Bioscience Methods 2025, Vol.16, No.3, 137-153
http://bioscipublisher.com/index.php/bm

Review and Progress Open Access

Mitochondrial Genome Evolution of Abalone and Its Applications in Species Identification

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Bioscience Methods, 2025, Vol.16, No.3 doi: 10.5376/bm.2025.16.0013
Received: 21 Mar., 2025
Accepted: 29 Apr., 2025
Published: 19 May, 2025

Preferred citation for this article:
Sun C.M., and Mai R.D., 2025, Mitochondrial genome evolution of abalone and its applications in species identification, Bioscience Methods, 16(3): 137-153 (doi: 10.5376/bm.2025.16.0013)

Abstract The genus *Haliotis* includes a variety of marine shellfish with important economic and ecological values. In recent years, the mitochondrial genome has received extensive attention in the study of abalone systematic classification and species identification due to its unique advantages. This study systematically sorted out the structural characteristics of the abalone mitochondrial genome, the genetic variation pattern and the evolutionary relationship between different species, focusing on the analysis of the role of SNP and non-synonymous mutations in adaptive evolution. Through the construction of a whole-genome phylogenetic tree, the lineage differentiation and geographical isolation correlation within the genus *Haliotis* were revealed, and the application potential of mitochondrial DNA markers (such as *COI* gene) in rapid species identification and traceability detection was evaluated. Combined with case analysis, the mitochondrial variation characteristics of abalone species in the southeast coast of China were compared, the genetic differences between introduced populations and local populations were evaluated, and the disputes over the classification of abalone in Japan, Australia and East Asia were discussed. The study shows that the evolutionary characteristics of the abalone mitochondrial genome are of great value for species identification, but different methods have their own advantages and limitations. This study aims to provide a basis for the systematic classification of the abalone genus, and to provide theoretical support for the scientific management of my country's abalone germplasm resources, the protection of genetic diversity and sustainable utilization, so as to improve the quality of abalone seed industry and ensure the ecological security of marine fisheries.

Keywords Abalone; Mitochondrial genome; Phylogeny; DNA barcode; Species identification

1 Introduction

The genus *Haliotis* is a large gastropod mollusk with great economic and ecological value in the ocean. Its meat is tender and nutritious, making it a popular seafood delicacy. It is farmed and harvested along the coasts of the world. According to statistics, more than 50 abalone species have been reported worldwide, and about 7 species are distributed along the coast of China, mainly including the wrinkled disc abalone (*Haliotis discus hannai*) and the variegated abalone (*H. diversicolor*), showing rich species diversity (Chen et al., 2016). Abalone not only has important fishery and aquaculture value, but is also an ideal material for studying the adaptive evolution and biogeographic distribution of shellfish. Some large abalone species have been listed as endangered species due to overfishing and resource decline. It is urgent to protect their genetic diversity. In recent years, China's abalone farming industry has developed rapidly and production has increased significantly. In 2018, production increased by about 14.5 times compared with 2003 (You, 2023). With the expansion of the abalone industry and the increase in cross-regional introduction activities, it is increasingly important to quickly and accurately identify abalone species and germplasm. This is not only related to the selection and breeding of fine varieties and aquaculture management, but also has practical significance for preventing species confusion, maintaining market order and protecting wild resources.

Due to its special genetic characteristics, mitochondrial DNA has been widely used in the classification and evolution of marine invertebrates. Compared with the nuclear genome, the mitochondrial genome has a compact structure (no introns), uniparental inheritance, and a small effective size, making it easy to obtain the full sequence, so it is often used to infer molecular phylogenetic relationships and species identification. The mitochondrial genome of mollusks such as abalone usually contains 13 protein-coding genes, 2 rRNA genes, and 22 tRNA genes,

with a total length of about 16 kb, and the gene organization is relatively conservative, which facilitates cross-species gene comparison and phylogenetic analysis. The mitochondrial gene mutation rate is high, and it can accumulate sufficient intra- and inter-specific variation to distinguish closely related species or populations (Li et al., 2021). Especially in shellfish classification, traditional morphological methods sometimes make it difficult to accurately identify species due to morphological plasticity or different developmental stages. The application of mitochondrial DNA sequences (such as *COI* gene barcodes) provides an efficient and reliable molecular means for identifying cryptic species, new species, and larval stage individuals (Jung et al., 2015; Szyp-Borowska and Sikora, 2019).

This study will systematically review the evolutionary characteristics of the abalone mitochondrial genome and its application progress in species identification, summarize the basic structure of the abalone mitochondrial genome, including characteristics such as gene composition, length, gene arrangement, and variation patterns in control regions and non-coding regions, analyze the distribution patterns of variation types (such as SNPs and InDels) in the abalone mitochondrial genome, explore the adaptive evolution that non-synonymous mutations may reflect, and compare the gene conservation and differences between different species. Based on the phylogenetic reconstruction results of the mitochondrial whole genome sequence, the phylogenetic structure and differentiation nodes of the main evolutionary branches within the genus *Haliotis* are explained, and the relationship between mitochondrial variation and geographical isolation is analyzed. Through specific case studies, the systematic relationships of abalone species populations in the southeastern coast of China are compared, the genomic differences between the introduced Japanese abalone and the local abalone are evaluated, and the phylogenetic relationships and taxonomic disputes between Japanese, Australian and East Asian abalone are discussed. This study hopes to provide a comprehensive and in-depth reference for abalone taxonomy and molecular identification research to promote abalone species protection, genetic breeding and sustainable development of the industry.

2 Structural Features of the Abalone Mitochondrial Genome

2.1 Genome composition, size, and gene order

The abalone mitochondrial genome is a closed circle, generally between 16 kb and 17 kb in length. For example, the mitochondrial genome of *Haliotis discus* is about 17 037 bp in length and contains 37 genes, including 13 protein-coding genes, 22 tRNA genes and 2 rRNA genes. This gene composition is consistent with the mitochondrial genomes of most invertebrates (especially gastropods), reflecting the high conservation of the mitochondrial genome. The gene arrangement of the abalone mitochondrial genome remains unchanged among different species: the relative order and chain polarity of the genes are basically the same. Studies have compared the mitochondrial genome arrangements of multiple abalone species and found that except for a few species (such as European abalone *H. tuberculata*), the mitochondrial gene arrangements of abalone species are all homologous, and no major rearrangements have occurred (Pu et al., 2020). It has been reported that the positions of $tRNA^{Ser}_{(AGN)}$ and $tRNA^{Phe}$ in the mitochondrial genome of *H. tuberculata tuberculata* have been swapped relative to other species, suggesting that the genome structure is extremely stable during the evolution of abalone, and only a few lineages have undergone minor rearrangements. This highly conservative gene arrangement facilitates the comparative alignment of mitochondrial genomes and the identification of homologous sequences in different abalone species. The GC content of the abalone mitochondrial genome is approximately 35%-41%. For example, the base composition of the mitochondrial genome of *H. asinina* is 35.3% A, 24.3% T, 13.3% G, and 27.1% C, showing the common characteristics of A+T bias (Mamat et al., 2025). The composition and organizational structure of the abalone mitochondrial genome are highly similar among species within the genus, which lays the foundation for subsequent interspecific variation and phylogenetic analysis.

