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Whole-Genome Analysis of Abalone Reveals Phylogenetic Relationships and Adaptive Evolution Mechanisms

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Abstract Abalone (Haliotis) molluscs play an important role in marine biodiversity and aquaculture, but their phylogenetic relationships and adaptive evolutionary mechanisms have not been fully elucidated. Whole genome sequencing in recent years has provided a new opportunity to study the systematic evolution and environmental adaptation of abalone. In this study, by comparing the whole genome data of multiple abalone species, we reconstructed the phylogenetic tree of the genus Abalone, estimated the divergence time, and analyzed the evolutionary characteristics of genome structure and function. The results showed that the abalone genome size ranged from 1.2 Gb to 1.8 Gb, encoding approximately 20 000 to 30 000 genes, with a repetitive sequence ratio of nearly 50%. Positively selected genes related to temperature, salinity, and immunity were identified, as well as the expansion of specific gene families involved in shell formation and metabolic pathways. These adaptive genomic changes may explain the adaptation of abalone to different environments such as high latitudes and tropical waters. Taking the representative species of Japanese abalone, African abalone and Australian green abalone as examples, this study deeply analyzed the adaptive characteristics in their genomes, including cold-adapted immune genes, multiple heat-resistant genes and shell morphology-related selection signals, and discussed the application prospects of abalone whole genome research in germplasm protection, molecular breeding and stress resistance breeding. This study provides a new perspective at the genome level for understanding the phylogeny and environmental adaptation of abalone, and also provides a scientific basis for abalone resource protection and genetic improvement.

Keywords Abalone; Whole genome; Phylogeny; Adaptive evolution; Genomic breeding

1 Introduction

The genus Haliotis includes more than 50 species of marine gastropod mollusks, which are distributed in tropical and temperate coastal areas around the world except the northern Atlantic Ocean. Abalone feeds on large algae in a benthic lifestyle and plays an important role in maintaining the balance of algae beds in marine ecosystems (Barkan et al., 2024). At the same time, abalone has been a famous "sea treasure" since ancient times. It is highly praised for its delicious meat and high nutritional value and is known as one of the "eight treasures of seafood". Due to its high economic value, abalone has also become an important farmed shellfish in the world. Especially in China, the abalone farming industry has developed rapidly in recent decades: as of 2020, China's annual abalone production is about 203 500 tons, accounting for more than 85% of the global abalone farming production. Among them, the main species is the wrinkled disc abalone (*Haliotis discus hannai*), which occupies the leading position in aquaculture production. While large-scale farming meets market demand, it also puts higher requirements on abalone resource protection and industry sustainability (Zhang et al., 2022). In recent years, factors such as illegal fishing, habitat destruction and warming sea water caused by climate change have caused a sharp decline in many wild abalone populations. About 20 of the world's 54 abalone species are at risk of extinction.

Although abalone is important, both in terms of classification and breeding, people do not know enough about its evolutionary relationships, and most previous studies rely on appearance and mitochondrial gene analysis. These methods are controversial and not accurate enough. The relationships between abalone species obtained by different studies are not the same, and the classification of some abalone is still uncertain. Species such as the nine-hole abalone have different views in molecular research, and traditional classification results do not match genetic data (Agius et al., 2024). Past genetic research has mainly focused on mitochondrial genes or a few nuclear genes, and the research content is relatively limited. Only in recent years have a few abalone genomes been measured, but most of them are only rough versions and are not detailed enough. These genomic data are far from enough, which affects our in-depth understanding of abalone genes and functions. For example, the first abalone genome was a draft version of the wrinkled abalone, which was not released until 2017. Later, the red abalone, black abalone, etc. also had genomes, but some tropical abalone and variegated abalone species have not yet had relevant data. Due to the lack of complete genetic information, it is also difficult for us to figure out how abalone adapts to different environments, such as temperature, salinity and pathogens. At present, the improvement of abalone's stress resistance and growth traits mainly relies on traditional breeding methods, and the genes and molecular mechanisms related to these traits have not been figured out. Now with the development of high-throughput sequencing technology, we have the hope of using the whole genome to study the evolutionary relationship of the abalone genus (Pu et al., 2020; Wang et al., 2023). By comparing the complete genomes of different abalone species, we can draw a clearer phylogenetic tree and find important genes related to environmental adaptation. However, research in this area is still in its infancy, and further improvement is needed in species selection and genome quality.

This study will use abalone whole genome data to systematically analyze the phylogenetic relationships and adaptive evolutionary mechanisms of abalone species, integrate the genomes of multiple abalone species published in the past five years (including *Abalone discus*, *Abalone japonensis*, *Abalone rubrum*, *Abalone nigromaculata*, *Abalone occidentalis*, etc.), conduct comparative analysis of their genomic structural characteristics, and construct a phylogenetic tree of abalone based on high-confidence single-copy orthologous genes, explore the evolutionary history and geographical diffusion process of abalone, identify genes and gene families that show rapid evolution in different environments, including candidate genes related to temperature, salinity adaptation and immune defense, as well as gene expansion related to shell mineralization, growth metabolism, and select three representative abalone species as cases for in-depth analysis. This study analyzes abalone phylogeny and adaptive evolution at the whole genome scale, providing an important reference for future functional genomics research and molecular breeding practices.

