic variation that only appears in a specific regional lineage, such as a specific mitochondrial haplotype or mutation site, which can be used to trace the origin and spread of the lineage. Taking the northern lineage of *Channa argus* as an example, its mitochondrial D-loop region has a unique deletion mutation, which is only found in populations north of the Yellow River, but not in *Channa argus* in the Yangtze River Basin and south of it. This deletion mutation can be used as a marker of the northern lineage to identify the source of suspicious released populations. Similarly, the South China lineage of *Channa maculata* carries a unique microsatellite repeat sequence that does not exist in other snakeheads in Southeast Asia, which can help distinguish the mitochondrial types of *Channa maculata* in South China and the closely related species *Channa* annan.

In terms of adaptive evolution, the environments of lineages in different regions are significantly different, so they may have accumulated genetic characteristics adapted to them. Although the nuclear genome plays a more important role in adaptive traits, the mitochondrial genome may also be involved in environmental adaptation because it controls energy metabolism. For example, the lineage of black snakehead in high-latitude areas may select more efficient energy metabolism mutations in mitochondrial genes to maintain activity in cold water temperatures. In contrast, snakeheads in tropical hypoxic waters (such as some small snakeheads in Thailand) may have substitutions in mitochondrial complex genes that are conducive to hypoxic respiration. Studies on plateau schizothorax have found that certain mutations in the mitochondrial *ND1* gene improve the efficiency of electron transfer, allowing it to effectively produce energy in a low-oxygen, high-altitude environment (Jin et al., 2018; Li et al., 2018). Similar mechanisms may also exist in the genus Snakehead: for example, the broad-fronted snakehead lives in a mountain stream environment where the water temperature is relatively low and food is scarce. It is speculated that its mitochondria may have evolved a more energy-efficient respiratory chain configuration. This needs to be further verified by comparing the mitochondrial gene functions of the broad-fronted snakehead and the plain snakehead.

The adaptive evolution of regional lineages is also reflected in behavior and life history. Most snakehead species in the Indochina Peninsula reproduce in the monsoon rainy season and have a strong adaptation to seasonal rhythms; while the black snakehead in northern China can survive under the ice in winter and have significant tolerance to low temperatures. The genetic basis behind these differences may partly come from mitochondrial regulation. For example, mutations in some regulatory regions of the control region sequence of the northern lineage of black snakehead may have changed the regulation of respiration at low temperatures, thereby improving the survival rate over the winter. Similarly, the rapid reproduction characteristics of the southern lineage of snakehead may be related to the improvement of mitochondrial replication rate and energy supply capacity.

6 Case Studies

6.1 Genomic comparison of *Channa argus* and *Channa maculata*

Channa argus and *Channa maculata* are the two most widely distributed and economically important snakehead fish in China. The two are morphologically similar, but their ecological habits and geographical distribution are different: *Channa argus* is cold-resistant and distributed in the north; *Channa maculata* prefers warmth and is only found in the south. Using mitochondrial genome data, we can deeply compare the genetic differences between these two closely related species. First, from the perspective of the whole genome sequence, the mitochondrial genome lengths of *Channa argus* and *Channa maculata* are very similar, both about 16.56 kb, and the gene content and arrangement are exactly the same (Xu et al., 2024). This indicates that they share the ancestral genome structure. However, in terms of sequence, there are obvious base differences between *Channa argus* and *Channa maculata*, and the difference rate of the mitochondrial full sequence is about 8%~9%. This difference is enough to distinguish them as two species. These differences are mainly concentrated in the control region and several coding genes: the sequence difference in the control region is more than 15%, while the difference in the *COX1* gene is less than 2%. Of particular note, some non-synonymous mutations are fixed between the two species. For example, several amino acid substitutions found in the *ND5* gene, all of which are one amino acid in *Channa argus* and another in *Channa maculata*, suggest that the two may differ in the function of complex I.

