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Mitochondrial Genome Sequencing in Marine Bivalves: Progress, Applications, Challenges and Future Directions

(Penjujukan Genom Mitokondria dalam Bivalvia Marin: Kemajuan, Aplikasi, Cabaran dan Hala Tuju Masa Depan)

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ABSTRACT

Mitochondrial genome sequencing has become a vital tool for understanding marine bivalve evolution, genetics, and adaptation. This review highlights advances from Sanger to next- and third-generation sequencing, which have improved the accuracy and efficiency of mitogenome studies. These developments have uncovered unique features such as doubly uniparental inheritance (DUI) and extensive gene rearrangements, deepening insights into bivalve evolution, phylogenetics, conservation, and aquaculture. Mitogenomics aids in species identification, population analysis, and selective breeding for traits like disease resistance. Despite challenges such as complex architectures, annotation gaps, and unusual inheritance like DUI, emerging technologies such as single-cell sequencing, CRISPR, and omics integration offer new opportunities. As data sharing and collaboration expand, mitochondrial genomics will continue shaping marine conservation and sustainable aquaculture.

Keywords: Aquaculture; bivalvia; conservation genetics; mitochondrial genome sequencing; phylogenetics

ABSTRAK

Penjujukan genom mitokondrion telah menjadi alat penting dalam memahami evolusi, genetik dan penyesuaian bivalvia marin. Ulasan ini mengetengahkan kemajuan daripada kaedah Sanger kepada penjujukan generasi baharu dan generasi ketiga yang telah meningkatkan ketepatan serta kecekapan kajian mitogenom. Perkembangan ini telah mendedahkan ciri unik seperti pewarisan seinduk berganda (DUI) dan penyusunan semula gen yang ketara, sekali gus memperkukuh pemahaman tentang evolusi bivalvia, filogenetik, pemuliharaan dan akuakultur. Mitogenom membantu dalam pengecaman spesies, analisis struktur populasi dan pembiakan terpilih bagi ciri seperti ketahanan penyakit. Meskipun berdepan cabaran berkaitan kerumitan seni bina genom, jurang anotasi dan corak pewarisan luar biasa seperti DUI, kemunculan teknologi baharu seperti penjujukan sel tunggal, CRISPR dan integrasi pelbagai omik menawarkan peluang baharu. Dengan perluasan inisiatif perkongsian data dan kerjasama penyelidikan, genom mitokondrion dijangka terus memacu kemajuan dalam pemuliharaan marin dan amalan akuakultur yang mampan.

Kata kunci: Akuakultur; bivalvia; filogenetik; pemuliharaan genetik; penjujukan genom mitokondria

INTRODUCTION

Marine bivalves, including clams, oysters, mussels, and scallops are ecologically and economically significant molluscs. As filter feeders, they support nutrient cycling, enhance water quality, and act as habitat engineers that promote marine biodiversity (Theuerkauf et al. 2021; Vaughn & Hoellein 2018). Their ability to bioaccumulate substances makes them valuable environmental bioindicators (Vaughn & Hoellein 2018). Economically, bivalves are important in aquaculture and fisheries, contributing food, building materials, and jewellery, with the shells market alone estimated at $5.2 billion, while their nutrient remediation services are worth $1.2 billion annually (Olivier et al. 2018). However, their productivity is increasingly threatened by climate change through changes in sea temperatures and environmental stressors (Masanja et al. 2023; Steeves et al. 2018; Welton et al. 2024).

Understanding bivalve genetics heavily relies on mitochondrial DNA (mtDNA), a maternally inherited, non-recombining molecule with a high mutation rate, making it a powerful tool for studying population structure, evolutionary history, and maternal lineages (Pakendorf & Stoneking 2005). Despite its small size, mtDNA encodes important genes involved in cellular respiration and protein synthesis (Ferreira & Rodriguez 2024), and its simple

structure aids genetic analysis. However, challenges such as heteroplasmy, nuclear inserts, and selection pressures can complicate analyses, requiring careful interpretation of mtDNA data (Ferreira & Rodriguez 2024; Pakendorf & Stoneking 2005).