2 Genome Architecture and Evolutionary Characteristics

2.1 Overview of abalone genome size and structure

The diploid chromosome number of abalone species is usually 2n=36 or 32, corresponding to 18 or 16 pairs of haploid chromosomes. The sequenced abalone genome size is in the range of about 1.2 Gb~1.8 Gb, with the characteristics of a medium genome size. For example, the size of the assembled genome of *Abalone discus* is about 1.86 Gb, with a GC content of about 40.5%; the draft genome of *Abalone africanus* is about 1.50 Gb; the genome of *Abalone cracherodii* is about 1.71 Gb; the genome of *Abalone cracherodii* is relatively small, about 1.2 Gb. Abalone genomes are generally high in proportion to repetitive sequences and transposons, which is one of the main reasons for the large size of mollusc genomes (Dale-Kuys et al., 2017). It is reported that the total amount of repetitive sequences in the genome of *Abalone discus* is more than 40%, of which scattered repetitive elements (such as LINE, class I and class II transposons) are the main ones. In terms of the number of predicted genes, the species are not much different, generally containing more than 20 000 coding genes. For example, the predicted genes of Haliotis discus are about 23 400; the green abalone genome has annotated about 27,700 protein-coding genes; the number of genes in the red abalone and black abalone genomes is also between 23,000 and 25 000. In terms of genome assembly quality, the latest research has improved from the initial short-read fragment assembly to chromosome-level assembly. For example, the tropical abalone *H. asinina* recently obtained the first high-quality genome at the chromosome level, with a total of 18 assembled chromosomes, which is consistent with the karyotype. The abalone genome structure has a similar composition framework to other gastropod mollusks, but also shows some family-specific characteristics, such as large-scale genome duplications, low GC content, and gene cluster distribution (Barkan et al., 2024). These characteristics may be related to the unique development and metabolism of mollusks, providing background information for subsequent in-depth research.

2.2 Gene content, repetitive elements, and unique genomic features

The abalone genome has a high gene content and relatively low gene density, reflecting its large genome size and rich non-coding regions. Compared with model mollusks (such as golden snails and oysters), many genes in the abalone genome have multiple copies or gene family expansion. For example, the Pacific abalone Haliotis discus has a very developed aquaporin gene family, with a total of 18 members identified, more than double the number of AQP family members in most mollusks (Jia et al., 2022; Zhao and Wu, 2024). These aquaporin genes may give abalone stronger water regulation ability under osmotic and temperature stress through gene duplication and functional differentiation. For another example, the matrix protein genes related to abalone shell formation show rich innovation and diversity. Transcriptome and proteome studies have shown that the mantle secretory protein (i.e., shell matrix protein) collections of different abalone species are significantly different, including a large number of genus-specific new genes and special repetitive domains (Xin et al., 2017). These low-complexity repeat sequences (RLCDs) are often generated through mechanisms such as slipped replication and gene recombination, thus promoting the expansion and diversification of the shell matrix protein family. In addition to the expansion of functional genes, there are also some unique repeat sequences in the abalone genome. For example, some abalone-specific transposable elements are only found in abalone and its closely related species, suggesting that they may play a unique role in the evolution of the abalone genome. It is worth noting that the genomic microsatellites and scattered repeat sequences of abalone are very rich. This provides material for population genetics and molecular marker screening, but also increases the difficulty of gene assembly and sequencing. With the application of third-generation sequencing and Hi-C technology, these complex regions can be more completely analyzed in the future, so as to better explore the potential functional elements in the abalone genome.

2.3 Evolutionary conservation and divergence in mollusks

Comparative genomic studies have shown that the abalone genome has retained some common characteristics in mollusks and has also evolved its own uniqueness. In terms of conservation, abalone shares a large number of ancient developmental genes and metabolic pathway genes with other mollusks (such as bivalve oysters, stone snails, etc.). For example, core genes related to shell mineralization, neural regulation, and energy metabolism are highly conserved among all groups of molluscs. This reflects that as an ancient evolutionary lineage, molluscs have a relatively stable genetic blueprint for basic life processes. However, there are significant differences between abalone and other molluscs in terms of genome size, gene family, and repetitive sequences. The bivalve oyster genome (~560 Mb) is much smaller than that of abalone, probably because it has undergone genome contraction and lower repetitive sequence content. However, the genome of some gastropods, such as the golden apple snail (>2 Gb), is larger than that of abalone, indicating that the genomes of gastropods with different ecological habits have experienced different expansion histories. Abalone also differs from other marine shellfish in terms of immune and stress-related genes. For example, the number of Toll-like receptor (TLR) families encoded in the abalone genome varies greatly among different species: the wrinkled abalone has 29 *TLR* genes, while the red abalone has 33 and the green abalone has only 16. This difference may reflect the different evolutionary strategies of each species to cope with their own pathogenic pressures. Compared with bivalves, gastropods such as abalone have a more complex neuroendocrine regulation system, which is reflected in the expansion of neuropeptide and hormone receptor gene families. For example, dozens of new neuropeptide precursor and receptor genes have been identified in the green abalone genome, which may play an important role in regulating reproduction and metabolism. The abalone genome has both commonalities and individuality with other mollusks.