Comparisons at the nuclear genome level also provide interesting insights. Recent chromosome-level whole genome sequencing and assembly of *Channa argus* and *Channa maculata* revealed that *Channa argus* has 24 pairs of chromosomes, while *Channa maculata* has 21 pairs. The *Channa maculata* genome has three fewer chromosomes, which is due to three chromosome fusion events in the *Channa maculata* genome. This difference highlights the degree of evolutionary divergence between *Channa argus* and *Channa maculata*, which is greater than what can be seen from mitochondrial data (Figure 1) (Ou et al., 2021). Chromosomal fusions may have profound effects on gene expression and adaptability. For example, it is speculated that *Channa maculata* may have expanded some immune-related genes, making its disease resistance better than that of *Channa argus*. Correspondingly, mitochondrial genome comparison also supports that *Channa maculata* may be more resistant to diseases: a mutation in the mitochondrial *Cyt b* gene of *Channa maculata* is reported to be related to the regulation of cell apoptosis, which may enhance the ability to cope with pathogens.

On the other hand, the advantage of *Channa argus* in low-temperature environments may partly come from its optimized mitochondrial function. Comparison of the whole mitochondrial genomes of *Channa argus* and *Channa maculata* found that there was a unique amino acid substitution in the *Channa argus* ATP synthase subunit 6 (*ATP6*) gene that was not found in *Channa maculata*, which was speculated to improve the efficiency of ATP synthase at low temperatures. Although this speculation needs experimental verification, its corresponding relationship with the stronger cold tolerance of *Channa argus* is worth further study. As a pair of sister species, *Channa argus* and *Channa maculata* have both high similarity and clear variation differences in mitochondrial genomes. These differences are not only indicators of molecular phylogeny, but may also correspond to differences in ecological adaptability between the two. The comparative case of *Channa argus* and *Channa maculata* shows that even for closely related species, variations at the mitochondrial and nuclear genome levels can accumulate to a significant degree and subtly affect the physiological and ecological characteristics of the species.

6.2 Phylogenetic positioning of samples from Guangxi, Yunnan, and Indochina

Guangxi and Yunnan in China are geographically connected to the Indochina region (Vietnam, Laos, Thailand, etc.) and are located in the tropical-subtropical transition zone with rich biodiversity. The phylogenetic tree constructed based on the mitochondrial whole genome sequence shows that the *Channa maculata* samples from Guangxi and Vietnam are clustered together with almost no genetic differentiation, which supports that they belong to the same species and the same lineage. This also confirms that Guangxi and the northern part of Indochina were geographically connected (such as the Red River Basin), allowing *Channa maculata* to be widely distributed. The broad-headed *Channa* samples in Guangxi and the broad-headed *Channa* (or closely related species) samples in northern Vietnam also clustered into one branch, belonging to the Gachua lineage. This shows that there is a continuous distribution of the broad-headed *Channa* lineage in the mountain stream environment of South China and Indochina, without obvious isolation.

In contrast, the situation in Yunnan is more complicated. Snakehead samples in western Yunnan (mainly *Channa* spp.) cluster with some samples from Thailand and Myanmar on the phylogenetic tree, but independent small branch lines also appear (Figure 2) (Tan et al., 2012; Hsu Htoo et al., 2025). For example, the *Channa* spp. in Xishuangbanna, Yunnan, and the *Channa* spp. in northern Thailand cluster into sister groups, suggesting that they were once connected through the Lancang-Mekong River system; while some snakehead samples in southeastern Yunnan (possibly *Channa sinensis* or its close relatives) are close to samples in southern China, indicating a connection with the Pearl River system. This reflects Yunnan's special status as a biogeographic intersection: its western water system belongs to the Southeast Asian basin, and its eastern water system belongs to the Pearl River basin in China. Therefore, the lineages of snakeheads in different regions are respectively connected to the lineages of the Indochina Peninsula or South China.