In bivalves, mitochondrial genome sequencing shows unique evolutionary traits, including variable gene order and adaptive genomic features, such as AT-rich regions and tandem repeats (Feng et al. 2021; Serb & Lydeard 2003). These variations aid adaptation to extreme environments and provide insights into evolutionary relationships and taxonomy (Ozawa et al. 2017; Plazzi, Puccio & Passamonti 2016). In recent decades, research on mitochondrial genome sequencing in marine bivalves has grown steadily, reflecting advances in sequencing technologies and growing interest in their ecological and economic significance. Figure 1 shows the increasing number of publications published on bivalve mitochondrial genomes between 2015 and 2024, highlighting this trend. Given the growing interest in mitochondrial genomics, this review explores recent advancements in mitochondrial genome sequencing technologies, their applications, challenges and future directions in marine bivalves.

DIVING INTO THE MITOCHONDRIAL GENOME OF MARINE BIVALVES

Bivalve mitochondrial genomes have various distinct features that set them apart from other metazoans. Notably, unlike most animals with strictly maternal mitochondrial inheritance, some bivalves exhibit doubly uniparental inheritance (DUI), a system where males and females transmit separate mitochondrial lineages via sperm and eggs, respectively (Figure 2). This mechanism has been documented in species from the orders *Mytiloida*, *Veneroida*, and *Unionoida* (Capt et al. 2020; Smith et al. 2023). Under DUI, males pass a unique M-type to their sons, while females pass the F-type to all offspring resulting in substantial genome size differences and sequence divergence between mitotypes, which can often surpass 50% (Capt et al. 2020; Wang, Li & Qi 2022). Furthermore, bivalve mitochondrial genomes frequently lack the *atp8* gene, which is otherwise commonly present in most other metazoans (Li et al. 2022).

Bivalve mitochondrial structure and gene organization also vary remarkably. For instance, rock scallops (Spondylidae) exhibit large gene rearrangements compared to their relatives in Pectinidae, while Teredinidae maintain conserved gene order despite high amino acid substitution rates (Li et al. 2023). Elements such as tandem repeats and AT-rich areas in the control region have a role in regulating replication and transcription, allowing for adaptability to extreme conditions like the deep sea (Yang et al. 2019). These characteristics reflect the evolutionary plasticity and ecological diversity of bivalve mitochondrial genomes.

Bivalves also encounter several genomic challenges, including heteroplasmy, repetitive regions, and overall structural complexity. Heteroplasmy, the presence of multiple mitochondrial genomes within an individual, is especially prevalent in DUI species, where different inheritance paths lead to significant amino acid divergence

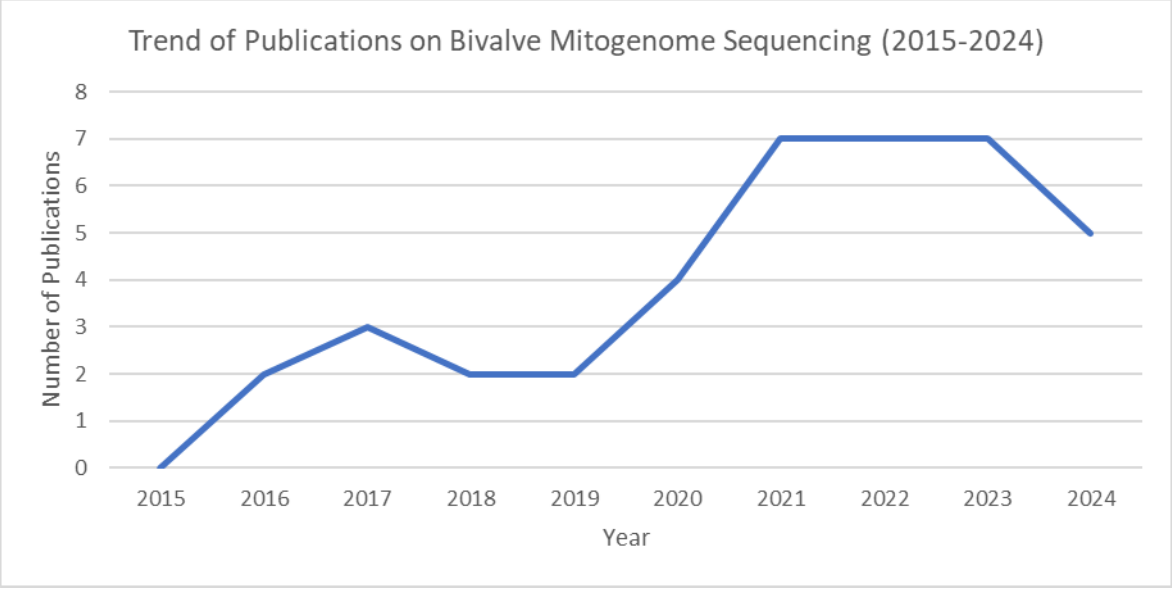

FIGURE 1. The trend of publications on bivalve mitochondrial genome sequencing from 2015 to 2024. The data highlights a steady increase in research output, peaking in 2021-2023, reflecting advancements in sequencing technologies and a rising interest in bivalve mitogenomic

