Electronic Journal of Biotechnology 76 (2025) 39–48

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage: www.elsevier.com/locate/ejbt

Review article

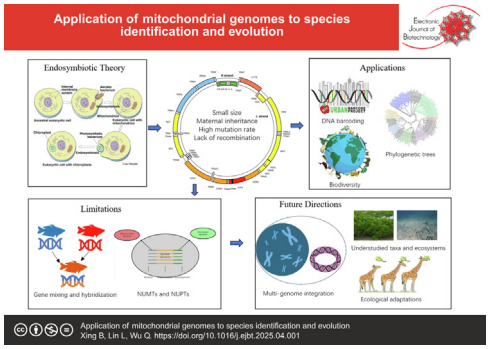
Application of mitochondrial genomes to species identification and evolution ☆

Bingpeng Xing [a,b,*], Liangyu Lin [c], Qiong Wu [a,d,*]

[a] Third Institute of Oceanography Ministry of Natural Resources, Fujian, China
[b] Observation and Research Station of Coastal Wetland Ecosystem in Beibu Gulf, Ministry of Natural Resources, Beihai, China
[c] Marine Academy of Zhejiang Province, Zhejiang, China
[d] Beijing Normal University, Beijing, China

GRAPHICAL ABSTRACT

Application of mitochondrial genomes to species identification and evolution.



ARTICLE INFO

Article history:
Received 1 October 2024
Accepted 3 April 2025
Available online 2 June 2025

Keywords:
Evolution
Evolutionary patterns
Maternal inheritance
Mitochondrial genomes
NUMTs
Phylogenetic relationships
Species classification
Species divergence

ABSTRACT

Mitochondrial genomes (mtDNA) have become invaluable in species classification and evolutionary studies due to their unique characteristics, including maternal inheritance, and high mutation rates. This review examines the application of mtDNA in tracing evolutionary history, elucidating phylogenetic relationships, and understanding mechanisms of species divergence. The evolution of mitochondrial DNA research from its initial focus on energy metabolism to its current role in biodiversity assessments highlights its significance in modern biology. Mitochondrial DNA barcoding, particularly utilizing the cytochrome *c* oxidase I (COI) gene, has revolutionized species identification, enabling rapid and accurate classification across diverse taxa. The article further explores the implications of mtDNA in understanding adaptive evolution, as genetic variations within mitochondrial genomes can reveal insights into how species respond to environmental pressures. However, challenges such as gene mixing, hybridization, and incomplete lineage sorting can complicate interpretations of mtDNA data. Thus, integrating mitochondrial with nuclear genome data is advocated to provide a comprehensive view of species relationships and evolutionary patterns. Future research directions emphasize the need for multi-genome studies,

Abbreviations: COI, Cytochrome *c* oxidase I; eDNA, Environmental DNA; EGT, Endosymbiotic gene transfer; ESUs, Evolutionarily Significant Units; mtDNA, Mitochondrial genomes; NUMTs, Nuclear mitochondrial DNA segments; NUPTs, Nuclear plastid DNA segments; rRNA, Ribosomal RNA; TIM, Translocase of the inner membrane; TOM, Translocase of the outer membrane; tRNA, Transfer RNA.

☆ Audio abstract available in Supplementary material.

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso

* Corresponding authors.
E-mail addresses: xingbpeng@gmail.com (B. Xing), wuqiong1127@gmail.com (Q. Wu).

https://doi.org/10.1016/j.ejbt.2025.04.001

investigations into ecological adaptations, and exploration of understudied taxa and ecosystems, which are crucial for enhancing our understanding of biodiversity and informing conservation strategies.

How to cite: Xing B, Lin L, Wu Q. Application of mitochondrial genomes to species identification and evolution. Electron J Biotechnol 2025;76. https://doi.org/10.1016/j.ejbt.2025.04.001.