2.2 Variation patterns in control and non-coding regions

In the mitochondrial genome, the control region (D-loop) is the longest non-coding region and is usually the region with the most sequence variation. The mitochondrial control region of abalone is rich in AT bases and contains replication origins and transcriptional regulatory elements. Its length and sequence may vary significantly between different species and even between different individuals of the same species. For example, in different individuals of *Haliotis diversicolor*, the length of the control region can vary by tens of bases, often due to changes in the

copy number of tandem repeat sequences. Studies have found that there are some repeat motifs and microsatellite sequences in the control region of abalone. The unstable expansion and contraction of these repeat units lead to polymorphism in the length of the control region. At the same time, the control region sequence evolves rapidly and often accumulates interspecies diagnostic mutation sites. For example, by comparing the control region sequences of multiple geographical populations of *Haliotis discus*, it was found that the base substitution frequency in the hypervariable region was significantly higher than that in the coding gene region, which can be used to identify subtle population genetic differentiation. In addition to the control region, there are also some extremely short non-coding spacers (usually only a few bp to tens of bp) in the abalone mitochondrial genome. Despite the short length of these spacers, base insertion/deletion (InDel) variations have also been observed between some species. For example, a comparison of mitochondrial genes of *H. diversicolor* from two geographical populations showed that there was a 5 bp spacer between the tRNA-His and nad5 genes, and a 1 bp insertion mutation was detected only in Vietnamese individuals (Xin et al., 2011). In general, the variation of abalone mitochondrial non-coding regions (especially control regions) is higher than that of coding regions, and the high polymorphism of their sequence length and composition provides useful markers for population genetic diversity analysis and discrimination of closely related species. However, since control region sequences are usually difficult to align and analyze, concatenated sequences of 13 protein-coding genes are more often used in phylogenetic studies to construct reliable phylogenetic trees.

2.3 Structural differences in tRNAs, rRNAs, and protein-coding genes

The abalone mitochondrial genome contains 22 tRNA genes, most of which are between 60-71 bp in length and have a typical cloverleaf secondary structure. The mitochondrial tRNA gene sequences of different abalone species are highly conserved, and most of the differences are point mutations in the mid-loop or anti-codon loop. However, the typical DHU arm phenomenon of individual tRNA genes losing was also observed in a few species, which is a common secondary structure simplification pattern of mitochondrial tRNA in mollusks. The lengths of the two mitochondrial rRNA genes (12S and 16S rRNA) do not vary much among abalone species, generally about 0.96 kb for 12S and about 1.3 kb for 16S. Their sequence conservation is relatively high, but rRNA genes of different species can still accumulate certain substitutions, especially in some highly exposed loop regions. Studies have shown that the partial sequence of the mitochondrial 16S rRNA gene can be used to distinguish different abalone species along the coast of China, and the sequence differences are significant (Zhang et al., 2022).

In contrast, due to the functional constraints of amino acids, the evolution of most sites of the 13 protein-coding genes remains conservative, and there are fewer non-synonymous substitutions between species. For example, the cytochrome oxidase subunit I gene (*COI*) is often used as a DNA barcode, and its sequence differs by more than 10% among closely related abalone species, but the intraspecific difference is usually less than 2%, which can effectively distinguish species. In the genus *Haliotis*, the lengths of each protein-coding gene are completely consistent or only slightly different between species, suggesting that these genes have hardly undergone frameshift mutations or large fragment deletions since their common ancestor. However, there are differences in the evolutionary rates of different coding genes: genes such as ATP synthase subunit 8 (*ATP8*) and NADH dehydrogenase 6 (*NAD6*) tend to evolve faster and accumulate relatively more non-synonymous substitutions, while genes such as *COI* and cytochrome b (*CYT b*) are more conservative. For example, by calculating the non-synonymous/synonymous substitution rate ratio (Ka/Ks) of the four species of the genus *Haliotis*, it was found that the Ka/Ks of the *NAD2*, *NAD6*, and *ATP8* genes were significantly higher than those of other genes, suggesting that these genes may have experienced relatively loose selection constraints or directional selection pressures. This result shows that different mitochondrial genes bear different functional constraints in the evolution of abalone, and the structural differences and variation patterns of some genes may be related to environmental adaptation (Tshilate et al., 2023). The components of the abalone mitochondrial genome differ to varying degrees among species: tRNA and rRNA genes are highly conserved, protein-coding genes are generally conserved but have mutation hotspots, and non-coding regions, especially control regions, are rich in variation, providing important clues for interspecies and population identification.

3 Genome Variation and Evolutionary Divergence

3.1 Distribution of SNPs, InDels, and base substitutions

The mitochondrial genome of abalone has accumulated a certain level of single nucleotide polymorphism (SNP) and insertion/deletion (InDel) variation in different species and populations. These variations are not randomly distributed throughout the genome, but have certain regularities. In general, the variation in the protein-coding gene region is mainly base substitution (SNP), and most of them are synonymous mutations, while InDel variation often occurs in the non-coding region. Taking the small abalone (*H. diversicolor*) as an example, a study determined the mitochondrial coding region sequence of the variegated abalone from southern China and Vietnam, and found that there were 111 SNPs in the whole genome, of which 94 occurred in the protein coding region and most of them were synonymous mutations. Only 7 variations were found in the non-coding control region, and the remaining 10 were located in the rRNA region (Xin et al., 2011). This shows that most of the variation in the abalone mitochondrial genome is concentrated in the coding region, but due to the redundant nature of the codons, the amino acid sequence is not significantly affected. Correspondingly, only a very small amount of amino acid differences were detected in the above-mentioned different geographical populations of *Haliotis diversicolor*, mainly distributed in some regions such as the ND series genes. InDel variation is relatively rare in the abalone mitochondrial genome and mostly occurs in non-coding spacer regions or control regions, which can be used as additional molecular markers. For example, in the mitochondrial control region of *Haliotis discus*, insertions/deletions of 1-2 bp in length were observed between different individuals, resulting in different haplotype lengths. The base substitution differences between different abalone species are more obvious. In general, the differences in mitochondrial sequences between species are mainly reflected in synonymous substitutions at the third codon position and variations in the control region, which together determine the genetic distance of the sequence. For example, the similarity of the mitochondrial genome sequences of the Chinese population and the Korean population of *Haliotis discus* is as high as more than 98.5%; while the mitochondrial sequences of two morphologically similar species (such as *Haliotis discus* and *Haliotis diversicolor*) differ by about 5%-8%, and dozens of fixed difference sites can often be detected on the DNA barcode (COI) fragment, which can clearly distinguish the two species. Interestingly, in artificial hybridization studies, the mitochondrial sequences of the first generation of abalone hybrids were completely derived from the mother, and no new mutations were detected, which is consistent with the law of strict maternal inheritance of mitochondria. Guo et al. (2019b) sequenced the mitochondrial genome of the hybrid offspring of *Haliotis discus* (mother) × *Haliotis nigromaculata* (father), and the results showed that the sequence consistency between the hybrid F1 and the mother *Haliotis discus* was as high as 99.40%, with only very small base differences. This further confirms that the variation of the abalone mitochondrial genome mainly comes from lineage accumulation rather than hybridization.