Specifically, Yunnan samples can be divided into two categories on the phylogenetic tree: one is adjacent to Southeast Asian species (such as *Channa fasciatus* or *Channa annanensis*), and the other is adjacent to Chinese species (such as *Channa maculata* and *Channa sinensis*). This indicates that Yunnan has preserved Snakehead lineages from both directions, which may be the result of multiple biological migrations and isolations in history. During the glacial period, the climate of the Yunnan Plateau was cool and humid, connecting the northern and southern habitats, allowing some South China lineages to spread westward; and the expansion of the South Asian tropical forests during the interglacial period facilitated the Southeast Asian lineages to enter Yunnan from the north.

6.3 Ecological adaptations influencing lineage evolution in key habitats

The evolutionary adaptation of Snakehead in different ecological environments is an important aspect of understanding its lineage formation. In typical aquatic environments (such as high-altitude mountain streams, low-oxygen swamps, cold lakes, etc.), Snakehead lineages often show special ecological and behavioral characteristics, suggesting that there are corresponding genetic adaptation mechanisms behind them. Taking the plateau mountain stream environment as an example, the members of the broad-fronted snakehead lineage living there are relatively small individuals, short life cycles, and early sexual maturity, reflecting the tendency of "r-strategy" to cope with environmental instability (Wang et al., 2023). These characteristics may be partly determined by genetics. In the mitochondrial genome of these fish, some mutations that enhance basal metabolism and reproductive rate may have been fixed, allowing them to reproduce rapidly in the resource-limited mountain stream environment. This adaptation mechanism is similar to the strategy of alpine cold-water fish to evolve higher reproductive investment to compensate for high mortality.

Some black fish (such as mud snakeheads) live in swamps or rice fields with poor water quality and low dissolved oxygen, and have evolved auxiliary organs for air breathing and certain amphibious capabilities. Mitochondria play a key role in this adaptation: effective anaerobic metabolism and a lower oxygen-consuming respiratory chain may be the key to their survival. Studies have found that black snakeheads that can survive in the air have a higher tolerance to lactic acid accumulation in their blood and muscles. It is speculated that their mitochondria may have adjusted the pyruvate metabolic pathway to adapt to frequent hypoxia. This suggests that lineages such as the black snakehead may have improved mitochondrial enzyme functions, such as the Cyt b complex reducing free radical production, and the *ND* gene product improving the stability of electron transfer under hypoxic conditions. Molecular evidence of this type of adaptation still needs to be obtained by comparing the mitochondrial functions of snakeheads in different environments.