in mitochondrial proteins (Le Cam et al. 2023; Lubośny et al. 2020). This complicates studies of mitochondrial biology and may interfere with species delimitation. For instance, in the *Aequiyoldia eightsii* species complex, amplification bias and mitochondrial heteroplasmy can mislead species identification (Martínez et al. 2023).

Structural complexity is further exacerbated by repetitive DNA. In the Antarctic bivalve, *Adamussium colbecki*, a large proportion of the genome comprises transposable elements and satellite DNAs, influencing genome evolution (Biscotti, Barucca & Canapa 2018). Similarly, the Pacific oyster, *Magallana gigas* (formerly *Crassotrea gigas*) has a genome rich in repetitive elements and structural variations, contributing to its genetic diversity and environmental adaptability (Qi, Li & Zhang 2021). These features complicate genome assembly and annotation, hindering a full understanding of bivalve genomic architecture (Gerdol et al. 2020; Smith 2021). Addressing these challenges requires continuous advancement in sequencing technologies and bioinformatic approaches.

RIDING THE GENOMIC WAVE: ADVANCEMENTS IN SEQUENCING TECHNOLOGIES

EARLY STUDIES USING SANGER SEQUENCING

The journey of mitochondrial genome began with the introduction of Sanger sequencing, also known as first-generation sequencing, pioneered by Frederick Sanger. This pioneering method enabled the accurate sequencing of entire genomes and remained the gold standard for over three decades due to its high precision in detecting single-nucleotide polymorphisms and small insertions or deletions (Arteche-López et al. 2021; Cheng, Fei & Xiao 2023; Hu et al. 2021). Despite its reliability, Sanger sequencing presents several limitations in terms of speed, throughput, and cost-efficiency. The process was labour-intensive and time-consuming, making it challenging to sequence large sample sets or complete mitochondrial genomes efficiently (Legati et al. 2021; Pareek et al. 2011). Moreover, this method typically generates relatively short read lengths, approximately 500 to 1,000 base pairs (bp), which restricts its utility in assembling complex genomes (Midha, Wu & Chiu 2019). Its performance also declined with degraded DNA samples, a common issue in research involving archival or ancient specimens (Timmermans et al. 2016).

TRANSITION TO NEXT-GENERATION SEQUENCING (NGS) TECHNOLOGIES

The emergence of next-generation sequencing (NGS) technologies marked a pivotal advancement in the field of genomics. Compared to Sanger sequencing, NGS technologies offer high-throughput, greater cost-efficiency, and enhanced accuracy. These advances have made it feasible to rapidly sequence complete mitochondrial genomes, a task that was once labour-intensive and expensive (Abicht et al. 2018; Harvey et al. 2019). These platforms enable simultaneous sequencing of multiple samples, thereby increasing efficiency and reducing per-sample costs (Harvey et al. 2019; Holt et al. 2021).

Among the most widely used NGS platforms, Illumina stands out for its short-read sequencing capabilities, generating millions of reads less than 300 nucleotides in length (Wick 2019). This high-throughput capacity makes it particularly well-suited for comprehensive genomic analyses (Harvey et al. 2019; Song, Yan & Li 2022). The development of NGS technologies has greatly advanced