Contents

1. Introduction

As an essential genetic material within cells, the mitochondrial genome (mtDNA) has found broad applications in eukaryotic species classification and evolutionary studies in recent years [1,2]. The structural and functional characteristics of mitochondrial DNA make it an ideal tool for investigating biological evolution and systematics [3]. Due to its relatively small size, high mutation rate, maternal inheritance, and lack of recombination, researchers can utilize mtDNA to trace species' evolutionary history, reveal phylogenetic relationships between species, and infer the timing and mechanisms of species divergence [4,5]. However, one limitation of mtDNA is that its usefulness in broader phylogenetic relationships (e.g., higher taxonomic levels such as orders or classes) can be limited, especially due to phenomena like substitution saturation, which may cause incorrect tree placements [6]. Research on mitochondrial genomes began in the 1960s, initially focusing on its role in energy metabolism [7]. With advances in molecular biology techniques, mtDNA gradually became a pivotal tool in evolutionary biology. Early studies demonstrated that mtDNA provides crucial genetic information that aids in understanding phylogenetic relationships among species [8,9]. During the 1980s and 1990s, the applications of mtDNA expanded significantly, particularly in species identification and taxonomic research, where mitochondrial genomes were widely used to analyze biodiversity and explore mechanisms of speciation [10]. In recent years, with the rapid development of high-throughput sequencing technologies, mitochondrial genome research has entered a new phase [11]. Researchers can now acquire large amounts of mtDNA data in a short time, allowing for large-scale phylogenetic analyses and species classification. This technological progress not only has improved the efficiency of obtaining mtDNA data but also has enabled more systematic and comprehensive studies on genetic variation and species adaptation [1]. The high mutation rate of mtDNA is particularly effective in revealing recent evolutionary events and population dynamics, providing strong support for understanding the historical evolution of species [12].

The application of mitochondrial genomes in species classification has demonstrated significant advantages, especially in complex and diverse biological groups [13]. Using mtDNA barcoding technology, researchers can rapidly and accurately identify species and construct phylogenetic trees. This technique has become a standard method in studies on highly diverse groups such as insects, fish, and birds [14,15,16,17,18]. By comparing specific regions of the mitochondrial genome, scientists can identify genetic differences between species, effectively addressing ambiguities and uncertainties in traditional classification methods. Furthermore, mtDNA has shown unique potential in studying adaptive evolution and ecological adaptation between species [19]. By analyzing the mitochondrial genomes of species in specific environments, researchers can uncover genetic variations associated with adaptive changes. These studies not only help us understand how species adapt to environmental pressures through genetic changes but also reveal the role of selective pressure in the evolutionary process. Recent studies have shown that mitochondrial genome analysis in understudied taxa such as nematodes can

uncover astonishing variations in mutation rates and adaptive strategies [20,21]. This highlights the potential for mitochondrial genomic research to reveal hidden biodiversity in lesser-explored ecosystems. Although mitochondrial genomes hold substantial value in species classification and evolutionary studies, their application is not without challenges. These include gene mixing, hybridization, and incomplete lineage sorting, which can lead to misunderstandings regarding species relationships [22]. Therefore, integrating mitochondrial genome data with nuclear genome data provides a more comprehensive view of species relationships, reducing the biases introduced by mtDNA and improving the accuracy of phylogenetic analysis [23].

As research methods and technologies continue to evolve, the application of mtDNA will further expand, offering new perspectives for understanding biodiversity, species formation, and evolutionary history. This review systematically analyzes the application of mitochondrial genomes in species classification and evolutionary studies, explores their advantages and limitations, and forecasts future research directions. Through this exploration, we aim to provide biologists with valuable references, promoting further development in this field.

2. Structure and evolutionary insights of the mitochondrial genome

2.1. Structure and characteristics of the mitochondrial genome

Mitochondrial DNA (mtDNA) is a vital genetic material found in the mitochondria of eukaryotic cells, primarily known for its key role in energy production through oxidative phosphorylation [24]. The fundamental structure of the mitochondrial genome is circular, a feature that it shares with the genomes of many prokaryotes. Compared to the linear chromosomes of nuclear DNA, the circular structure offers several advantages in terms of stability and replication efficiency [25]. In most animals, the mitochondrial genome typically ranges from 15,000 to 20,000 base pairs, significantly smaller than the nuclear genome. A typical animal mitochondrial genome contains 37 genes, which can be divided into three categories: protein-coding genes, transfer RNA (tRNA) genes, and ribosomal RNA (rRNA) genes (Fig. 1) [26]. Specifically, the mitochondrial genome encodes 13 protein-coding genes, which are essential for the electron transport chain and ATP synthesis. These genes include subunits of various enzyme complexes involved in oxidative phosphorylation, such as ATP synthase, cytochrome *c* oxidase, and NADH dehydrogenase. The small and efficient coding structure of the mitochondrial genome reflects its adaptation to specific metabolic functions following endosymbiotic integration [27].