3.2 Non-synonymous mutations and potential adaptive implications

Although most mitochondrial gene mutations are functionally neutral or nearly neutral, a small number of non-synonymous mutations may play a role in adaptive evolution. Abalone is distributed in vast seas from tropical to temperate zones. Different species have different ecological environments such as water temperature and food. Some changes in the mitochondrial genome may be related to environmental adaptation. For example, a study compared the mitochondrial gene sequences of various temperate and tropical marine mollusks and found that the mitochondrial genome GC content of species living in higher water temperature environments was slightly increased, which is believed to help improve the stability of mRNA secondary structure, thereby improving the efficiency of protein synthesis under high temperature conditions. A similar trend was also observed in abalone: the average GC content of mitochondrial gene sequences of tropical species (such as ear abalone *H. asinina*) was higher than that of temperate species (such as European abalone), which is speculated to be a molecular adaptation to water temperature differences (Zhang et al., 2025). Amino acid substitutions in specific mitochondrial protein genes may affect the performance of enzyme complexes. For example, the amino acid variation of NADH dehydrogenase subunit genes among different abalone species is mainly concentrated in certain transmembrane regions, and variations in these regions may change the function of proton pumps to adapt to different metabolic needs. The selective pressure analysis of 13 mitochondrial protein genes in four species of the genus *Haliotis*

showed that most genes were under strong purifying selection, with Ka/Ks far less than 1, but the *ND2*, *ND6* and *ATP8* genes showed an increase in Ka/Ks ratios in some evolutionary branches. This suggests that these genes may have positive selection sites, which promote the adaptation of species to specific environments. For example, ATP synthase plays a key role in energy metabolism. Although the small subunit of ATP8 is short in length, it evolves rapidly. Changes in its specific amino acids may affect the stability of the enzyme complex under different temperatures or pH conditions (Deng et al., 2019). For another example, cytochrome oxidase (COX) subunits are extremely conserved among abalone species, but some amino acid substitutions have been detected in a few heat-resistant species. It is speculated that these substitutions improve the catalytic efficiency of the enzyme at high temperatures.

It should be pointed out that it is still challenging to identify adaptive mutation sites in mitochondrial genes because genetic drift and pedigree history often mask selection signals. However, with the accumulation of more genome sequences and environmental data of abalone species, researchers have begun to apply methods such as phylogenetic generalized least squares to incorporate evolutionary tree information into the analysis to distinguish which molecular changes are significantly associated with environmental factors. Current evidence supports some associations between non-synonymous variations in the abalone mitochondrial genome and adaptive evolution. For example, temperature gradients may drive subtle adjustments in the base composition and protein sequence of some mitochondrial genes, thereby improving the adaptability of aerobic metabolism to different water temperatures (Zhang et al., 2025). In the future, through larger-scale comparative genomic studies and functional experiments (such as measuring the activity of different mutant enzymes), it is hoped that the adaptive significance of specific mutations will be clarified.

3.3 Conserved and divergent regions among abalone species

Abalone species are evolutionarily closely related, and their mitochondrial genomes are generally highly conserved, but there are still clear genetic differences between different species. This feature of "differences in overall similarity" provides an important basis for abalone classification and species identification. In terms of conservation, all abalone species share the same set of mitochondrial genes, the gene order is almost completely consistent (except for the aforementioned individual exceptions), and the nucleotide and amino acid sequences of many key genes are highly similar. For example, the green abalone (*H. laevigata*) and the red abalone (*H. rubra*) are both closely related species from Australia. The similarity of their mitochondrial genome sequences is about 92%, and most genes have only a few base differences between the two species. For another example, the mitochondrial sequences of the Chinese population of *Haliotis discus* and the population native to Japan/Korea are almost identical, with a similarity of more than 98.5%. These high similarities reflect that the genetic information of the common ancestor of the abalone genus has been largely preserved during the differentiation process. However, different species have also accumulated enough variation to distinguish them from each other. Especially in the COI gene fragment commonly used in DNA barcoding, the genetic distance between different abalone species is usually more than 10%, while the intraspecific distance is generally less than 2%. Therefore, the COI sequence can reliably distinguish the species identity of abalone (Figure 1) (Chiappa et al., 2022). Not only COI, but other genes or regions in the mitochondrial genome also provide genetic signals to distinguish species. For example, 16S rRNA fragments or ND4 genes with large differences can be selected as auxiliary markers to improve the accuracy of identification.

A notable pattern of differentiation is that according to the phylogeny of mitochondrial sequences, abalones on the west coast of the Pacific Ocean (such as *Haliotis discus*, *Haliotis diversicolord*) and abalones in the Atlantic and Europe (such as *Haliotis tuberculata*) belong to different evolutionary lineages and have a large genetic distance from each other. This is consistent with the transmission pathways and reproductive isolation in geological history. In addition, species in the same geographical area are often more closely related to each other. For example, several species distributed near the coast of China and Japan (*Haliotis discus*, *Haliotis diversicolord*, etc.) are clustered into one branch, while species in Australia (*Haliotis tuberculata*, etc.) are clustered into another branch (Mamat et al., 2025). These differences indicate that different abalone species have accumulated species-specific mitochondrial DNA variations during long-term evolution, thus forming a clear differentiation pattern. The

mitochondrial genome of the abalone genus has a highly conserved skeleton, but the differences at the base level are sufficient to delineate species boundaries. High conservation ensures that we can use universal primers to amplify the corresponding genes of different species for direct sequence comparison; and sufficient differences ensure that sequence analysis can identify species. This balance between conservation and difference makes the mitochondrial genome an ideal molecular tool for abalone species identification and phylogenetic analysis. In practical applications, it is only necessary to determine the sequence of one or a few highly variable region genes in the abalone mitochondrial genome, and then compare it with the sequence library of known species to determine the species identity and analyze the kinship.

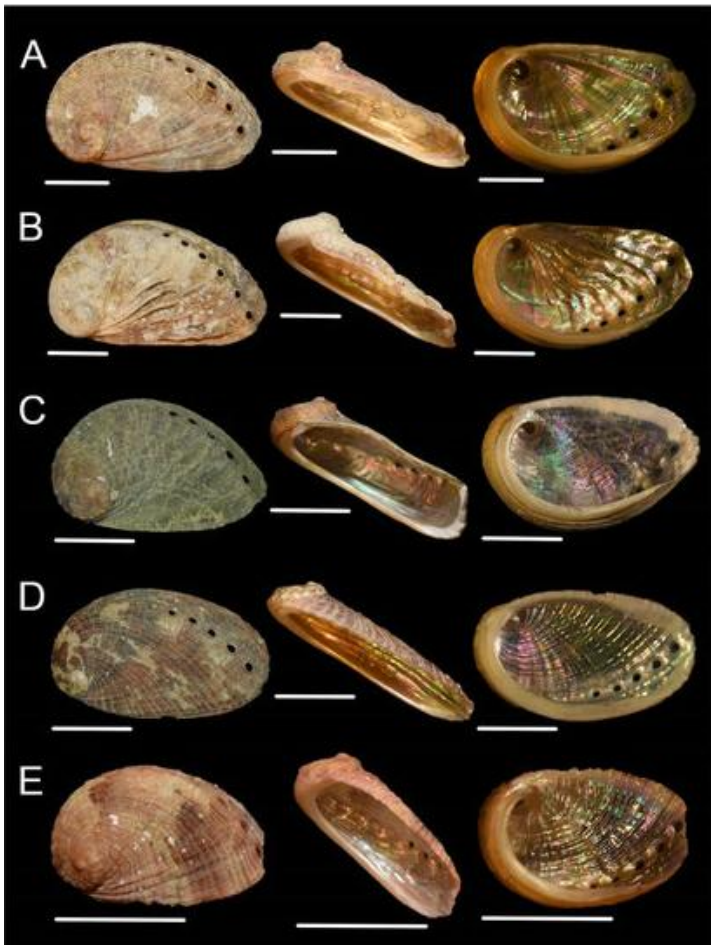

Figure 1 Abalone shell sample. (A) *Haliotis tuberculata tuberculata* BAU 1391. (B) *Haliotis tuberculata tuberculata* with lamellae (formerly *Haliotis lamellosa*) BAU 676.3. (C) *Haliotis mykonosensis* BAU 657.3. (D) *Haliotis tuberculata coccinea* BAU 717.3. (E) *Haliotis stomatiaeformis*, juvenile BAU 699. All scale bars are 1 cm (Adapted from Chiappa et al., 2022)