3 Phylogenetic Reconstruction Using Whole-Genome Data

3.1 Phylogenetic tree inference across abalone species

Tshilate et al. (2023) collected high-quality genome sequence data of multiple representative species of the genus Abalone (including *H. discus*, *H. japonicus*, *H. variegated*, *H. red*, *H. black*, *H. green*, etc.), and used phylogenetic genomics methods to reconstruct their phylogenetic relationships (Figure 1). By selecting tandem orthologous single-copy genes, this study obtained spliced sequences containing about hundreds of conserved proteins, and constructed a phylogenetic tree using the maximum likelihood method. The results support that Abalone species can be divided into several major branches (clades) (Nam et al., 2017; Botwright et al., 2019; Guo et al., 2019). For example, the analysis showed that *H. discus* and *H. discus* were clustered first, and the two formed the North Pacific abalone branch; while the Australian green abalone (*H. laevigata*) and the South African abalone (*H. midae*) formed the Southern Hemisphere abalone branch. The American species such as red abalone (*H. rufescens*), black abalone (*H. cracherodii*) and pink abalone and white abalone form the American abalone branch, which is distantly related to the Asian-Oceanian abalone. The whole genome phylogenetic tree is generally consistent with the traditional classification results based on mitochondrial DNA, but some details have been revised. For example, this study supports the view that the disc abalone group along the coast of Japan is a geographical population of the wrinkled disc abalone, rather than an independent species. For another example, the nine-hole abalone (*H. diversicolor*) and its subspecies form an independent small branch, suggesting that its relationship with the wrinkled disc abalone is more distant than previously thought. This suggests that the nine-hole abalone may be classified as a different subgenera within the genus. It is worth mentioning that the support rate of each major branch in the phylogenetic tree of this study is very high, thanks to the reliable signals provided by the massive sites of the whole genome. In contrast, previous trees based on single genes or mitochondrial genomes may have insufficient support for some nodes due to systematic uncertainty. The camel-eared abalone (*H. asinina*) was clearly placed at the base of the abalone phylogenetic tree and separated from other temperate abalone branches early. This is consistent with the geographical distribution, that is, tropical abalone may represent an ancient evolutionary lineage of the genus Abalone (Barkan et al., 2024). Phylogenetic reconstruction based on whole genome data provides a clearer picture of the species relationship of the genus Abalone and provides a new basis for taxonomy and evolutionary biology research.

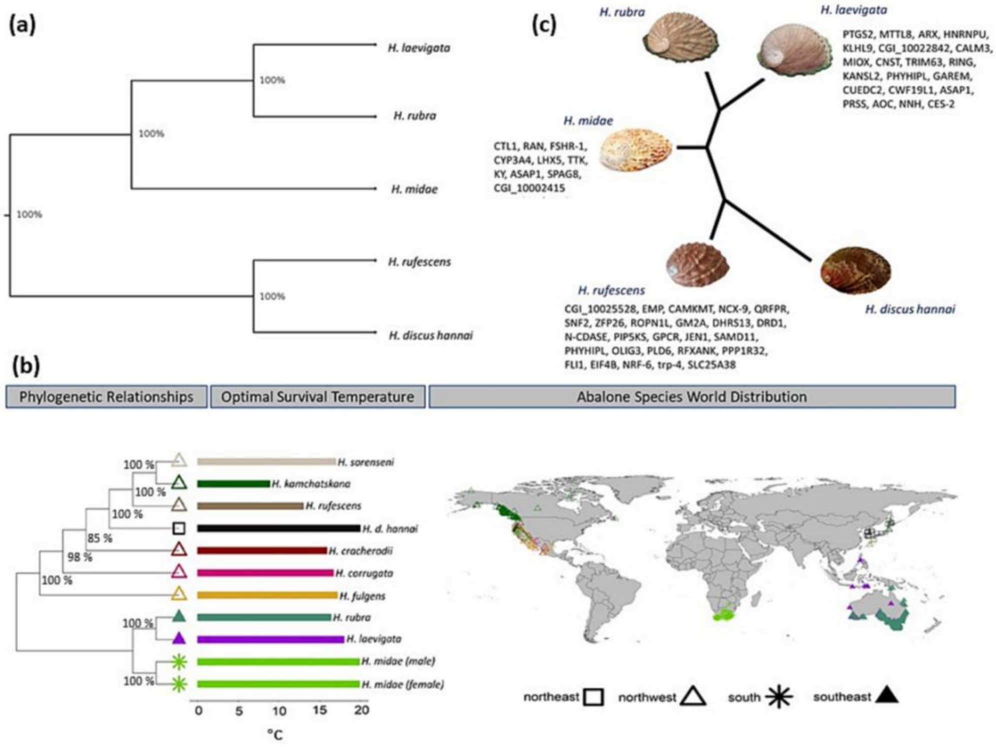

Figure 1 (a) Orthologous gene-based phylogenetic relationship in select abalone species, showing the close-relatedness among *H. midae*, *H. rubra* and *H. laevigata* and bootstrap values based on 100 pseudo-replicates given at the branching node. (b) Phylogeographic distribution of abalone species, based on whole genome SNP phylogenomic relationships (bootstrap values calculated on 100 pseudo-replicates given at the branching node), in relation to their optimum mean temperatures. The genome-wide SNP phylogeny places the South African abalone close to other southern abalones. Species occurrence information are from the Global Biodiversity Information Facility (GBIF) and optimal temperature values were retrieved from SeaLifeBase (https://www.sealifebase.se). (c) Positively selected genes (PSGs) identified with genes involved in immune defence, sensory response, and reproduction in selected abalone species) (Adopted from Tshilate et al., 2023)

3.2 Molecular clock analysis and divergence time estimation

With the help of phylogenetic trees and combined with molecular clock models, this study further estimated the differentiation time of the main evolutionary lineages of the genus Abalone. Using fossil records and the differentiation time of other gastropods as calibration points, this study infers that the genus Abalone originated in the Late Cretaceous about 100 million years ago, and then experienced multiple species radiations in the Paleogene and Neogene. For example, the divergence between abalones in the northern Pacific (Halion discus, *Abalone japonensis*, etc.) and abalones in the southern hemisphere (*Abalone occidentalis*, *Abalone occidentalis*, etc.) occurred approximately at the end of the Cretaceous about 70 million years ago. Botwright et al. (2019) also obtained a similar differentiation time estimate (about 71 million years ago) by comparing the genomes of *Abalone occidentalis* and *Abalone occidentalis*. This suggests that the early differentiation of the abalone genus may be related to plate movement and ocean separation. Later, during the Miocene, regional radiation of abalone occurred along the coasts of different continents. For example, this study estimates that the common ancestor of the species of the American abalone branch (red abalone, black abalone, etc.) lived about 30 million years ago. During the Miocene warm period, changes in ocean circulation may have contributed to the formation of these species (Tshilate et al., 2023).