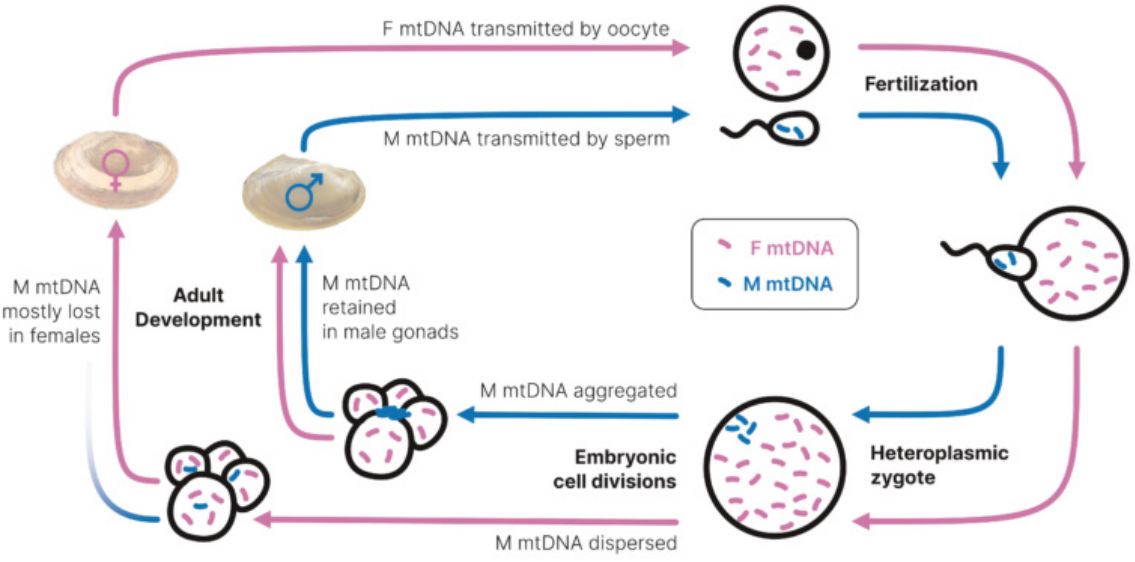

FIGURE 2. The generalisation of DUI of mitochondria in bivalves (Smith et al. 2023)

mitochondrial genome research by enabling the rapid and cost-effective sequencing of entire mitochondrial genomes (Hu et al. 2021; Kumar, Cowley & Davis 2019). Furthermore, NGS has improved the detection and characterization of mitochondrial heteroplasmy and large deletions, offering greater accuracy than traditional sequencing approaches (Legati et al. 2021). However, it has limitations in resolving complex genomic regions because of its shorter read length (Calus, Ijaz & Pinto 2018).

EMERGENCE OF THIRD-GENERATION SEQUENCING METHODS

Third-generation, or long-read sequencing, represents a significant progression in genomic technologies, offering significant advantages for resolving complex mitochondrial genomes. Unlike earlier sequencing technologies, recent innovations, particularly those from Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT), offer long reads that can cover tens to thousands of kilobases. This capability proves to be advantageous for sequencing areas with complex structures, like the long non-coding and tandem-repetitive regions found in mitochondrial DNA (Kinkar et al. 2021; Midha, Wu & Chiu 2019).

A key benefit of long-read sequencing is the capacity to overcome the limitations of short-read technologies, especially in accurately resolving repetitive elements and detecting major genomic rearrangements. These platforms yield more contiguous and accurate assemblies, which are essential for reconstructing the full structure of mitochondrial genomes (Jung et al. 2019; Kraft & Kurth 2020; Kumar, Cowley & Davis 2019). For instance, ONT technology has enabled the complete characterization of mitochondrial non-coding regions, showing structural features that were previously difficult to resolve (Kinkar et al. 2021).

Furthermore, third-generation sequencing can detect epigenetic modifications and sequence full-length transcripts without requiring assembly, offering deeper insight into genome biology and function (Athanasopoulou et al. 2022; Logsdon, Vollger & Eichler 2020). These capabilities are especially advantageous for mitochondrial genome research, as they simplify the assembly process and reduce the error rates associated with short-read sequencing (Kumar, Cowley & Davis 2019; Pareek et al. 2011). As a result, long-read sequencing has become an essential tool for exploring the complexity, functionality, and evolutionary dynamics of mitochondrial genomes (Fukasawa et al. 2020; Kinkar et al. 2021; Midha, Wu & Chiu 2019). In the context of bivalve research, the selection of sequencing technology should be guided by its suitability for mitochondrial genome assembly (Table 1).