4 Phylogenetic Reconstruction and Lineage Differentiation

4.1 Construction of mitochondrial genome-based phylogenetic trees

With the development of molecular biology technology, the mitochondrial whole genome sequences of more and more abalone species have been determined, providing rich data for phylogenetic research. The phylogenetic tree constructed based on the mitochondrial whole genome (usually taking the concatenated sequence of 13 protein-coding genes) has significantly higher resolution and credibility than the tree constructed with a single gene. Recent phylogenetic analysis covers the main abalone species in the world, and the results support that the genus *Haliotis* is an evolutionary branch of a monophyletic origin, and it can be divided into several branches. For example, Mamat et al. (2025) reconstructed a phylogenetic tree using the whole genome sequences of 8 abalone species, showing that the Northwest Pacific species such as *Haliotis discus*, *Haliotis diversicolord*, and Japanese black abalone (*H. discus discus*) are clustered into one branch, while in contrast, the green abalone and red abalone of Australia are clustered into another branch, and the ear abalone of the Central Pacific and the Mexican

abalone of the Eastern Pacific are each in their own branch. In this phylogenetic tree, the topological structure between species has a close correspondence with their geographical distribution. For example, the tropical species of ear abalone (*H. asinina*) first merged with another tropical species of sheep abalone (*H. ovina*), and then formed a branch with the small abalone (*H. diversicolor*) in the warm temperate zone; this branch then merged with large temperate species such as red abalone (*H. rubra*) and European abalone (*H. tuberculata*). This result is generally consistent with the judgment of the distance between species based on traditional morphological classification, and is also consistent with previous research results based on genes such as 16S and COI. It is worth noting that the support rate (such as Bootstrap value) of whole genome phylogenetic analysis is generally high, and many key branches have received > 90% bootstrap value support, which shows that mitochondrial whole genome data is sufficient and reliable for analyzing the deeper systematic relationships of the genus *Haliotis*. In addition, some previously controversial taxa have also been clarified on the molecular phylogenetic tree. For example, regarding the question of whether the Taiwan nine-hole abalone and the mainland variegated abalone belong to the same species, the whole genome data show that the genetic distance between the two is very close, and they are mixed in the phylogenetic tree, which supports that they actually belong to different geographical populations of the same species. In contrast, another small abalone in the Indo-Pacific region (produced in Indonesia) is significantly different from the Taiwanese nine-hole abalone, and evidence supports its identification as an independent species (possibly *H. squamata*), a finding also reflected by the length of the long branches on the phylogenetic tree. It can be seen that the phylogenetic analysis based on the whole mitochondrial genome provides a clear branching structure and quantitative support for the evolutionary relationship between abalone species, laying the foundation for solving taxonomic problems and discovering potential new species. In the process of constructing a phylogenetic tree, commonly used methods include maximum likelihood (ML) and Bayesian inference (BI), and multiple sequence alignment and model selection are used to ensure the reliability of the results. Due to the base composition bias and evolutionary rate heterogeneity of mitochondrial genes, it is usually necessary to partition different genes and apply corresponding substitution models during analysis to improve the accuracy of the phylogenetic tree (Liu et al., 2018; Zhao and Wu, 2024).

4.2 Divergence nodes and lineage clustering

The phylogenetic tree of the abalone genus reveals several major evolutionary branches and their differentiation nodes. This information can help us reconstruct the evolutionary history and geographical diffusion process of the abalone genus. According to mitochondrial genetic evidence, the genus *Haliotis* may have originated in the Tethys Ocean in the Middle Paleozoic and then spread to the coasts of the world. The base node of the phylogenetic tree divides the abalone species into two major lineages: one is the Atlantic-Eastern Pacific lineage, including European abalone and American abalone, and the other is the Indo-Pacific lineage, including various abalones in Asia and Oceania (Mamat et al., 2025). This differentiation node occurred approximately in the early Cenozoic, coinciding with the geological events of the closure of the Tethys Ocean and the isolation of the oceans from each other. Within the Indo-Pacific lineage, it can be further divided into several clusters, such as: East Asian cluster (*Haliotis discus*, *Haliotis diversicolord*, Japanese abalone, etc.), Oceania cluster (Australian green abalone, abalone red, etc.) and East Pacific cluster (Mexican abalone, etc.). The differentiation node ages of these clusters may correspond to changes in ocean climate or ocean current patterns. For example, the common ancestor of the East Asian cluster may have spread through the Kuroshio system in the Pliocene, while the differentiation of the Australian cluster is related to the South Pacific warm current. Phylogenetic cluster analysis shows that species close to each other on the phylogenetic tree often have similar geographical distributions and ecological habits. This confirms the role of geographic isolation and adaptive radiation in abalone evolution. For example, large temperate abalone (*Halion discus*, European abalone, etc.) cluster together, while small tropical abalone (*Haliotis scrofa*, etc.) form another cluster, suggesting that environmental selection pressure shapes the genetic convergence of species with similar ecological niches (Zhang et al., 2025).

4.3 Correlation between mitochondrial variation and geographic distribution

The spatial pattern of abalone species and populations shapes the distribution characteristics of their mitochondrial genetic variation. By analyzing the association between mitochondrial variation and geographical factors, the

effects of isolation and gene flow on the genetic structure of abalone can be revealed. At the species level, abalones in different geographical regions show obvious genetic differentiation. For example, the mitochondrial sequences of the *Halion discus* off the coast of China and the red abalone in California are very different, corresponding to the long-term geographical isolation on both sides of the Pacific Ocean (Cook, 2025). Even within the region, geographical barriers can lead to genetic differentiation of populations. Taking the cultured population of *Haliotis discus* in China as an example, there is a significant difference in the mitochondrial haplotype frequency between the Dalian population in the north and the Fujian population in the south, and the FST value indicates that the two populations have a certain degree of genetic separation. This may be due to the long distance along the coast of China and the separation of cold and warm currents. For example, the Taiwan Strait was once a land bridge during the glacial period, and the abalone populations on different sides may have experienced isolation in history, thereby accumulating genetic differences. Mitochondrial DNA sequence analysis also revealed some interesting phenomena, such as genetic mixing between introduced populations and local populations. China introduced *Haliotis discus* seedlings from Japan for culture at the end of the 20th century. After many generations of reproduction, the mitochondrial genetic diversity of the current coastal cultured population is slightly lower than that of the original Japanese population, but it remains highly similar overall. This indicates that the initial maternal genetic contribution of the introduced population was dominant and did not hybridize with the local abalone.