Similarly, the differentiation between African abalone and Australian abalone was roughly 15 million years ago, which coincides with changes in the Indian Ocean-Atlantic water system. It should be pointed out that due to the limited reliable fossil calibration, there is a certain uncertainty in the molecular clock inference, but the overall time frame is consistent with geological events. According to the time tree constructed in this study, the evolutionary trajectory of the abalone genus can be depicted: the genus originated in the Tethys Sea and then split into two major lineages in the north and south with continental drift. The northern hemisphere lineage diversified in the northwest Pacific, and the wrinkled abalone group appeared; the southern hemisphere lineage evolved into warm-resistant species in South Africa and Australia. The rise and fall of sea levels and changes in ocean currents during the glacial period have shaped the recent distribution pattern of species. For example, the Quaternary glacial period caused some abalone populations to be isolated, thus forming closely related species. The evolutionary history of abalone is closely related to changes in the global marine environment.

3.3 Revisions in abalone taxonomy based on genomic evidence

Based on the high-resolution phylogenetic tree and differentiation time information obtained in this study, some revision suggestions can be made for the classification of the genus Abalone. Given that the genomes of the wrinkled disc abalone (from Hokkaido, Japan to the northern coast of China) and the Japanese abalone (*H. discus*) are very different, it is supported to regard the two as the same species or at least very closely related evolutionary units. Traditionally, Japanese abalone is sometimes considered to be a nominative subspecies of the wrinkled disc abalone. The results of this study further confirm that its taxonomic status should be merged, which will simplify the classification system of the genus Abalone. The phylogenetic tree of this study shows that the nine-hole abalone (*H. diversicolor*) and its variants (such as the variegated abalone *H. diversicolor* supertexta) form an independent branch and are far away from the wrinkled disc abalone, supporting its treatment as an independent species or subgenus. For a long time, the classification of *Haliotis diversicolor* and Haliotis discus has been controversial. Based on genomic evidence, *Haliotis diversicolor* can be classified as a new sub-classification unit such as "*Haliotis diversicolor*" to reflect its genetic independence.

For some newly described species in recent years, such as the yellow and pink abalone (*H. corrugata*, *H. fulgens*, etc.) in Mexico, the genomic analysis of this study supports the validity of their respective species. Although these species are morphologically similar, their genomic differences are clear and their species status should be retained (Vega-García et al., 2015; Mares-Mayagoitia et al., 2023). This study found that although the green abalone (*H. laevigata*) and black-lipped abalone (*H. rubra*) in Australia overlap geographically, their genomes are significantly different, which proves that they are not the same species, but a pair of different species known to hybridize naturally. This is also reflected in aquaculture practice - the fertile offspring of green abalone and black abalone are used for breeding, but the genomic differences between the parents still maintain the species boundaries (Kube et al., 2023). Phylogenetic studies based on the whole genome provide more solid evidence support for abalone taxonomy. This study suggests that future revisions of abalone classification should fully refer to genomic data and reasonably divide species and subspecies boundaries. For example, the abalone genus can be divided into the North Pacific subgenus, the Southern Hemisphere subgenus, etc. according to genetic distance, thereby reflecting its systematic evolutionary pattern.

4 Adaptive Evolutionary Mechanisms

4.1 Identification of positively selected genes related to temperature, salinity, and immunity

Abalone can live in a variety of waters, from cold high-latitude waters to warm tropical waters. Different species of abalone have very different adaptability to the environment. Some are more tolerant to high temperatures, while others are more adaptable to low temperatures or salinity changes. In this study, we compared the whole genome of abalone and found some key genes related to temperature and salinity adaptation. This study also used positive selection analysis and found signs of accelerated evolution in some genes related to stress response. For example, genes encoding heat shock proteins (HSPs) and molecular chaperones showed positive selection signals in tropical abalone. This may indicate that changes in these genes help proteins become more stable and more heat-resistant. In the cold-adapted Japanese abalone, some antifreeze protein genes may have played a role. These genes may have become more numerous or their functions have been enhanced, but this needs further verification. Regarding salinity adaptation, some genes that regulate water and salt inside and outside the cell, such as Na+/K+-ATPase and organic matter transport proteins, have also changed at certain sites. Some gene families may also become larger. This may help abalone maintain homeostasis in environments with large salinity changes (Creencia and Noro, 2018).

In addition to temperature and salinity, abalone's defense against pathogens is also an important aspect of their adaptation to the environment. We found sequence differences in some immune-related genes in different abalone species. For example, the Toll-like receptor (*TLR*) genes related to innate immunity are more numerous in temperate abalone, while in some tropical abalone, some *TLR* genes have become pseudogenes or even completely disappeared. All abalone lack a gene called MAVS. This gene is critical in many vertebrates, mainly to help fight viruses. Although abalone does not have MAVS, their RIG-I-like receptor (*RLR*) genes have undergone significant changes, which may initiate antiviral responses in other ways (Agius et al., 2024). This shows that abalone has formed its own defense method in long-term evolution, perhaps by adjusting the pathway of signal transmission to compensate for the lack of MAVS. Some immune genes related to colistin response and phagocytosis, such as TRAF, also showed adaptive changes at specific sites, indicating that these genes may have also undergone positive selection.