In addition to the protein-coding genes, the mitochondrial genome includes 22 tRNA genes, which are crucial for translating mitochondrial mRNA into proteins. tRNA molecules are responsible for transporting specific amino acids to the ribosome, facilitating the assembly of mitochondrial proteins during protein synthesis. The mitochondrial genome also encodes two rRNA genes (16S and 12S rRNA), which are integral components of the mitochondrial ribosome and play a critical role in the translation of mitochondrial mRNA [28,29]. Another key component of the mitochondrial genome is the control region, often referred to as the D-

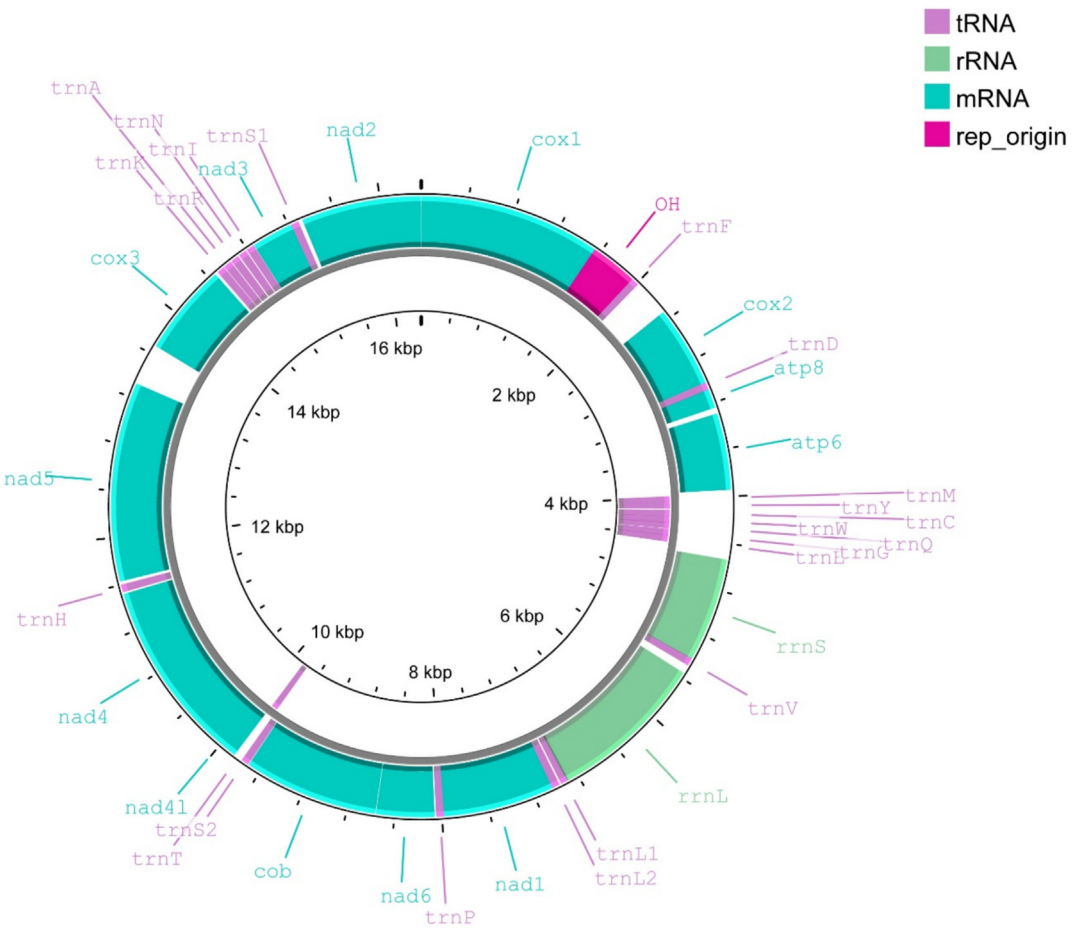


Fig. 1. Structure of a typical mitochondrial genome.

loop. This non-coding region plays a vital role in regulating the replication and transcription of mitochondrial DNA [7]. The control region contains essential elements for replication and transcription initiation, including promoters and termination signals [30]. The presence of conserved sequences within the control region allows the binding of specific transcription factors and the recruitment of mitochondrial RNA polymerase, ensuring the proper expression of mitochondrial genes [31]. The D-loop region is also notable for its high variability, which provides advantages in evolutionary studies. Variation in the control region offers insights into population genetics, species divergence, and evolutionary history. Analyzing the genetic diversity of the control region enables researchers to investigate lineage-related transitions and historical processes [32].