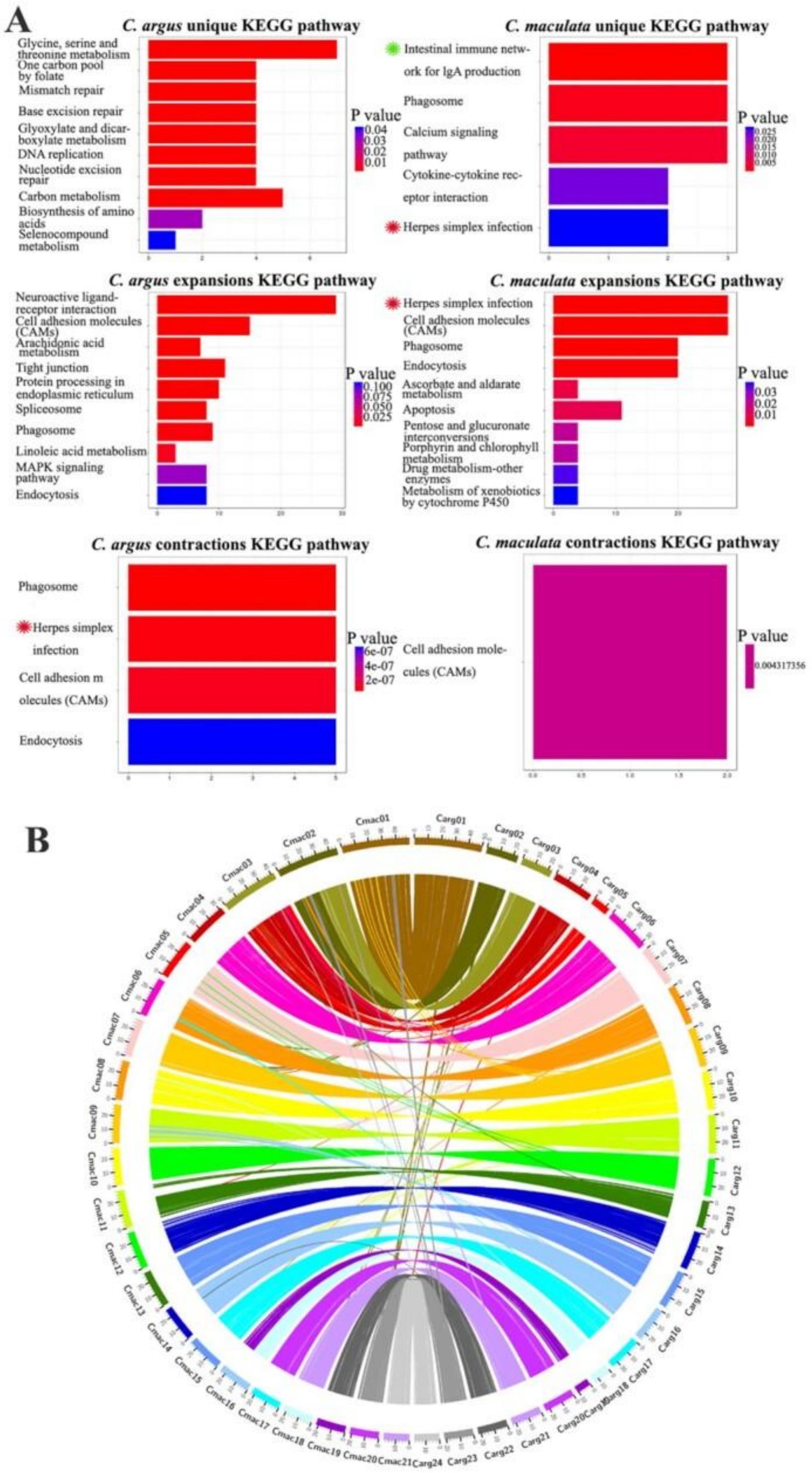

Figure 1 Comparative analysis of the *C. argus* and the *C. maculata* genomes (Adopted from Ou et al., 2021)

Image caption: (A) KEGG enrichment analysis of the unique, expansion, and contraction gene families. The ordinate is KEGG terms, the abscissa is the number of genes in the pathway, and the colour represents the corresponding P-value. Left, enrichment result for *C. argus*; right, enrichment result for *C. maculata*, same asterisks indicate same terms. (B) There was a high collinearity between the 2 species. Chr 2 and 3 of *C. argus* correspond to Chr 2 of *C. maculata*, Chr 4 and 5 of *C. argus* correspond to Chr 3 of *C. maculata*, Chr 18 and 19 of *C. argus* correspond to Chr 16 of *C. maculata* (Adopted from Ou et al., 2021)