TABLE 1. A comparative overview of the most widely used sequencing technologies and their suitability for mitochondrial genome assembly in bivalves

Sequencing technology	Read length	Accuracy	Cost	Best use case	References
Sanger	Short (800-1,000 bp)	High	High	Small-scale, targeted sequencing	Runnel et al. 2022; Schloss et al. 2016
Illumina	Short (< 300 bp)	High	Low	High-throughput, whole-genome sequencing	De Maio et al. 2019; Zhang et al. 2021
PacBio	Long (> 10,000 bp)	High	Medium to high	De novo genome assembly, structural variation in bivalves	Runnel et al. 2022, Weirather et al. 2017; Ferrarini et al. 2013
ONT	Long (> 10,000 bp)	Low, can be improved through post-sequencing corrections	Low	Real-time, portable sequencing	Weirather et al. 2017; De Maio et al. 2019; Volden et al. 2018

GENOMES BENEATH THE WAVES: APPLICATIONS IN MARINE BIVALVES

PHYLOGENETICS AND EVOLUTION

Mitochondrial genome sequencing has significantly advanced our understanding of bivalve phylogenetics and evolutionary history. A comprehensive analysis of 100 mitochondrial genomes by Plazzi, Puccio and Passamonti (2016) showed clade-specific patterns, identifying both conserved domains and divergent genes such as *atp6*, *nad2*, *nad4L,* and *nad6*. Their findings also demonstrated a correlation between genome rearrangement and evolutionary rates, outlining three major evolutionary phases, which are the origin of bivalves, the branching of palaeoheterodonts, and a second radiation that led to today's biodiversity.

In the Veneroida order, sequencing efforts have refined phylogenetic relationships in the genus *Donax*, aiding species identification and aquaculture efforts (Fernández-Pérez et al. 2017). Similarly, work on Tridacninae clams has clarified relationships among *Tridacna* species, uncovering distinct evolutionary paths and laying groundwork for future taxonomic and conservation studies (Tan et al. 2021).

Mitochondrial genomes also offer insight into deep-sea lineages such as *Bathymodiolus* mussels and Vesicomyid clams, showing evolutionary adaptations to extreme environments (Ozawa et al. 2017; Yang et al. 2019). Within Mytilidae, mitogenomic analyses have elucidated subfamily relationships and lineage diversification (Gaitán-Espitia et al. 2016; Lee et al. 2019).

POPULATION GENETICS

Mitochondrial DNA (mtDNA) markers, such as the cytochrome c oxidase subunit I (COI) gene, are widely used to assess population structure and genetic diversity in bivalves due to their maternal inheritance and high mutation rate. For instance, Jiang et al. (2024) used mtDNA markers to examine eight populations of *Nerita yoldii* along the Chinese coastline. The findings showed high haplotype but low nucleotide diversity, suggesting past bottlenecks and genetic differentiation influenced by ocean currents and gene flow dynamics.

Similarly, Hui et al. (2016) applied mitochondrial and microsatellite markers to study *Tridacna crocea* across the Indo-Malay Archipelago. Their analysis uncovered consistent population structures, with clear differentiation in the Java Sea, highlighting the utility of mtDNA in tracing maternal lineages, connectivity, and historical dispersal. These findings support the role of mtDNA in informing conservation strategies for marine bivalves.

CONSERVATION AND BIODIVERSITY

Mitochondrial DNA (mtDNA) plays a vital role in identifying endangered species and shaping conservation strategies by showing genetic diversity and population structure. Katsares et al. (2008) used COI and 16S rDNA markers to study the endangered *Pinna nobilis* across Greece, finding high haplotypic diversity but low population differentiation, likely due to passive larval dispersal. These insights inform protection strategies and highlight the potential for aquaculture and population management (Katsares et al. 2008; Petit-Marty, Vázquez-Luis & Hendriks 2020).

In *Lampsilis rafinesqueana*, a freshwater mussel, mtDNA and RAD sequencing showed population divergence shaped by historical biogeographic events, such as glaciation and river system shifts (Hein, Farleigh & Berg 2024). This genetic structure helps define conservation units and manage inbreeding or outbreeding risks, supporting more effective recovery plans. Overall, mtDNA sequencing offers a powerful tool for monitoring biodiversity and guiding species conservation.

AQUACULTURE AND BIOTECHNOLOGY

Mitochondrial DNA (mtDNA) markers play an important role in bivalve aquaculture because they are usually inherited maternally and do not recombine, making them reliable for tracing broodstock lineages and reducing the risk of inbreeding (Harrison 1989; Liu & Cordes 2004). In oysters and related species, the unusual system of doubly uniparental inheritance (DUI) offers opportunities to study both lineages but also requiring careful marker choice (Fernández-Pérez et al. 2018; Iannello et al. 2021).