2.2. The endosymbiotic theory and its implications for mitochondrial genomes

The endosymbiotic theory provides a foundational framework for understanding the origin of mitochondrial genomes and their role in eukaryotic evolution [33]. Initially proposed by Russian botanist Constantin Mereschkowsky in 1905 and later expanded by Lynn Margulis in the 1960s, this theory posits that mitochondria originated from free-living proteobacteria that were engulfed by a host cell, leading to a mutually beneficial relationship [34]. Over time, the engulfed bacteria evolved into mitochondria, which became indispensable for the host cell's bioenergetic processes [35]. Key evidence supporting this theory comes from the structural and genetic similarities between mitochondria and their prokaryotic ancestors [36]. Mitochondria possess a double membrane, circular DNA, and ribosomes similar to those found in proteobacteria, supporting their endosymbiotic origin [33,36]. Moreover, endosymbiotic gene transfer has played a pivotal role in mitochondrial evolution [37]. During this process, many genes originally present in the mitochondrial genome were transferred to the nuclear genome, resulting in the streamlined mitochondrial genomes today [33,37]. Recent advancements in molecular phylogenetics have provided further insights into the endosymbiotic origin of mitochondria [38]. Comparative genomic analyses have identified homologous genes between mitochondrial and α-proteobacterial genomes, strengthening the hypothesis that mitochondria evolved from an ancestral α-proteobacterium [39]. Moreover, discovering intermediary forms, such as the Rickettsiales group of bacteria, highlights potential evolutionary links between extant proteobacteria and mitochondria [40]. Experimental studies on protein import machinery, such as the translocase of the outer membrane (TOM) and inner membrane (TIM), reveal highly conserved mechanisms, suggesting a shared evolutionary origin. These systems were likely adapted from the endosymbiont's original protein transport machinery to enable communication and metabolic integration with the host cell [41]. The incorporation of proteobacteria as mitochondria allowed early eukaryotic cells to utilize oxygen more efficiently for energy production, giving them a competitive advantage in an increasingly oxygenated environment. This bioenergetic innovation supported the development of larger and more complex cells, enabling processes such as phagocytosis, intracellular transport, and genome expansion [42]. Additionally, mitochondria are involved in essential cellular functions beyond energy metabolism, including apoptosis, calcium signaling, and the synthesis of key metabolites such as iron-sulfur clusters, underscoring their multifaceted role in eukaryotic life [43].

The endosymbiotic theory has broad implications for understanding the evolution and diversification of eukaryotes [33,36,37]. The acquisition of mitochondria not only provided the bioenergetic foundation for eukaryotic complexity but also likely influenced the diversification of eukaryotic lineages. Studies on secondary and tertiary endosymbiosis in algae further demonstrate how similar processes have contributed to the evolution of plastids and other organelles [44]. These events highlight the recurring role of endosymbiosis as a driving force in evolutionary innovation and underscore its importance in the evolutionary history of life on Earth [27]. As research techniques continue to advance, novel approaches such as single-cell genomics, high-resolution imaging, and machine learning algorithms are expected to uncover additional details about the endosymbiotic process [45]. These methods may help identify previously unknown intermediate stages in the evolution of mitochondria and further elucidate the genetic and biochemical pathways involved in their integration into the host cell. By analyzing mitochondrial genomes, researchers can trace evolutionary lineages, uncover the timing of endosymbiotic events, and explore the functional adaptations that have enabled mitochondria to thrive within eukaryotic cells [46]. The integration of this evolutionary perspective into mitochondrial genome studies not only enhances our understanding of the origins and functions of mitochondria but also provides broader insights into the evolutionary dynamics of eukaryotic cells.

3. Applications of mitochondrial DNA

3.1. Mitochondrial DNA in species identification and biodiversity conservation

In addition to its role in energy production, mtDNA serves as a crucial genetic marker in phylogenetic studies [12]. Long-term research has revealed several unique features of mtDNA that distinguish it from nuclear DNA, making it particularly valuable for evolutionary studies. One of the most prominent characteristics of mtDNA is maternal inheritance, where DNA is passed down exclusively from the mother to the offspring [47]. This feature enables researchers to trace maternal lineages and study genetic relationships between individuals within populations. Since the mitochondrial genome provides valuable insights into historical biogeography and adaptive evolution, mtDNA is commonly used to study speciation events, population structure, and the impact of environmental factors on genetic diversity. Another key characteristic of mtDNA is its relatively high mutation rate [48]. This rapid accumulation of mutations allows researchers to investigate recent evolutionary events and assess genetic diversity among closely related species. The high mutation rate makes mtDNA especially useful for studying population structure and speciation processes, particularly in species with shorter generation times [49]. Furthermore, unlike nuclear DNA, mitochondrial DNA does not undergo recombination. The absence of recombination ensures that mtDNA maintains a consistent genetic lineage across generations, providing a clear genetic marker for tracing ancestry and evolutionary relationships [5].