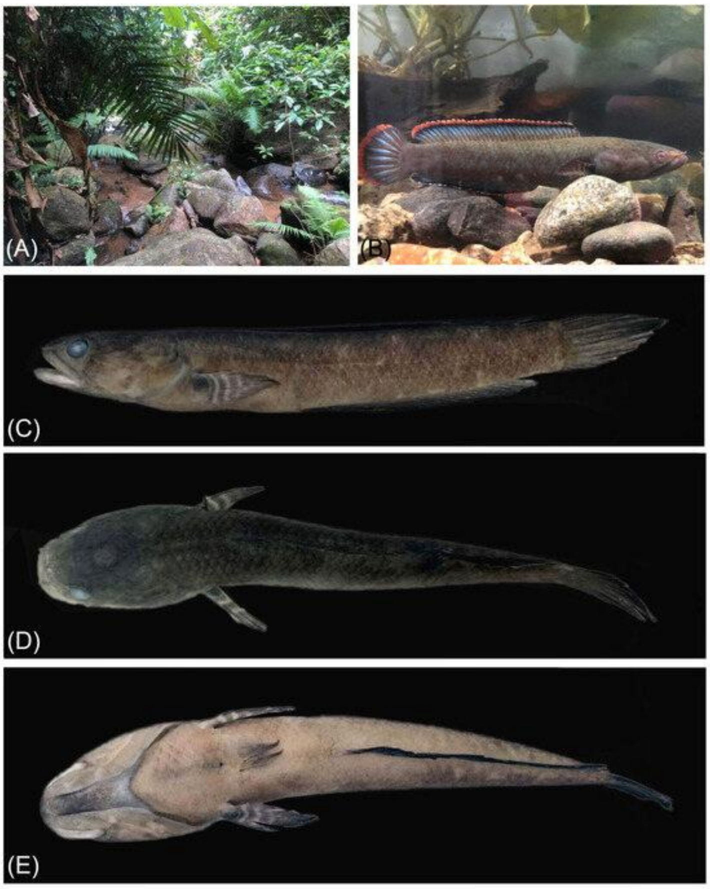

Figure 2 (A) Collection site and natural habitat of *Channa shingon* in Kachin State, Myanmar; (B) lateral view and coloration of a live specimen in an aquarium; and vouchered specimen views showing; (C) lateral with pectoral fin pattern; (D) dorsal; and (E) ventral with lower side of head and thorax perspectives of *C. shingon* (Adopted from Hsu Htoo et al., 2025)

In cold lake environments, snakeheads represented by the northern lineage of the black snakehead show strong cold resistance. They can still survive when the water temperature is close to freezing in winter, while the spotted snakehead enters a paralyzed state when it is below 10 °C. This difference is likely to be derived from long-term natural selection, which has accumulated mutations in the black snakehead that ensure that cells can still produce energy at low mitochondrial temperatures. For example, the fatty acid composition of the mitochondrial membrane of the muscle of the black snakehead may have changed (partially unsaturated), reducing the membrane phase transition point; some mitochondrial enzymes such as ATP synthase may have improved the catalytic efficiency at low temperatures. These changes are inseparable from the support of genomic variation, among which mutations related to the mitochondrial genome should play a certain role. Ou et al. (2021) found through whole genome analysis that *Channa argus* and *Channa maculata* have different gene expression responses to low temperatures: *Channa argus* brain and liver rapidly activated a large number of differentially expressed genes at low temperatures. This suggests that *Channa argus* is genetically equipped with a mechanism to respond quickly to low temperatures.

7 Concluding Remarks

Mitochondrial genome variation plays an important role in analyzing the phylogenetic relationships of the genus Snakehead. On the one hand, the whole mitochondrial genome sequence provides rich information for constructing a high-resolution phylogenetic tree, which clarifies the complex lineage relationships within the genus Snakehead. Many branch arrangement problems that were difficult to solve based on limited gene fragments in the past have been answered with the help of whole genome data, such as identifying new sister species pairs and clarifying the affiliation of major lineage groups. The systematic relationships revealed by mitochondrial variation are consistent with geographical distribution and morphological characteristics, which improves the credibility of taxonomy. On the other hand, mitochondrial variation also provides clues for studying species adaptability. Although its direct impact is limited, through the mitochondrial differences of different lineages, we can infer some possible ways for species to adapt to the environment and point out the direction for further functional research. For example, the molecular basis behind traits such as cold tolerance of black snakehead and disease resistance of spot snakehead can be inferred through lineage-specific mutations.

Mitochondrial DNA as a maternal genetic marker can also help reveal population historical dynamics, such as glacial refuges and expansion routes. In the phylogeographic study of the genus Snakehead, the unique haplotypes in different regions and their phylogenetic positions provide evidence for reconstructing the history of species diffusion. For example, the haplotype diversity of the northern lineage of *Channa argus* is low and concentrated, suggesting that it may have experienced a rapid expansion after the glacial bottleneck; while the haplotype differentiation of *Channa argus* in Southeast Asia is deep, indicating that the region may be its long-term evolutionary center. These inferences all rely on the maternal genetic information recorded by mitochondrial variation. It can be said that mitochondrial genome variation is an indispensable part of the study of the phylogeny and evolution of the genus Snakehead. However, it should also be recognized that it is not omnipotent. In some recent events of rapid differentiation, mitochondria may have incomplete sequencing problems; for hybrid species, it can only reflect the history of the maternal side. Therefore, when using mitochondrial variation to analyze systematic relationships, we need to interpret it with caution and try to combine nuclear gene data to obtain a more comprehensive perspective.