4.2 Gene family expansions in shell formation and metabolic pathways

The precious value of abalone is not only reflected in the quality of the meat, but also in its strong and beautiful shells. The abalone shell is composed of aragonite calcium carbonate and organic matrix, which has evolved into a complex biomineralization mechanism. Genome analysis found that the gene family involved in shell formation has expanded and diversified significantly in abalone. For example, genes encoding nacre organic matrix proteins (such as velvet snail protein, gelatin protein, etc.) have multiple copies and variant forms in the abalone genome. These genes are usually highly expressed by the mantle, and their products are secreted into the shell to regulate crystal growth (Mann et al., 2018; Sharker et al., 2021). Comparison of the mantle "secretome" of different abalone species shows that even closely related species have very different sets of shell matrix proteins.

Shell proteins of a large number of gene families were independently innovated in each lineage. For example, the proline-rich shell protein family found only in the wrinkled abalone was replaced by another set of serine-rich proteins in the green abalone. This phenomenon of "same function, different sequence" indicates that the shell formation-related gene family has undergone rapid divergent evolution in different lineages of abalone. Gene family expansion is not limited to structural proteins. This study also noted that some metabolism and detoxification-related genes are particularly abundant in abalone. For example, the multifunctional oxidase gene

(CYP450 family) has many members in the abalone genome, which may be related to its broad-spectrum diet and detoxification needs. Another example is that abalone also has extra copies of enzyme genes involved in amino acid degradation and urea cycle, which is speculated to be related to its high-protein seaweed diet. It is particularly noteworthy that abalone's neuroendocrine and growth hormone regulatory genes also have an expansion trend. For example, more than 40 neuropeptide precursors were identified in the green abalone genome, which is more than other mollusks. These neuropeptides may have redundant and diverse functions in regulating gonad development and growth metabolism. For another example, abalone also has a relatively rich insulin-related peptide and receptor genes, which contribute to its growth rate and regulation of glucose and lipid metabolism. The expansion of the shell structure and metabolism-related gene family reflects the functional enhancement of abalone to adapt to its ecological niche: the hard shell requires a complex matrix protein network to support it, and rapid growth and movement require an efficient metabolic regulation system. These genomic characteristics are consistent with the evolutionary lifestyle of abalone occupying the coastal reef ecological niche and eating nutrient-rich macroalgae.

4.3 Adaptive structural variants and genomic islands of divergence

The genetic changes of abalone do not only occur in a single gene. On a larger scale, their genomes have also undergone some adjustments, such as rearrangements and structural changes. These changes appear in some particularly obvious areas, namely "hot spots". When comparing the genomes of different abalone species, this study found that some chromosome segments changed particularly quickly, and some regions were highly diverse. For example, in the comparison between the wrinkled abalone and the nine-hole abalone, several large regions showed structural changes, some of which were reversed in gene order (called inversion) and some of which were moved in position (called translocation). These large genomic changes may be one of the reasons for the separation of the two species. Because these changes make it difficult for genes in related regions to recombine, that is, they cannot be freely combined as before, so that the differences between the two species are fixed more quickly. This study also used the method of population resequencing to analyze the genomic differences of wrinkled abalone in different regions of China. The results showed that some regions on the chromosome had particularly high F_st values (this is an indicator of the difference between two populations). These "differentiation islands" often contain genes related to environmental adaptation (Huang et al., 2021). For example, on chromosome 7, there is a region that is very different between northern and southern abalone. There are some genes related to immune response and stress response. This result shows that these regions may be subject to selection pressures from different environments, such as different water temperatures and water quality, and are the key to the adaptation of the group to the environment.

Similar situations also occur in the comparison between green abalone and black abalone. In the gene region that controls shell color and shape, the allele frequencies of the two species are different. This difference may be due to natural selection. Further analysis found that changes in some traits are related to changes in genome structure. For example, studies have shown that the green abalone genome lacks a gene that can regulate pigment deposition that black abalone has, which may explain the difference in their shell color (Botwright et al., 2019). There are some particularly variable regions in the abalone genome, such as microsatellites and VNTR (variable tandem repeats). These regions vary in length in different species, and many of them appear in the place where genes are regulated, which may affect gene expression, that is, whether the genes are turned on or off, thereby affecting the adaptability of abalone. The evolution of the abalone genome is not just a matter of slowly changing genes one by one, but also a large segment of changes together. These changes may have played a big role in the process of species formation and adaptation. For example, some areas may prevent different species of abalone from interbreeding (creating reproductive isolation), or they may help them adapt to new environments more quickly.

5 Case Studies: Phylogenomics and Adaptation in Representative Species

5.1 *Haliotis discus hannai*: high-latitude adaptation and immune responses

Japanese abalone (*Haliotis discus hannai*) is native to the cold temperate waters of the North Pacific, including northern China, the Korean Peninsula and the coast of Japan. It is currently the abalone species with the highest aquaculture production in China and Northeast Asia, and its adaptability to low-temperature environments and disease resistance have attracted much attention. Through the analysis of the Japanese abalone genome and population data, this study obtained some genetic characteristics of this species' adaptation to high-latitude environments. In terms of temperature adaptation, a series of cold-resistance-related genes in the Japanese abalone genome have specific changes compared with other species. For example, genes encoding antifreeze proteins show unique amplification in Japanese abalone. This type of protein can prevent the formation of tissue ice crystals at low temperatures and improve cell cold tolerance. Studies have shown that antifreeze active substances exist in the hemolymph of high-latitude abalone, and their genetic basis may be related to the expansion of antifreeze protein genes found in this study. The immune genome characteristics of Japanese abalone are also worth noting. Due to the disease stress in the aquaculture environment, breeding disease-resistant strains has become one of the main goals.