Maternal inheritance, high mutation rate, and lack of recombination make mtDNA an ideal candidate for phylogenetic analysis and species identification [5,50]. The mitochondrial cytochrome *c* oxidase I (COI) gene has become the standard barcode gene for species classification and identification, playing a significant role in taxonomy, species identification, and biodiversity research [51,52]. It is widely used in species identification across various biological groups, from insects and fish to mammals and birds [51]. DNA barcoding based on the COI gene has revolutionized how we document biodiversity and identify species, particularly in groups that have not been extensively studied [14,51].

Moreover, mitochondrial DNA barcoding is not limited to species identification; it also plays a crucial role in tracking biodiversity changes, detecting invasive species, and studying evolutionary processes [8,51]. By creating comprehensive barcode libraries for

different taxonomic groups, researchers can generate extensive reference datasets that serve as benchmarks for future species identification efforts. Overall, mitochondrial DNA is not only a fundamental tool for species identification but also provides essential support for understanding evolutionary processes, monitoring biodiversity, and advancing conservation efforts [21,23]. As the technology continues to evolve, its integration with genomics, environmental DNA analysis, and other genomic methods will further expand its applications in biodiversity research and conservation management [53].

3.2. Application of mitochondrial genomes in phylogenetics

The mitochondrial genome has played a significant role in studies. Researchers can deeply investigate evolutionary relationships, species divergence, and adaptive mechanisms using mitochondrial genome data to construct phylogenetic trees [3,9,54,55]. This approach is particularly suitable for studying species that undergo significant changes over short periods, providing valuable information about the origin and evolutionary processes of species. In many studies, complete mitochondrial genome sequences have been used to build phylogenetic trees, helping to identify relationships between species [54,56,57]. For example, by analyzing the mitochondrial genomes of different fish species, researchers have uncovered the evolutionary history of species and their relationships across various groups [58]. This method provides insight into the relationships between species and helps identify new species and correct classification errors [50].

Mitochondrial genomes are also valuable in studying species divergence and evolutionary relationships. By comparing the mtDNA of different species, researchers can infer the timing and mechanisms of species divergence [59]. PCR amplification and sequencing techniques are used to obtain the mtDNA sequences of target species, typically including the COI gene and the control region (D-loop) [25,58]. Bioinformatics tools are then employed to compare the mtDNA sequences of different species and determine genetic differences. Researchers can construct phylogenetic trees based on these comparisons using methods such as maximum likelihood, Bayesian inference, or neighbor-joining. Through molecular clock theory, combined with the mutation rate of mtDNA, genetic differences can be translated into a time scale [48]. This process allows scientists to estimate the timing of species divergence and identify the environmental factors influencing these divergences.

3.3. Research on endosymbiotic gene transfer

The endosymbiotic theory proposes that mitochondria and chloroplasts originally originated from free-living bacteria, which were captured by host cells through an endosymbiotic event and have long coexisted with the host cells [36,37]. During this process, the initially free-living symbiotic bacteria formed a symbiotic relationship with the host cells, eventually evolving into modern mitochondria and chloroplasts. Over time, the vast majority of genes originally belonging to the symbiotic bacteria were transferred to the host's nuclear genome, a process known as endosymbiotic gene transfer (EGT) [37]. EGT is a crucial process in genomic evolution, significantly influencing the formation of biological diversity and species adaptation, and playing an essential role in the evolution of eukaryotes [60]. The size of mitochondrial and chloroplast genomes has significantly reduced, retaining only a few genes directly involved in energy metabolism or other key functions [61]. For example, the mitochondrial genome retains only about 13 genes, which encode key proteins involved in the electron transport chain and ATP synthesis. These gene products work closely with proteins encoded by the host nuclear genome to jointly maintain cellular oxidative phosphorylation functions, supporting the cell's energy needs [62]. In comparison, nearly all other genes of the mitochondria and chloroplasts have been transferred to the nuclear genome [60,63]. This transfer not only reduced the size of the organellar genomes but also enhanced the host's ability to regulate these genes and reduced the mutation burden on the mitochondrial genome, as mitochondria typically have a higher mutation rate [63].