However, some studies have shown that natural hybridization between the introduced *Haliotis discus* and the local abalone discus was detected in some areas, and the mitochondria of their offspring were the same as those of the *Haliotis discus* mother, but the nuclear DNA showed recombinant characteristics (Yang et al., 2023). This suggests that we need to conduct a joint analysis of mitochondrial and nuclear genes to deeply evaluate the impact of introduction on the genetic pattern of local abalone. In general, the geographical pattern of mitochondrial variation supports isolation-induced differences: distant populations differ greatly, and adjacent populations are more similar. For example, the mitochondrial haplotype network of the abalone populations in East Asia is star-shaped, with the central type shared in all places, while the marginal type is regionally specific (Li et al., 2021). This shows that although there is a certain gene flow among populations in various places, local genetic differentiation has still occurred in the long run. Looking at the species level, the black abalone and the wrinkled abalone in Japan basically have no mitochondrial haplotype sharing in the cross-distribution area, suggesting that their reproductive isolation is effective (Hsu and Gwo, 2017). On the contrary, the Taiwan Jiukong and the mainland abalone share most haplotypes, indicating that gene exchange may have occurred in history through the cross-strait transport of planktonic larvae. The Mantel test found that the mitochondrial genetic distance between abalone populations is often positively correlated with the geographical distance, but the degree of influence varies from species to species. The longer the geographical isolation time and the greater the distance, the greater the difference in abalone mitochondria, which is consistent with the isolation-induced differentiation pattern in the marine environment. At the same time, ocean currents and human activities can also break the simple distance-gene relationship. For example, the artificial introduction of Japanese wrinkled abalone has made the East China population and the native Japanese population very different (Guo et al., 2019a). Therefore, when analyzing the geographical association of mitochondrial variation, it is necessary to comprehensively consider marine geographical barriers, ocean current paths, biological life history and human factors.

5 Development of Mitochondrial Markers for Species Identification

5.1 Screening and validation of core diagnostic genes

Molecular identification of abalone species usually relies on differences in mitochondrial gene sequences, of which the most commonly used are mitochondrial encoded genes, such as the *COI* gene (i.e., animal DNA barcode standard fragments). To ensure the accuracy and efficiency of identification, it is necessary to screen out core marker genes that are sensitive to species differences and easy to amplify and sequence. A large number of studies have shown that *COI* gene fragments have a high success rate in abalone species identification, with species identification efficiency reaching more than 95%. For example, by determining the abalone COI sequence, researchers found that almost all abalone species have a "barcode gap" on this gene, that is, the genetic distance

within the species is significantly smaller than the distance between species. Ran et al. (2020) also confirmed that the COI barcode can correctly classify about 98% of the samples into species in a study of 11 species of shellfish (including abalone) along the coast of China. Therefore, *COI* has been widely regarded as the preferred gene for abalone species identification. However, in some cases a single COI fragment may not be sufficient to solve all problems. For example, for closely related species pairs (such as Taiwan's nine-hole abalone and mainland abalone), the COI sequence difference is very small (about 1%), and identification ambiguity may occur. For this reason, it is possible to consider introducing other mitochondrial gene fragments as an auxiliary means. The 16S rRNA gene is a common choice. Its evolutionary rate is slightly slower, but the secondary structure region is obviously different, and it has good discrimination for some closely related species. In addition, the cytochrome b (cyt b) gene is often used in fish classification and also shows high interspecific variation in abalone, making it suitable as a supplementary marker. In order to screen the best species identification gene combination, researchers usually evaluate the interspecific genetic distance and intraspecific variation level of different genes, as well as the convenience of amplification and sequencing success rate. The results often show that the combination of COI plus 16S can significantly improve the reliability of identification, and the credibility is extremely high when the identification conclusions of the two are consistent (Kannan et al., 2020). When the two are inconsistent or in doubt, a third marker (such as ND1 or cyt b) can be added for verification. In addition to traditional sequencing methods, some studies have developed species-specific PCR identification techniques based on core mitochondrial genes. For example, specific primers for *Haliotis rubripes*, *Haliotis diversicolor*, *Haliotis ocellaris*, etc. are designed, and the sizes of amplified products of different species are distinguished by multiplex PCR, so as to quickly identify the species components in mixed samples. There are also studies using high-fidelity probe qPCR methods to detect specific abalone DNA in processed aquatic products and distinguish *Haliotis rubripes* from other abalone raw materials (patent CN109680077B). The selection of core mitochondrial genes needs to take into account both discrimination and practicality. At present, COI is undoubtedly the first choice, and combining other markers can improve the accuracy in tricky situations. With the popularization of abalone mitochondrial genome sequencing, more highly variable markers can be discovered based on whole genome information in the future, such as specific fragments of the control region or species-specific SNPs in the nuclear genome, but these need to be verified by a large number of samples in practical applications. At this stage, the species identification strategy developed around mitochondrial core genes can meet most scientific research and law enforcement needs.

5.2 DNA barcoding applications in abalone taxonomy

DNA barcoding technology refers to a method of identifying species using standard short gene sequences. Since Hebert et al. proposed using mitochondrial COI fragments as universal animal barcodes, this technology has been widely used in species diversity research and monitoring of fish, mollusks, etc. In abalone, DNA barcoding technology has shown great application potential. First, it provides an objective and quantifiable standard for species identification. By establishing an abalone barcode sequence library, any sample of unknown origin (whether it is larvae, fragments or even processed products) can be sequenced to determine the species identity. For example, for imported dried abalone and other aquatic trade products, barcode sequence comparison can be used to identify their species and reveal whether there is a phenomenon of cheap abalone impersonating precious abalone, thereby safeguarding consumer rights (Senathipathi et al., 2024). Secondly, DNA barcoding technology is also used in the discovery of cryptic abalone species. Some abalone that were previously believed to belong to the same species based on morphology may show obvious differentiation after barcode analysis, suggesting the existence of new species or evolutionary lineages that have not been recognized. Taiwan's nine-hole abalone and some Indonesian abalone are identified as different evolutionary groups through COI barcode differences (>10%) (Hsu and Gwo, 2017). Thirdly, barcode technology can assist in the management of abalone seedlings and cultured strains. Since the morphology of different species of abalone seedlings is extremely similar in the larval stage, DNA barcodes can be used to quickly screen the composition of larvae in the nursery pond to ensure the purity of the introduced species or seedlings. In genetic breeding, barcodes can also be used to verify the maternal species of hybrid offspring to track breeding pedigrees. It is worth mentioning that with the development of high-throughput sequencing, DNA barcoding technology has derived a variety of new forms, such as

metabarcoding, which can be used for environmental DNA monitoring. By extracting environmental DNA from aquaculture water or sediments and amplifying barcode sequences such as COI, it is possible to detect whether there is recruitment of wild juvenile abalone or invasion of alien abalone in the aquaculture area (Li et al., 2021). This non-invasive monitoring method is very beneficial for aquaculture ecological management and wild resource protection. In addition, mini-barcoding technology can solve the problem of DNA fragmentation in processed products by selecting shorter barcode sequences to achieve species identification, which has been proven to be feasible in dried abalone and canned products. In general, the application prospects of DNA barcoding technology in the identification of abalone species are very broad, and it can play a unique role in scientific research, industrial supervision and resource protection. Of course, to fully realize its potential, it is necessary to establish a complete reference database and standard operating procedures. At present, a large number of species barcode sequences including abalone have been entered into GenBank and BOLD systems around the world, but it is still necessary to ensure that the sequences are accurately annotated and cover all species. With the advancement of these works, DNA barcoding will surely become a powerful tool for the study and management of abalone species diversity.