This study compared the immune genes of Japanese abalone and other abalone and found that Japanese abalone has more types of immune-related genes, especially in some innate immune factors, such as pattern recognition receptors and lysozymes. For example, Japanese abalone has up to 29 *TLR* genes, which is currently the largest number in abalone (Zou et al., 2023). This may indicate that it is more sensitive and has more ways to identify pathogens. In the NF-κB signaling pathway, some immune regulatory genes of Japanese abalone, such as Rel, have some unique amino acid changes compared with other abalone. These changes may affect their ability to regulate immune responses (Sun et al., 2022). Through population genetic analysis, it can be seen that some gene loci related to disease resistance have undergone significant changes in the artificial breeding process of Japanese abalone. The frequency of some alleles has increased significantly, which may be deliberately retained by people during the breeding process. Most of these genes are related to the production of mucus and the enhancement of skin barrier function, which may help improve antibacterial ability.

In terms of nutrition and metabolism, Japanese abalone also has its own characteristics. In order to adapt to the cold season, they may have developed a special way of energy storage. Their fat synthesis and decomposition related genes are expressed differently from warm water abalone. This may help them maintain basic body functions in the low temperatures of winter. From the phylogenetic analysis, Japanese abalone, Korean abalone and Japanese disc abalone actually belong to the same branch, and their genomes are very different.

5.2 Haliotis midae: genomic insights into warm-temperate tolerance

African abalone, also known as South African abalone, is native to warm waters in southern Africa. Compared with Japanese abalone, African abalone has a stronger tolerance to high temperature environments, and its optimal growth temperature is significantly higher. In the context of global warming, African abalone has attracted attention as a high-temperature resistant variety. This study analyzed the adaptive characteristics of the African abalone genome. This study compared the protein-coding sequences of African abalone and Japanese abalone, and identified multiple genes that were positively selected in the African abalone branch. A considerable number of them are related to heat stress and protein homeostasis maintenance. For example, some members of the heat shock protein 70 (HSP70) family have specific amino acid substitutions in African abalone, which may enhance their folding aid function at high temperatures. For example, enzymes involved in antioxidant defense (such as superoxide dismutase SOD, peroxidase GPx, etc.) show higher copy numbers or gene expression potential in the African abalone genome, helping to clear high temperature-induced reactive oxygen damage (Shiel et al., 2015).

Some metabolic genes in the African abalone genome have changed their regulatory sequences, allowing their metabolic rates to be maintained at high temperatures. For example, this study found that the promoter region of the mitochondrial respiratory chain complex protein gene of the African abalone has a unique cis-regulatory element, which may enhance the stability of its mitochondrial function under high temperature conditions. This is consistent with the observation that South African abalone can still maintain high vitality in the hot season (Tripp-Valdez et al., 2019). In terms of immunity, the pathogenic environment faced by African abalone is slightly different from that of temperate abalone, and its immune genome is also different accordingly. This study noted that the African abalone genome lacks the mitochondrial antiviral signaling protein MAVS, which is consistent in all abalone. However, African abalone may make up for this defect by evolving different RIG-I-like receptor pathways to cope with viral diseases that are more prone to outbreaks under higher water temperature conditions. Some phagocytosis and inflammatory response-related genes (such as NOD-like receptors and inflammasome components) of African abalone carry unique alleles, which are speculated to be related to their disease resistance spectrum. This study conducted a gene family expansion analysis on African abalone and found that gene families related to energy metabolism and environmental information processing are particularly abundant in its genome. For example, African abalone has extra copies of photopigment genes and circadian rhythm genes, which may help it perceive and adapt to diurnal changes and light conditions in coastal shallow water environments. These characteristics have improved the adaptability of African abalone to conditions such as high temperature during the day and low oxygen at night. Through the above analysis, this study preliminarily outlined the genetic basis of African abalone's heat-resistant adaptation, that is, to resist high temperature stress by strengthening heat shock response, antioxidant and metabolic regulation. This information is of reference value for the future breeding of high-temperature tolerant abalone varieties. For example, molecular marker-assisted selection can be carried out for key genes such as HSP70, thereby genetically improving the heat tolerance of farmed abalone.

5.3 Haliotis laevigata: Local selection signals in shell morphology and stress response

The Australian green abalone is a large abalone species unique to Australia, known for its beautiful green shell and large size. Green abalone often inhabits environments with turbulent water and moderate temperatures, and has certain stress resistance and unique shell shape. This study used population genomics methods to detect local selection signals related to shell morphology and stress resistance in the green abalone genome. This study compared the frequency distribution of green abalone genes in different geographical populations and found that several gene regions had undergone significant differentiation under the action of selection pressure. For example, in the QTL region that controls shell shape and thickness, this study detected significant differentiation signals. These regions contain genes that regulate mantle development, such as bone morphogenetic protein BMP pathway genes and shell matrix protein genes (Sandoval-Castillo et al., 2018). It is speculated that in an environment with high waves and fast currents, individuals with flatter and thicker shells have a higher survival rate, so the favorable variants of these genes are positively selected and enriched in the population.

Similarly, local selection related to stress resistance was also found. Green abalone may experience combined stress of high temperature and hypoxia in summer. This study found a selection region related to hypoxia tolerance on its chromosome 12, which is rich in heme-binding proteins and mitochondrial respiration regulatory genes. Individuals carrying specific mutations may be better able to tolerate temporary hypoxia in the intertidal zone, and these mutations therefore increase in frequency in wild populations. It is worth noting that this study compared the genomic differences between green abalone and its closely related species, black-lipped abalone (H. rubra), and found that some trait QTL regions in the hybrid offspring of the two showed hybrid vigor signals. For example, a gene region that affects growth rate is a single allele in green abalone and different in black abalone, but the hybrid offspring has better growth performance when they have both alleles (Figure 2) (Kube et al., 2023).