The process of EGT led to the formation of nuclear mitochondrial DNA segments (NUMTs) and nuclear plastid DNA segments (NUPTs) [64]. NUMTs and NUPTs are gene fragments that have been transferred from the mitochondria and chloroplasts to the nuclear genome, with varying degrees of retention and degeneration within the nuclear genome [65]. With the discovery of NUMTs and NUPTs, researchers have gradually recognized the complex regulatory relationship between nuclear DNA and organellar DNA. During evolution, the mitochondrial and chloroplast genomes progressively shrank, and through EGT, the majority of genes were transferred to the nuclear genome [65]. This process not only altered the structure of the organellar genomes but also formed a close functional connection between the nuclear and organellar genomes. The functions of mitochondria and chloroplasts are no longer entirely controlled by their genomes but rely on genes transferred to the nuclear genome [63]. Both genomes cooperate through a regulatory relationship to maintain cellular energy metabolism and other physiological processes. In this collaborative regulatory relationship, the nuclear genome is responsible for regulating the expression of organellar genes and also managing the genetic material of the organelles. The mutation rate of organellar genomes is higher, whereas nuclear genomes typically have lower mutation rates, which makes the nuclear genome play a key role in coordinating and regulating the organellar genomes [66]. The insertion of NUMTs and NUPTs plays a bridging role in this regulatory network; they not only serve as carriers of genetic information exchange during evolution but also may regulate the expression of functional genes, maintaining the coordination between organelles and the nuclear genome within the cell [64,65].

The transfer of NUMTs and NUPTs is not only an important marker of genomic evolution but it also has a profound impact on the adaptive evolution of the host species [64]. First, NUMTs and NUPTs indicate long-term genetic exchange and co-evolution between the organellar and nuclear genomes [67]. This genetic exchange promotes the adaptive improvement of the host genome. For instance, NUMTs and NUPTs may enhance the host cell's ability to respond to environmental stress through gene recombination and regulation [68]. Species living in deep-sea or extreme environments may have sequences of NUMTs and NUPTs that have undergone adaptive variations to cope with high pressure, low oxygen, or extreme temperatures [55]. This adaptive variation is not limited to changes in gene structure but also includes functional changes, helping the host cell maintain coordination between the organelles and the nuclear genome in harsh environments [69]. Moreover, the presence of NUMTs and NUPTs can provide additional regulatory elements and genetic diversity, promoting the survival of host species in changing environments, and thereby fostering adaptive evolution. Studies have shown that certain insertion sequences of NUMTs may regulate the expression of nuclear genes, improving the functional coordination between organelles and the host, which plays a significant role in the long-term evolution of species [64,70].

4. Limitations of mitochondrial DNA

Despite its significant value in species classification and phylogenetic studies, mtDNA presents certain limitations. Key challenges include gene mixing, hybridization, and incomplete

lineage sorting, all of which may result in misinterpretation of species relationships.

4.1. Gene mixing and hybridization

Gene mixing and hybridization refer to the genetic exchange between different species [71]. These phenomena are particularly common among closely related species, and they often confuse mtDNA sequences, complicating the inference of species relationships. Such occurrences are typically associated with species' biogeographic distributions, reproductive behaviors, and overlapping ecological niches [72]. In many cases, species in overlapping areas may interbreed, leading to offspring with mixed genetic material. This genetic mixing can create a complex genetic background, making species identification via mtDNA more difficult [73]. For example, certain fish species, such as salmon and trout, frequently reproduce in the same waters, resulting in gene mixing between their genomes [74]. While this hybridization may increase mtDNA diversity, it also complicates the phylogenetic analysis of these species [75]. Similarly, gene mixing is common among amphibians, such as frogs and toads, which share similar environments and engage in hybridization [76]. The offspring of such events may exhibit both morphological and genetic traits from both parent species, further complicating species classification.