5.3 Framework for rapid identification and traceability systems

In order to better apply molecular identification technology to abalone species monitoring and aquatic product traceability, it is necessary to build a set of rapid, accurate and economical comprehensive detection systems. Based on the current research progress, we put forward the following suggestions: First, establish a reference database and detection standards for DNA barcodes of abalone species. At the national or industry level, mitochondrial COI and other sequences of various abalone species should be collected, an authoritative reference sequence library should be constructed, and standard PCR amplification and sequencing protocols should be formulated. In this way, when an unknown sample is sent for inspection, each laboratory can operate according to a unified process, and the identification conclusion can be obtained by comparing the sequencing results with the database. Second, develop on-site rapid detection tools for abalone species. In law enforcement or market supervision, in order to improve the detection efficiency, a rapid method that does not require sequencing can be used. For example, ARMS-PCR primers are designed based on species-specific SNP sites to achieve species specificity of the amplified product. Real-time fluorescence PCR technology can also be applied to complete the simultaneous detection of multiple species within a few hours using specific probes. For example, a patented method has developed fluorescent quantitative PCR using the mitochondrial sequence specific to the wrinkled abalone, which can determine whether the sample contains wrinkled abalone components with one click. Third, use high-throughput genotyping technology to strengthen the traceability of abalone products. For high-value abalone species (such as Australian abalone and Japanese abalone), it is possible to consider developing a typing detection platform based on SNP chips or microsatellites to achieve species identification and origin certification for individual or batch products. Peng et al. (2021) have constructed an abalone SNP chip containing 60K markers, which can be simplified for species identification in the future. Chip testing can type thousands of loci simultaneously within a few hours, which improves accuracy. Fourth, combine molecular identification technology with information technologies such as blockchain to form a full traceability system for abalone products. Specifically, DNA identity tags are assigned to each batch of abalone during the breeding or fishing stage (through typing records), and the test verification results are uploaded at each link of the product circulation. Consumers can obtain authenticity information such as species and origin by scanning the product QR code. Finally, to improve the rapid identification system in practice, cost and convenience must also be considered. For example, for grassroots supervision, portable PCR equipment and kits can be promoted, which can be operated without complex laboratory conditions. At present, some companies have launched on-site DNA detection kits for aquatic products, which contain pre-freeze-dried PCR reagents. Only sample lysis solution needs to be added to amplify and the results can be read through test strips, which is very suitable for rapid screening. We believe that with the continuous maturity of molecular detection technology, it is feasible and necessary to establish a comprehensive system covering abalone species identification and product traceability. This will help combat seafood trade fraud, protect the reputation of geographical indication products, and safeguard the sustainable use of abalone resources.

6 Case Studies: Regional Population Phylogeny and Identification

6.1 Comparative analysis of abalone from Southeastern China

The southeastern coast of China (including the coasts of Fujian and Guangdong) is a world-famous abalone farming and distribution area, mainly including the local abalone (commonly known as "nine-hole abalone") and the introduced abalone. Molecular comparative studies of abalone resources in this area help to understand the genetic structure of different species and populations. First, the genetic diversity level of each cultured population of local abalone is generally high, but there is a slight differentiation between different farms. Through mitochondrial COI and control area analysis, it was found that several cultured abalone populations in the coastal area of Fujian shared most haplotypes, showing a close relationship, but there were also some population-specific haplotypes, which may be due to different seed sources. In general, abalone can basically be regarded as a large genetic exchange group in the southeastern coast, and no obvious regional differentiation has yet appeared. In contrast, the genetic diversity of the introduced abalone population is slightly lower than that of local abalone due to its short farming history. The average nucleotide diversity of mitochondrial haplotypes of the wrinkled abalone population in some farms in Fujian is about 0.002,0, which is lower than 0.003,5 of the variegated abalone population in the same period. This may be caused by the introduction bottleneck and artificial breeding. However, from the perspective of molecular pedigree, most of these wrinkled abalone populations are mixed with the original populations in Japan or the north on the same lineage. For example, a study comparing the wrinkled abalone cultured in Fujian with the original population in Shandong pointed out that the COI sequences of the two had only 0-1 base differences and no differentiated branches were formed (Ren et al., 2017). This shows that the wrinkled abalone in the southeast coast basically continues the genetic background of the introduced original species and has not yet significantly differentiated from local abalone or other introduced batches.

On the other hand, in natural sea areas, there are still sporadic wild variegated abalone populations in the southeast coast (mainly in Taiwan Shoals and other places). Studies have found that there is no systematic difference in mitochondrial DNA between wild variegated abalone and farmed populations, but nuclear gene markers such as microsatellites show that unique alleles appear in wild populations. This suggests that when protecting local wild resources, its uniqueness should be considered to avoid genetic confusion of escaped individuals from aquaculture. Based on these molecular evidences, it can be seen that although the abalone species (*Haliotis diversicolor* and *Haliotis discus hannai*) in the southeastern coast of China exist in the same area, they are clearly genetically bounded and not mixed: the *Haliotis diversicolor* populations are clustered together, while the *Haliotis discus hannai* are classified as another group with the northern population. The COI sequence difference between the two is more than 20 bases, which can be accurately distinguished (Figure 2) (Bachry et al., 2019).

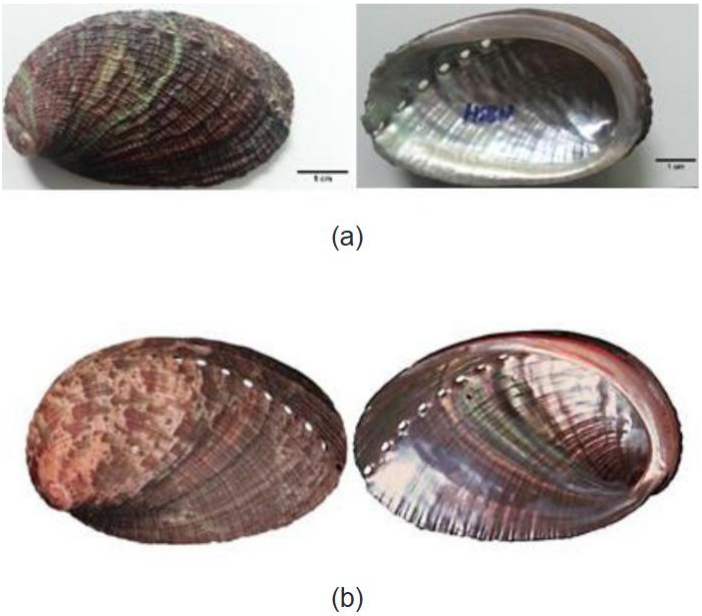

Figure 2 (a) Shell of *H. diversicolor squamata* and (b) shell of *H. diversicolor* (Adopted from Bachry et al., 2019)

In practical applications, this molecular difference has been used to identify the species of seedlings and products. For example, by sequencing the COI of abalone samples in the Fujian market, it was quickly determined whether it was a local *Haliotis diversicolor* or an introduced *Haliotis discus hannai*, and it was found that some of the so-called "local abalone" were actually foreign *Haliotis discus hannai*, which improved market transparency. The species diversity of abalone in the southeastern coast of China is mainly reflected in the coexistence of local *Haliotis diversicolor* and foreign *Haliotis discus hannai*. Mitochondrial DNA analysis can effectively compare their population genetic characteristics, providing a basis for regional resource management and variety optimization.