Beyond lineage tracing, mtDNA genes such as 16S rDNA and cytochrome b are widely used to assess population structure, genetic diversity, and demographic history in bivalves (Fernández-Pérez et al. 2018; Lee et al. 2021). These data help identify distinct stocks for conservation and management. Although evidence directly linking mtDNA haplotypes to stress or disease resiliencies still limited, mitochondrial variation is increasingly recognized as a useful indicator of environmental adaptation (Wu, Sainz & Shadel 2021). In this way, mtDNA complements nuclear genomic approaches like genotyping-by-sequencing (GBS) and genome-wide association studies (GWAS), which remain central for identifying complex traits, by adding insights into maternal lineages, population structure, and adaptive responses.

DRIFTING IN UNCERTAINTY: CHALLENGES IN MITOCHONDRIAL GENOMICS

Mitochondrial genome sequencing in marine bivalves poses both general and lineage-specific challenges. On the universal side, the high abundance of repetitive elements, sequencing biases, and annotation errors remain major obstacles in genome assembly and curation (Smith 2021; Tørresen et al. 2019). These issues are compounded by inconsistencies across sequencing platforms and bioinformatic pipelines, which can lead to misidentified genes and database errors that skew downstream analyses (Celaj et al. 2014; Raghavan et al. 2022; Salzberg 2019).

In bivalves, however, additional mitochondrial-specific complexities arise. These include heteroplasmy and doubly uniparental inheritance (DUI), ambiguous start/stop codon, and potential nuclear gene transfers, all of which hinder accurate annotation and require careful manual curation (Klirs et al. 2024; Lang et al. 2023; Lucentini et al. 2020). The lack of standardised workflows and limited availability of high-quality reference genomes further amplify these problems, underscoring the need for unified protocols to improve reproducibility and comparability in bivalve mitochondrial genomics (Baeza, Minish & Michael 2024; Raghavan et al. 2022).

ANCHORING THE FUTURE: THE NEXT WAVE IN MITOCHONDRIAL GENOMICS

EMERGING TECHNOLOGIES

The advancement of technologies such as single-cell sequencing and CRISPR-based tools is poised to transform mitochondrial genomics for marine bivalves. Single-cell sequencing allows for the high-resolution exploration of mitochondrial genome heterogeneity within individual cells, offering new insights into complex inheritance systems like doubly uniparental inheritance (DUI) (Breton et al. 2009; Le Cam et al. 2023). Meanwhile, CRISPR-based technologies open pathways for targeted editing and functional analysis of mitochondrial genes, potentially showing their roles in adaptation and evolution, particularly in extreme environments like the deep sea (Yang et al. 2019).

INTEGRATION WITH OTHER OMICS

Integrating mitochondrial genomics with transcriptomics, proteomics, and metabolomics offers a holistic view of marine bivalve biology. This multi-omics approach can show how mitochondrial function is linked to gene expression, cellular metabolism, and environmental adaptation. For instance, such integration has helped clarify the role of mitochondria in energy metabolism and deep-sea adaptation in vesicomyid bivalves (Yang et al. 2019). It also deepens our understanding of mitochondrial genome evolution across bivalve species (Plazzi, Puccio & Passamonti 2016).

DATA SHARING AND COLLABORATION

Progress in mitochondrial genomes of marine bivalves heavily relies on open-access databases and collaborative efforts. Public repositories of mitogenome sequences enhance comparative and phylogenetic studies, as shown in frameworks for various bivalve families (Lee et al. 2019; Li et al. 2022). Collaboration accelerates discoveries by facilitating data exchange, which is especially vital for understanding complex systems like DUI that require broad species and population data (Stewart et al. 2021).

CONCLUSIONS

Recent advancements in mitochondrial genome sequencing have significantly enhanced our understanding of marine bivalve evolution, genetics, and adaptation. High-throughput technologies have showed unique inheritance patterns and genome structures, supporting efforts in taxonomy, conservation, and aquaculture. These insights aid species identification, genetic diversity assessment, and selective breeding. Looking ahead, emerging tools such as single-cell sequencing, CRISPR, and integrative omics are expected to deepen our understanding of mitochondrial function. As collaborative research and data sharing expand, mitochondrial genomics will continue to shape the future of marine conservation and sustainable aquaculture.

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