4.2. Incomplete lineage sorting

Incomplete lineage sorting occurs when certain portions of a genome fail to accurately reflect the true divergence of a species [77]. This lack of clear genetic differentiation, often caused by gene flow and genetic mixing due to overlapping ecological niches, can lead to misinterpretations in species classification and phylogenetic analysis [78]. In some cases, while mtDNA sequences may indicate close relatedness between species, the overall genetic structure might be influenced by various historical factors [79]. For instance, in some bird species, mtDNA sequences may reveal similar genetic markers. Still, significant differentiation is observed in nuclear genomes, suggesting that mtDNA may not fully capture the evolutionary history of species, particularly when gene flow and hybridization are present [80]. Moreover, mtDNA can provide robust phylogenetic signals when analyzing closely related species (e.g., genus or family level). Still, broader taxonomic relationships, such as higher orders, may not be as accurately resolved due to incomplete lineage sorting and saturation of mtDNA markers [20,21]. Incomplete lineage sorting may lead to erroneous classification results. In ecological studies, relying solely on mtDNA for species identification may overlook these complex genetic relationships, affecting our understanding of species diversity and ecosystem function. Therefore, recognizing the significance of incomplete lineage sorting is crucial for accurate biological classification and ecological conservation [81].

4.3. Substitution saturation

Substitution saturation is a critical limitation in mitochondrial phylogenetic studies, particularly when analyzing deeper branches or distant evolutionary relationships [82]. When mtDNA sequences accumulate too many mutations, they may become saturated, meaning further mutations no longer contribute meaningful information about phylogenetic relationships. This phenomenon is especially prevalent in the third codon positions of protein-coding genes, where synonymous substitutions often accumulate rapidly [20]. Recent studies demonstrate that third codon positions can lead to conflicting phylogenetic signals, especially in closely related species, and result in incorrect tree placements. This saturation can severely affect the accuracy of tree estimation, leading to misleading results if not properly accounted for [16]. In some cases, removing saturated sites or using degenerative methods to account for synonymous substitutions can help mitigate these issues.

4.4. The challenges of NUMTs and NUPTs

Although NUMTs and NUPTs provide valuable insights into genomic evolution and reveal the complex genetic exchanges between organellar genomes and the host nuclear genome, their presence also presents significant challenges in genomic analysis and phylogenetic studies [70]. The high similarity between NUMTs and mtDNA in species identification often leads to misinterpretations [83]. Specifically, in mitochondrial DNA barcoding techniques, NUMTs may be mistaken for mitochondrial genes, leading to incorrect species relationship inferences or overestimating biodiversity [84]. DNA barcoding for species classification typically relies on mitochondrial genes, particularly the COI gene, as the standard barcode gene. However, the similarity between NUMTs and mtDNA makes this method prone to interference in certain cases, especially when the sequences of NUMTs overlap extensively with mitochondrial genes [85]. In such instances, researchers may incorrectly classify NUMTs as genuine mtDNA sequences, resulting in misidentification of species [84].

In phylogenetic tree construction, the presence of NUMTs can also significantly affect the structure of the phylogenetic tree, leading to erroneous inferences about the phylogenetic relationships between species [86]. If the insertion of NUMTs is not effectively recognized, they can create false phylogenetic relationships between species, distorting the evolutionary history of the species [64]. For example, in deep phylogenetic analyses, failure to account for NUMTs can lead to structural similarities between the phylogenetic trees of multiple species, misguiding researchers in understanding the correct evolutionary relationships between species. This interference is especially problematic when dealing with highly diverse groups, potentially leading to overestimated species diversity and even the misidentification of non-existent species. In addition to species identification and phylogenetic studies, NUMTs also complicate population genetic analyses. When comparing mtDNA sequences across different populations to assess gene flow and genetic diversity, the presence of NUMTs can interfere with the analysis of the population [85].

5. Future research directions

5.1. Multi-genome integration research

Multi-genome integration research has become a significant trend in modern biology, particularly in phylogenetics and evolutionary biology [87]. Combining mtDNA with nuclear genome data provides a more comprehensive understanding of species' evolutionary history [88]. While mtDNA, with its maternal inheritance and high mutation rate, effectively reflects the divergence between closely related species, relying solely on mtDNA can overlook complex evolutionary patterns. The nuclear genome, as a combination of genetic information from both parents, offers a broader genetic background. Therefore, integrating these two genome types allows for a more holistic understanding of species evolution and ecological adaptation. Through multi-genome integration research, scientists can correct misleading conclusions derived from single-genome studies, particularly in gene flow or hybridization [89]. Nuclear genome data can help reveal complex phylogenetic relationships and trace the genetic exchange history between species [90]. For instance, closely related species may appear to have similar phylogenetic relationships based on mtDNA, but nuclear gen-

ome differences suggest they have undergone independent evolution for a significant time [91]. Furthermore, multi-genome integration enhances the identification of Evolutionarily Significant Units (ESUs), population units with distinct genetic traits [92,93]. ESUs are vital for species conservation strategies, as they represent populations with unique ecological functions or evolutionary potential [93]. Multi-genome approaches can also be applied to ecosystem and community-level studies, where integrating the genomes of different species within a community enables scientists to explore species interactions and adaptations to environmental changes more thoroughly [92].