6.2 Genomic divergence between introduced and native populations

With the increase in global aquaculture exchanges, the genetic differences and potential impacts of introduced abalone species and local related species have attracted much attention. A typical case of China's abalone industry is the introduction of *Haliotis discus hannai* and the co-culture of local *Haliotis diversicolor*. Using mitochondrial genome and nuclear DNA markers to evaluate the differences between the two will help aquaculture management and species protection. In terms of mitochondrial DNA, the introduced *Haliotis discus hannai* and local *Haliotis diversicolor* have significant sequence differences, and the COI genetic distance is about 12%-15%, which is much higher than the general intraspecific differences. Therefore, mitochondrial markers can clearly distinguish between two different sources of abalone in the cultured population. For example, it has been reported that a small amount of *Haliotis diversicolor* mitochondrial haplotypes were detected in samples from a farm in Guangdong, suggesting that interspecies co-culture or mismixing occurred. The evaluation of differences in the nuclear genome is more complicated. *Haliotis discus hannai* and *Haliotis diversicolor* have different nuclear chromosome numbers: the former has 2n=36, and the latter has 2n=32. It is reported that hybridization can produce infertile offspring. The purity of the cultured population can be analyzed by high-throughput genotyping. For example, the application of 60K SNP chip detection found that most of the cultured individuals of *Haliotis discus* were clustered into the same category as the original Japanese population, but some individuals deviated from the principal component analysis, indicating that they may contain the gene components of *Haliotis diversicolor*. Further using the method of genome resequencing, the researchers found that about 2% of the nuclear genome fragments in two cultured populations of *Haliotis discus* in Fujian had the alleles of *Haliotis diversicolor*, indicating that hybridization may have occurred unintentionally during the culture process (Holland et al., 2022). Although these hybridization signals are weak, they should be taken seriously because continued gene introgression may change the genetic characteristics of the introduced species.

In order to maintain the homozygous excellent traits of the *Haliotis discus*, farmers should avoid co-culture with *Haliotis diversicolor* and regularly test the purity of the strain through molecular markers. Another aspect worth evaluating is the genetic differences in adaptability between the two species. The wrinkled abalone is native to the cold temperate zone, while the variegated abalone is native to the subtropical zone. Their genomes may have differences related to temperature tolerance (Zhang et al., 2025).

By comparing the enzymes encoded by the mitochondrial genes of the two, there are indeed differences in some heat resistance properties. For example, the variegated abalone COX enzyme has better tolerance to high temperatures than the wrinkled abalone (Xu et al., 2020). This also explains why the wrinkled abalone is prone to stress and even death during the high temperature period in summer, while the variegated abalone is more resilient. In aquaculture practice, the wrinkled abalone and the variegated abalone are sometimes crossbred to combine the advantages of both. However, due to the mismatch of chromosomes in distant hybridization, the survival rate and fertility of the offspring are very low (Hsu and Gwo, 2017; Li et al., 2024).

Therefore, the more feasible strategy at present is to maintain the purity of the species, carry out targeted breeding separately, and meet the ecological needs of different species through environmental regulation. The differences in the genome between the introduced wrinkled abalone and the local variegated abalone are significant. The mitochondrial DNA can be easily distinguished, and the nuclear DNA also shows high heterogeneity. On the one hand, this difference gives us molecular tools to regulate seedlings and product varieties, and on the other hand, it

reminds us to be cautious in dealing with the mixing of the two varieties to prevent genetic contamination and economic losses. From a conservation perspective, while the large-scale cultivation of *H. discus hannai* should be carried out, the genetic resources of local abalone should be retained, such as building isolated original breeding farms or protected areas to prevent it from being replaced in competition with foreign species.

6.3 Phylogenetic and taxonomic assessment of Japanese, Australian, and East Asian abalones

There have been some disputes in the taxonomy of abalone, focusing on species with close geographical distribution or similar morphology. For example, Japanese abalone includes two main types, *H. discus hannai* and *H. discus black*, Australian abalone includes green abalone and black-lipped abalone, and there are also *H. discus* and *H. variegata* in the coastal areas of East Asia. This section discusses the relationship and taxonomic treatment of abalone in these regions based on mitochondrial pedigree information. First, Japanese *H. discus hannai* and Japanese black abalone (*H. discus discus*) have long been regarded as different subspecies of the same species, but there are different opinions on taxonomy. Molecular phylogenetic results show that although the two abalones form a sister group relationship, they are still clearly distinguished in branches, and the genetic distance reaches the typical species level (Hsu and Gwo, 2017). In particular, they each form a monophyletic branch without haplotype confusion, which supports the view that they are independent species. Chinese scholars have also found in aquaculture practice that the survival rate of hybrid offspring of the two is extremely low, which further confirms the taxonomic distinction from the perspective of reproductive isolation. Therefore, in recent years, most researchers tend to formally recognize the Japanese black abalone (also known as the true abalone) as an independent species *H. discus*, while retaining the original scientific name of the wrinkled disc abalone *H. hannai.* In contrast, the controversy between the Taiwan nine-hole abalone and the mainland variegated abalone is just the opposite - they were once considered different species, but are now mostly merged into the same species. The Taiwan nine-hole abalone (traditionally called *H. diversicolor supertexta*) is slightly different from the mainland variegated abalone in shell shape and growth rate, which has led some scholars to advocate that it is a subspecies or even species-level distinction. However, molecular evidence has repeatedly shown that the mitochondrial sequences of the two are almost indistinguishable and intertwined in the phylogenetic tree. For example, a comparison of 16S and COI sequences of Taiwan and mainland abalone revealed that the interspecific genetic distance was only about 1%, far lower than the typical species difference. Classical genetic studies such as isozymes and electrophoretic markers have also failed to clearly separate the two. Therefore, the current mainstream view tends to believe that Taiwan abalone is only a geographical population, not an independent species, and its species name supertexta can be reduced to a synonym or infraspecific taxonomic unit of *H. diversicolor* (Yang et al., 2023).

The taxonomic controversy of Australian abalone mainly involves the interspecific relationship between green abalone (*H. laevigata*) and black-lipped abalone (*H. rubra*). They are distributed adjacently and have natural hybridization zones. At one time, some people suggested that they may belong to a highly variable population. However, mitochondrial DNA shows that the sequence similarity between the two is about 92%, and the interspecific differences are significant. Although hybridization occurs, the frequency is low and mostly one generation, without fusion of the two gene pools (Cook, 2025). The phylogenetic tree divides green abalone and black-lipped abalone into different branches, supporting that each is a species. It is also worth noting that there are several local species in Australia, such as *H. conicopora*, which are closely related to the above two species. The latest whole genome study found that they had a second contact after the ice age, and some genes showed selective introgression. This reminds us that in addition to distinguishing by morphology and mitochondria in classification, we should also monitor the exchange of nuclear genes to fully understand the species boundaries. By comparing the mitochondrial lineages of Japanese, Australian and East Asian abalone, many taxonomic disputes can be clarified. Molecular evidence generally supports the following viewpoints: Japanese wrinkled abalone and black abalone should be different species; Taiwan's nine-hole abalone and mainland variegated abalone should be considered the same species; Australian green abalone and black-lipped abalone are independent species, although there is limited hybridization in nature. For some other controversial taxa, such as the blue abalone and red abalone in Mexico, and the population division of rare abalone in South Africa, the same method can also be used for evaluation.