This suggests that by hybridizing green abalone with other species, favorable mutations of different species can be combined to explore hybrid vigor. This has also been practiced in Australian abalone farming (green abalone × black abalone hybridization is used to cultivate fast-growing hybrid abalone). Local genomic selection studies of Australian abalone have revealed key regions that affect shell morphology and stress resistance. The genes in these regions can be used as targets for molecular breeding to improve the shell color, shell shape and environmental adaptability of farmed abalone. For example, through molecular marker selection, abalone populations can be made to have thicker shells and more resistant to pressure and heat, thereby improving aquaculture output and risk resistance. Case studies of Australian abalone have shown that analyzing local adaptation from a genome-wide perspective has important implications for the targeted breeding of economic traits.

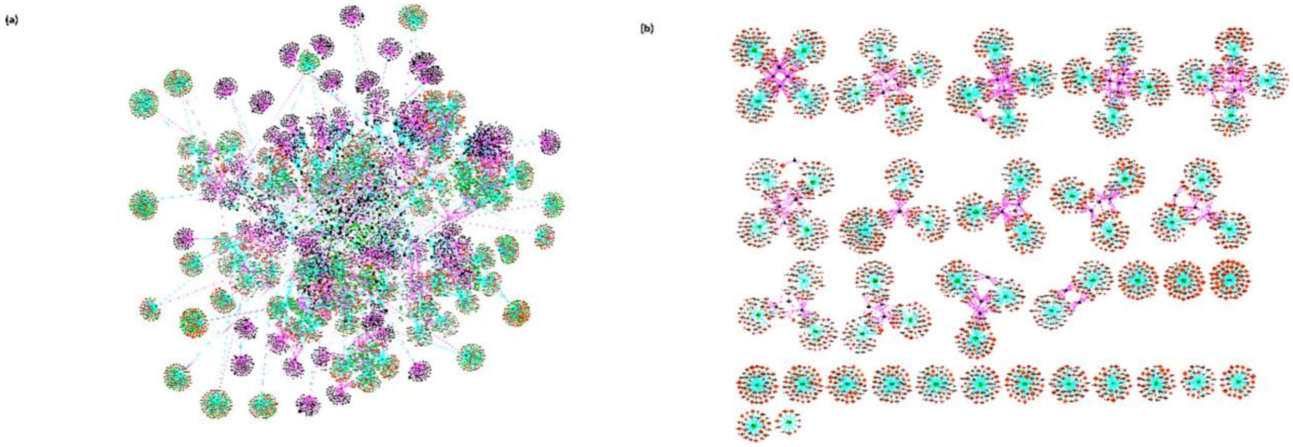

Figure 2 Pedigromics (Adopted from Kube et al., 2023)

Image caption: A random sample of 3 646 *H. rubra*, 3157H. leavigata and 2 967 hybrid progeny with total weight at 2.5 years plus their ancestor are represented in a. while 2 967 hybrid progeny and their 64 *H. leavigata* sires and 46H. rubra dams are represented in b. Each node represents one animal from the population. The node colours are black for *H. rubra*, green for *H. leavigata* and orange for hybrid and their size are based on total weight at 2.5 years. Male, female, and unknown sex are squares, triangles, and diamonds, respectively. The connection line (edge) colour is blue for progeny and sire and pink for progeny and dam (Adopted from Kube et al., 2023)

6 Applications in Conservation and Breeding

6.1 Identification of genetic markers for assisted selection

With the advancement of abalone genome research, a large number of genetic markers and functional genes have been identified, which can be used to accelerate the molecular breeding process of abalone. Traditional abalone breeding is mainly based on phenotypes such as growth rate and survival rate, which has a long cycle and high cost. Based on whole genome data, we can now develop high-density molecular markers (such as SNP chips) for whole genome selection breeding. For example, the research team of Xiamen University developed the abalone 40K breeding chip using the whole genome sequence of the wrinkled abalone, implemented genomic selection for growth and stress resistance traits, and greatly improved the breeding efficiency (Liu et al., 2022; 2024). Genomic markers can also assist in parentage identification and pedigree construction, solving the problem of difficult pedigree tracking in large-scale artificial breeding of abalone. In this study, we identified several molecular markers associated with traits. For example, specific loci related to shell color and shell thickness were found, which can be used to breed strains with bright shell color and excellent shell shape.

For example, the identification of alleles significantly associated with high temperature resistance survival rate can be used to screen heat-resistant individuals in the high-temperature breeding environment in the south. Incorporating these genomic markers into the breeding program can select potential excellent individuals in advance at the larval stage or even the fertilized egg stage, thereby shortening the generation interval and increasing genetic gain. Molecular markers also help maintain the genetic diversity of the cultured population. Long-term artificial breeding of abalone is prone to inbreeding and germplasm degradation. Monitoring the allele richness and inbreeding coefficient of the population through molecular markers can guide parent selection to avoid excessive inbreeding. Genomic marker technology provides new tools and ideas for abalone molecular breeding. The transition from empirical breeding to data breeding will effectively improve the speed and accuracy of abalone breeding. Looking to the future, with the reduction of sequencing costs and the maturity of analytical methods, genomic selection breeding is expected to be widely used in marine aquaculture shellfish such as abalone.

6.2 Whole-genome insights into hybrid vigor and disease resistance

Hybridization between different species or strains of abalone often produces hybrid vigor. For example, the hybrid offspring of green abalone and black abalone show faster growth and stronger stress resistance. Using genomic information, this hybrid vigor can be better mined and utilized. First, through genome comparison, this study can identify complementary alleles between parental species. For example, this study found that green abalone and black abalone have different alleles in some growth-related genes and each has its own advantages. Then, when both alleles are present in the hybrid offspring, a superposition effect may occur to improve the trait. This study can select parent combinations in a targeted manner to maximize hybrid vigor. Genomic analysis can also be used for background selection and backcross breeding of hybrid offspring (Xiao et al., 2021; Sui et al., 2024). If the offspring produced by hybridization have undesirable traits, they can be tracked by molecular markers, and the unwanted chromosome fragments can be eliminated by backcrossing while retaining beneficial genes. This is similar to the molecular design breeding idea in crops, and can also be tried in abalone breeding.