Although the mitochondrial genome provides a lot of useful information, the methods and data based on this study also have certain limitations. First, mitochondrial DNA only represents maternal inheritance and cannot reflect processes such as paternal gene flow and recombination. This may be misleading in some hybridization or gene penetration situations. For example, if two snakehead species have hybridized, mitochondria may only show a monophyletic relationship and mask the complex history of gene exchange. Therefore, the mitochondrial phylogenetic tree alone may not be able to fully reveal the species evolutionary network. In the genus Snakehead, although no new lineages formed by interspecific hybridization have been clearly reported, it does not rule out that there has been gene exchange in history, especially in geographical intersection areas. Solving this problem requires the introduction of nuclear genome markers (such as SNPs, nuclear gene sequences, etc.) for auxiliary verification. Secondly, the existing data coverage is still incomplete. There are dozens of species in the genus Snakehead, but sequencing studies are mainly focused on a few species with high economic value or wide distribution. Many rare species and newly described species lack genome data. For example, the mitochondrial genomes of several new species discovered in Myanmar in recent years have not yet been published. These gaps limit our understanding of the full picture of the genus Snakehead and cause species sampling bias in phylogenetic analysis. In the future, it is necessary to further determine the mitochondrial genomes of all known species in the genus Snakehead and conduct research on suspected cryptic species to improve the phylogenetic tree. In addition, the population-level sampling within some species is also insufficient. For example, in the complex lineage of the broad-headed *Channa*, there are few representative samples from different regions, and key lineage branches may be missed. Increasing population sampling will help depict the fine-scale phylogenetic geographical structure. On the technical level, mitochondrial genome analysis itself also has sources of error. For example, PCR amplification sequencing may produce errors, and there are difficulties in aligning sequences in tandem repeat regions. These need to be improved through higher-quality sequencing (such as third-generation sequencing to directly obtain complete circular sequences) and more optimized bioinformatics methods. Molecular clock calibration relies heavily on fossil records and evolutionary rate assumptions, and node ages may differ significantly under different assumptions. Therefore, we should be cautious about the inference of evolutionary time, and combine multiple genes and calibration points for cross-validation when necessary.

Looking to the future, the phylogenetic and evolutionary research of the genus Snakehead is expected to achieve more in-depth results driven by the integration of genomics and ecological methods. On the one hand, the application of metagenomics and comparative genomics will enable us to go beyond mitochondria and understand the evolution of the genus Snakehead from a whole genome scale. Using nuclear genome data obtained by high-throughput sequencing, phylogenetic trees containing tens of thousands of sites can be constructed to better solve complex situations such as rapid radiation and hybridization. For example, by comparing the whole genomes of *Channa argus* and *Channa maculata*, we can find differences in nuclear genes related to temperature adaptation, thereby supporting the speculation of mitochondrial analysis. At the same time, the whole genome haplotype network can accurately depict the historical dynamics of populations and provide a powerful tool for phylogenetic geography. On the other hand, incorporating ecological and environmental data into the evolutionary analysis framework can reveal the correspondence between genomic variation and ecological factors. For example, combining climate models and distribution data, the impact of glacial climate change on the spread of the Snakehead lineage can be deduced, and the location of climate refuges can be verified using population genetic data. For another example, physiological experiments on snakehead under different environmental conditions can be carried out to measure indicators such as oxygen consumption and cold resistance, and to conduct association analysis with the genotypes of different lineages to understand the adaptive significance of genetic variation from a functional perspective. Environmental DNA (eDNA) technology can also be used to detect the distribution and spread of snakehead species in the environment. Combined with phylogenetic information, it will help us understand the source and spread path of invasive species (such as black snakehead overseas) and formulate management strategies. Future research can also focus on the direct association between genes and traits. For example, through genome-wide association analysis (GWAS), mitochondrial or nuclear DNA markers related to growth, tolerance and other traits can be found, and hybrid offspring of different lineages can be cultivated for comparison to dissect species differences at the genetic level. This also has practical significance for genetic improvement in aquaculture. At present, China has successfully bred hybrids of *Channa argus* × *Channa maculata* for aquaculture. If we can identify which mitochondrial and nuclear gene combinations are beneficial to growth and stress resistance, it will guide the breeding of new varieties.