7 Concluding Remarks

The mitochondrial genome of the abalone genus is highly conserved and highly variable, making it an ideal molecular tool for species identification. It is precisely because the abalone mitochondrial genome retains a stable genetic composition and arrangement among species that we can use common molecular methods to obtain the corresponding sequences of different species and conduct reliable comparative analysis. At the same time, the sequence differences accumulated by different species (especially the differences in gene fragments such as COI and 16S) are sufficient to be used as species "barcodes" for identification. In the past decade, a large number of studies have demonstrated the accuracy and practicality of abalone species identification methods based on mitochondrial genes. For example, for young abalone or processed abalone products with extremely similar morphology, traditional methods cannot determine their species, while mitochondrial DNA sequence comparison can give a clear species conclusion. The evolutionary characteristics of the abalone mitochondrial genome, such as the combination of rapidly evolving control regions and highly conserved coding genes, provide the possibility of multi-level identification: sensitive distinction between species/populations can be achieved through highly variable regions, and the stability of comparisons and the consistency of traceability can be ensured by using conservative regions.The maternal monophyletic nature of abalone mitochondrial inheritance simplifies genetic interpretation and avoids the complexity brought about by nuclear gene recombination. This means that in practical applications, the use of mitochondrial markers can often directly and clearly reveal the sample's ownership. For example, if the mitochondrial haplotype of *Haliotis discus* is detected, it can be determined that its maternal species is *Haliotis discus*. The evolutionary pattern of the abalone mitochondrial genome provides valuable value for species identification: its degree of difference is moderate, and it can be stably distinguished at the species level; its molecular characteristics are clear, which facilitates the development of diverse detection methods. It can be foreseen that as more abalone species' mitochondrial genomes are sequenced and reference databases are improved, molecular identification will play an increasingly important role in abalone classification and resource management.

Although the mitochondrial genome has outstanding advantages in the systematic classification and identification of abalone, various molecular methods also have their own limitations, which need to be fully understood. The main advantages of mitochondrial DNA markers are: high mutation rate, strong resolution, simple experimental operation and low cost, which are suitable for large-scale sample screening. In particular, the popularity of COI barcodes has enabled non-professional laboratories to easily carry out species identification. In addition, as a maternal genetic marker, the mitochondrial genome plays a unique role in tracing the maternal origin of hybrid offspring. For example, we can confirm the maternal component of abalone hybrid populations through mitochondrial haplotypes.

In contrast, nuclear DNA markers are more complicated and need to consider parental inheritance and allele separation. They are often not as intuitive as mitochondria for interspecies identification. However, the mitochondrial method also has inherent limitations. First, mitochondrial DNA represents only the maternal genetic history and cannot reflect hybridization or paternal gene introgression. Once interspecies hybridization occurs, the offspring will carry the maternal mitochondria, so that the identification results may not match the morphological or nuclear gene information. Second, the mitochondrial genome sometimes has the problem of incomplete pedigree sorting between closely related species, which may lead to inconsistency between the single gene tree and the actual species tree (i.e., "DNA pedigree is inconsistent with species pedigree"). Although this situation is rare in abalone, the molecular tree results still need to be interpreted with caution. Third, there is the possibility of mitochondrial pseudogenes inserted into the nuclear genome (NUMTs), which will interfere with PCR and sequencing results and need to be avoided through primer design and analysis. Finally, different molecular methods have their own preferences: sequencing is accurate but time-consuming, suitable for laboratory identification; rapid PCR is convenient but requires pre-designed specific primers and probes, and is usually targeted at limited species. New technologies such as SNP chips and high-throughput sequencing can provide massive data, but the cost is high and the data analysis is complex, and they are generally used for research rather than daily identification.

Therefore, in specific applications, the appropriate method should be selected according to the goal. If it is a scientific research investigation, the most complete information can be obtained through whole genome sequencing/resequencing, from which mitochondrial sequences and nuclear genes can be extracted and analyzed together to obtain robust conclusions. For general species identification, COI barcode sequencing can be used first; for batch screening in customs and market supervision, specific PCR or portable detection equipment can be used to quickly lock suspicious samples. In summary, mitochondrial molecular markers provide strong technical support for abalone species identification, but we must also combine nuclear gene and morphological evidence to comprehensively evaluate the results. Different methods have their own strengths, and the accuracy and reliability of identification can be improved by complementing each other's strengths. For example, for samples with questionable identification results, 16S or nuclear gene ITS fragments can be further sequenced for verification to exclude the possibility of misjudgment of a single marker. As long as we give full play to the advantages of various methods and avoid their limitations, we can establish a robust and reliable abalone species identification system.

Looking to the future, abalone systematic classification and genetic research will develop in the direction of comprehensive multi-source information and interdisciplinary cross-cutting. In terms of taxonomic research, with the acquisition of more molecular data (including mitochondrial and nuclear genomes), we have the opportunity to re-examine the systematic position and species division of the abalone genus. Perhaps hidden lineages or taxa that need to be split/merged will be discovered. To this end, future taxonomic revisions should integrate molecular phylogenetic trees, morphological and anatomical characteristics, biogeographic and ecological information, and adopt an "integrated taxonomy" approach to give a more natural species definition. At the mitochondrial genome level, in the future, it may be possible to deeply understand the genetic mechanism of abalone adaptive radiation by analyzing the selection pattern and evolutionary dynamics of whole genome sequences. For example, the study of mitochondrial gene function in species in different temperature zones is expected to reveal their energy metabolism adjustment strategies and provide a basis for the selection and improvement of abalone germplasm in response to climate change. In terms of species identification and resource protection, we expect molecular monitoring to play a greater role. Governments and scientific research institutions can establish a DNA archive of abalone germplasm resources and implement long-term molecular monitoring of wild and farmed populations. Once signs of genetic diversity decline or invasion of foreign genes are found, timely measures (such as stocking or isolation) can be taken to prevent genetic decline and germplasm contamination.

At the same time, genetic technology can also be used to assist genetic selection, such as improving stress resistance traits through molecular marker-assisted selection (MAS) to achieve the cultivation of excellent varieties. From the perspective of conservation biology, more attention should be paid to the protection of abalone genetic diversity in the future. Under the pressure of global climate change and human activities, some abalone species (such as black abalone, South African abalone, etc.) face survival challenges. Molecular methods can be used to assess the genetic health of their populations and guide the establishment of conservation populations or genetic management plans with sufficient genetic diversity. For example, sequencing analysis can be used to determine the haplotype types that are preferentially retained in each conservation population to maximize the evolutionary potential of the species. In short, mitochondrial genome research will continue to be an important part of abalone systematic classification and species identification in the future, but we also need to expand our vision to the whole genome level and ecological environment level. Through multidisciplinary collaboration, we hope to fully reveal the evolutionary story of abalone and develop scientific and effective strategies to protect and utilize this precious marine biological resource.

Acknowledgments

We thank Mr Y. Ding from the Institute of Life Science of Jiyang College of Zhejiang A&F University for his reading and revising suggestion.

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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