In the disease-resistant breeding of farmed abalone, genomic technology has brought new methods. Whole genome association analysis (GWAS) or artificial selection experiments can be used to find the unique genetic variants in disease-resistant abalone. For example, if the genomes of surviving and dead abalone are compared after the abalone is infected with pathogens, it is possible to find sites related to disease resistance. The genes near these sites are likely to be candidate genes for disease resistance. With this genetic information, we can introduce disease-resistant genes into the farmed abalone population through hybridization. If policies allow, gene editing methods can also be used to directly improve them. At present, researchers have established a CRISPR/Cas9 editing platform on the wrinkled abalone, which can knock out or knock in certain genes. In the future, this method can be used to verify whether certain genes are useful, and it may also develop into a new breeding method. However, when using these technologies for hybridization or improvement, we must also be careful not to only pursue disease resistance. We must avoid excessive selection, which will lead to a decrease in genetic diversity, which will make abalone more susceptible to disease or worse adaptability to the environment. Genomic tools can also be used to monitor these risks. The addition of genomic technology makes abalone breeding more scientific and more targeted. After figuring out which genes are related to disease resistance, stress resistance, and fast growth, we can combine the genes that perform well from different abalone varieties to breed new varieties with stronger comprehensive abilities.

6.3 Implications for abalone resource conservation and climate adaptation

The whole genome study of abalone is not only helpful for aquaculture, but also useful for protecting wild abalone resources. Genomic data can help to formulate conservation plans more scientifically. For example, by analyzing the genomes of abalone in different regions, we can see how big the genetic differences are between them. If the differences are obvious, they can be divided into different conservation units (evolutionarily significant units, ESUs) and managed separately. For abalones such as the black abalone (*H. cracherodii*), which are already rare, genome studies have found that their populations are too small and their genetic diversity is also declining. This shows that when protecting them, in addition to protecting their habitats, some abalone should be artificially propagated and released to increase genetic diversity and reduce the risk of inbreeding. The genome can also help find which abalone individuals have good genes for adapting to the environment. For example, if the seawater in an area is getting hotter and hotter, abalone with "heat-resistant genes" can be preferentially selected for release to increase their chances of survival.

Now, climate change is having an increasing impact on coastal ecosystems, and abalone is one of the species that bears the brunt. We can use genome monitoring methods to see if the genes of abalone have changed with environmental changes. For example, by comparing the DNA of abalone collected at different times, we can see whether the alleles of certain genes have become more or less, so as to understand whether they are adapting to higher water temperatures or seawater acidification. This method can be used as a "genetic early warning indicator" to help predict the future of abalone and facilitate early protection measures. Genomic technology can also be used in law enforcement and management. The problem of abalone smuggling and illegal fishing is now serious. We can use genetic fingerprints to determine which species the abalone products are and where they come from. In this way, law enforcement agencies can more effectively combat illegal trade (Vargas-Peralta et al., 2022).

7 Conclusion

This study used the whole genome data of abalone to deeply analyze the evolutionary relationship and adaptation mechanism between different species. We constructed a phylogenetic tree containing multiple representative species, clarified the taxonomic structure of the genus Abalone, and proposed some taxonomic revision suggestions. Molecular clock analysis showed that the differentiation of abalone in the geological history was highly correlated with plate movement and climate change events. Through comparative genome and selection analysis, we identified several key genes related to environmental adaptation, including heat shock proteins for temperature tolerance, antifreeze proteins, transport proteins for regulating salinity, and receptors and signaling molecules with special changes in immune pathways. We also observed that the shell matrix proteins that control shell structure have independent expansions in different abalone lineages, indicating that their shell formation mechanisms are diverse. In addition, structural variations and hotspots in the genomes of different species indicate that the adaptive evolution of abalone occurs not only at the gene level, but also in large fragments of genome structure.

The results of this study provide strong support for the protection and breeding of abalone resources. In terms of protection, we revealed the genetic structure of different species and populations, which is helpful to define more scientific protection units and formulate reasonable germplasm exchange strategies to maintain genetic diversity and adaptation potential. In terms of breeding, the functional genes such as heat resistance and disease resistance we identified provide targets for molecular marker-assisted breeding, which can improve breeding efficiency and accuracy. We also found some gene regions related to hybrid vigor, which can be used to optimize parental combinations and cultivate strains with better comprehensive traits. Combining whole genome selection and genome monitoring methods is expected to promote abalone from traditional empirical breeding to precision breeding, and timely evaluate the genetic health of the population, prevent inbreeding and germplasm degradation, so as to achieve a win-win situation of industry quality improvement and sustainable resources.

Although this study has made initial progress, there are still many directions worth exploring in abalone genomics. The specific functions of positively selected genes and expansion families need to be verified by transcriptome analysis, gene knockout and other means; more resequencing data of wild and farmed populations will also help reveal the impact of human activities on the genome; the introduction of long-read sequencing and Hi-C technology will improve the quality of genome assembly and discover more key variants. In addition, this study found that abalone lacks the *MAVS* gene, and the alternative path of this immune mechanism deserves attention. In the future, functional research should be strengthened to explore the unique immune strategy of abalone. At the same time, cross-species comparisons and genomic breeding practices should also be promoted simultaneously, such as constructing selection indices and conducting gene editing experiments. As research deepens, we are expected to have a more comprehensive understanding of the evolution and adaptation process of abalone, promote its efficient breeding and resource protection, and contribute to the development of marine ecology and industry.

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Conflict of Interest Disclosure

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

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