Acknowledgments

We are grateful to Dr. W. Zhou for his assistance with the serious reading and helpful discussions during the course of this work.

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.

References

Alam M., Andriyono S., Sektiana S.P., Rahman M.M., and Kim H.W., 2019, The molecular characterization of complete mitochondrial genome of spotted snakehead fish *Channa* punctata (Bloch 1793), Mitochondrial DNA, 4(2): 547-548.
https://doi.org/10.1080/23802359.2018.1553520

Fan W., Zhang L., Su J., Luo Y., Jiao X.L., Huang Z.P., Zhao H., Zhao Z.M., Duan Y.L., Li Q., Du J., Zhuo T., Su Q.S., Wu J., Zhou J., 2022, Genetic diversity of two color morphs of Northern snakehead (*Channa argus*) unveiled by the mitochondrial DNA D-loop region, Mitochondrial DNA, 7(3): 515-520.

Fang C., Zeng F., Chen S., Li S., Yang Y., Lin W., Liu Y., Cheng Peng C., and Yang H., 2024, Gender impacted gut microbiota and growth performance in the blotched snakehead (*Channa maculata*), Microorganisms, 12(5): 871.
https://doi.org/10.3390/microorganisms12050871

Garg R.K., and Dohre S., 2024, Evaluation of molecular genetic diversity and DNA barcoding of Rita gogra from Narmada River revealed through mitochondrial *COX1* gene sequencing, Asian Journal of Biological and Life Sciences, 12(3): 587.
https://doi.org/10.5530/ajbls.2023.12.77

Hsu Htoo B., Laskar B., Lee S., Vu S.V., Phyo P.M.M., Thitsar P., Kim H.W., and Kundu S., 2025, Unified morphological and genetic analyses confirm the existence of the dwarf snakehead *Channa shingon* (Anabantiformes: *Channidae*) in Kachin State Myanmar, Fishes, 10(3): 100.
https://doi.org/10.3390/fishes10030100

Jin Y., Wo Y., Tong H., Song S., Zhang L., and Brown R.P., 2018, Evolutionary analysis of mitochondrially encoded proteins of toad-headed lizards *Phrynocephalus* along an altitudinal gradient, BMC Genomics, 19: 1-11.
https://doi.org/10.1186/s12864-018-4569-1

Kamran M., Yaqub A., Malkani N., Anjum K.M., Awan M.N., and Paknejad H., 2020, Identification and phylogenetic analysis of *Channa* species from riverine system of pakistan using *COI* gene as a DNA barcoding marker, Journal of Bioresource Management, 7(2): 10.
https://doi.org/10.35691/jbm.0202.0135

Li M., Yang X., Ni X., and Fu C., 2023, The role of landscape evolution in the genetic diversification of a stream fish *Sarcocheilichthys parvus* from Southern China, Frontiers in Genetics, 13: 1075617.
https://doi.org/10.3389/fgene.2022.1075617

Li R., Wang G., Wen Z., Zou Y., Qin C., Luo Y., Wang J., and Chen G., 2018, Complete mitochondrial genome of *Channa siamensis* and its phylogenetic consideration, Genes and Genomics, 41(2): 147-157.
https://doi.org/10.1007/s13258-018-0746-5

Liu M.-Y., Yin J., Han J., Ren J., and Yang S., 2020, *Channa argus* BMH from Baima Hu lake: sequencing and phylogenetic analysis of the mitochondrial genome, Mitochondrial DNA, 5: 2413-2415.
https://doi.org/10.1080/23802359.2020.1775144

Ou M., Huang R., Yang C., Gui B., Luo Q., Zhao J., and Chen K., 2021, Chromosome-level genome assemblies of *Channa argus* and *Channa maculata* and comparative analysis of their temperature adaptability, GigaScience, 10(10): giab070.
https://doi.org/10.1093/gigascience/giab070

Rüber L., Tan H.H., and Britz R., 2020, Snakehead (Teleostei: *Channidae*) diversity and the Eastern Himalaya biodiversity hotspot, Journal of Zoological Systematics and Evolutionary Research, 58(2): 356-386.
https://doi.org/10.1111/jzs.12324

Sholihah A., Delrieu-Trottin E.L., Condamine F., Wowor D., Rüber L., Pouyaud L., Agnèse J.F., and Hubert N., 2021, Impact of *Pleistocene eustatic* fluctuations on evolutionary dynamics in Southeast Asian biodiversity hotspots, Systematic Biology, 70(5): 940-960.
https://doi.org/10.1093/sysbio/syab006

Tan M.P., Jamsari A.F.J., and Siti Azizah M.N., 2012, Phylogeographic pattern of the striped snakehead *Channa striata* in Sundaland: ancient river connectivity geographical and anthropogenic signatures, PLoS ONE, 7(12): e52089.
https://doi.org/10.1371/journal.pone.0052089

Wang F., and Zhao F., 2024, Zebrafish as a model for studying ciliary development and disease, International Journal of Marine Science, 14(5): 332-340.
https://doi.org/10.5376/ijms.2024.14.0037

Wang G.L., Chen L., and Mai R.D., 2024, Toxicological studies of fish and fish cells in vitro and in vivo, International Journal of Marine Science, 14(6): 359-367.
https://doi.org/10.5376/ijms.2024.14.0040

Wang J., Li C., Chen J., Wang J., Jin J., Jiang S., Yan L., Lin H.D., and Zhao J.L., 2021, Phylogeographic structure of the dwarf snakehead (*Channa gachua*) around gulf of tonkin: historical biogeography and pronounced effects of sea-level changes, Ecology and Evolution, 11(17): 12583-12595.
https://doi.org/10.1002/ece3.8003

Wang J., Xu W.; Liu Y.; Bai Y., Liu H., 2023, Comparative mitochondrial genomics and phylogenetics for species of the snakehead genus *Channa* Scopoli 1777 (Perciformes: *Channidae*), Gene, 857: 147186.

Xu T., Zhang W., Li Y., Wang J., Bai Y., and Liu H., 2024, The Complete mitogenomes of two species of Snakehead fish (Perciformes: *Channidae*): genome characterization and phylogenetic analysis, Diversity, 16(6): 346.
https://doi.org/10.3390/d16060346

Zhang D.R., Hui H., Yu G.H., Song X.Q., Liu S., Yuan S., Xiao H., and Rao D., 2020, Shared response to changes in drainage basin: Phylogeography of the Yunnan small narrow-mouthed frog *Glyphoglossus yunnanensis* (Anura: *Microhylidae*), Ecology and Evolution, 10: 1567-1580.
https://doi.org/10.1002/ece3.6011

Zhang X., Zhu X., Chen K., Zhao J., Qing L., and Hong X., 2015, Comparison of the northern snakehead (*Channa argus*) and blotched snakehead (*Channa maculata*) and their reciprocal hybrids based on complete mitochondrial DNA sequences, Mitochondrial DNA, 26(5): 805-806.
https://doi.org/10.3109/19401736.2013.855902

Zhou A., Xie S., Liu S., Sun Z., Wang Z., Zhang Y., Chen Y., Luo M., and Zou J., 2019, Genetic diversity of northern snakehead (*Channa argus*) based on complete mitochondrial *COI* gene sequences, Mitochondrial DNA, 4(3): 599-602.
https://doi.org/10.1080/23802359.2018.1561222