In parallel, the functional study of NUMTs provides a new perspective for understanding the endosymbiotic events and the subsequent processes and mechanisms of gene transfer [60,70]. Although significant progress has been made in the research on NUMTs and NUPTs, their functions and roles remain important areas for future investigation [28,37]. Future studies should delve deeper into genomics and epigenetics to uncover the potential regulatory roles of NUMTs and NUPTs. In particular, it is essential to explore whether NUMTs play a crucial role in gene expression regulation and whether they contribute to coordinating interactions between the organelles and the nuclear genome [70]. Additionally, future research should focus on how NUMTs and NUPTs undergo adaptive variations under different environmental pressures, helping host species improve their ability to adapt to extreme environments. With the continued advancement of high-throughput sequencing technologies, researchers will be able to more efficiently detect and analyze the functions of NUMTs and NUPTs, further promoting the integration of genomics, phylogenetics, and evolutionary biology. Overall, multi-genome integration offers a more precise and comprehensive evolutionary perspective, especially for species classification, evolutionary patterns, and adaptive evolution studies [92].

5.2. Ecological adaptation studies

The mitochondrial genome plays a critical role in ecological adaptation, and future research must focus more on how mitochondrial genomes influence species adaptation in specific environments. Species often undergo genetic variations in response to environmental factors such as climate, food resources, and predator pressure [94]. These genetic variations are not only reflected in nuclear genomes but also in mitochondrial genomes. By studying mitochondrial genome expression patterns and mutations, researchers can uncover how species adapt genetically to different environments [50]. Since the mitochondrial genome is directly involved in energy metabolism, it is particularly important for responding to ecological pressures. For example, in extreme environments like high altitudes or polar regions, the mitochondrial genomes of certain species may undergo selective pressures that enable more efficient oxygen utilization or cold tolerance. By analyzing these genetic changes, scientists can better understand species adaptation mechanisms to environmental change. Furthermore, studying the impact of mitochondrial genomes on ecological adaptation can help predict how species will respond to future environmental changes [95].

5.3. Understudied taxa and ecosystems

Despite the broad applicability of mtDNA in species classification and evolutionary studies, many taxa and ecosystems remain underexplored, especially those with high biodiversity but limited genomic data. Complex environments such as mangroves, coral reefs, and deep-sea ecosystems are home to a variety of species, yet the mitochondrial genomic data for these groups are still sparse [96]. These ecosystems, which are critical for maintaining global biodiversity and ecosystem services, are often neglected in genomic studies. For example, mangrove ecosystems host a unique array of species that have adapted to brackish, high-salinity conditions [97]. Mitochondrial genome analysis could provide valuable insights into how these species have evolved to cope with such extreme environments [98]. Similarly, coral reefs, one of the most biologically diverse ecosystems, also harbor species whose mitochondrial genomes remain underexplored. These species have evolved specialized adaptations to survive in nutrient-poor, high-temperature conditions, and their mitochondrial genomics could reveal new mechanisms of adaptation. The deep-sea environment, which presents even more extreme conditions such as high pressure, low temperatures, and low oxygen, is another ecosystem that remains poorly studied in terms of mitochondrial genomics [55]. Species inhabiting the deep sea, such as certain fish, invertebrates, and cephalopods, have developed unique mitochondrial features to survive in these harsh conditions. For instance, some species show mitochondrial adaptations that enable efficient oxygen utilization in low-oxygen environments, a critical trait for survival at great depths [55,99]. However, due to the challenges of sample collection and the extreme nature of the deep sea, mitochondrial genomic data from these species are limited. The few studies that have been conducted suggest that deep-sea species possess mitochondrial genomes with unique mutations that allow them to thrive in such extreme environments. In addition to these ecosystems, tropical rainforests, which harbor a wide diversity of microfauna such as amphibians and insects, are also understudied in terms of mitochondrial genomics. Many rainforest species exhibit mitochondrial adaptations that support their survival in humid, fluctuating environments [100]. Understanding the mitochondrial genomes of these species could offer insights into their evolutionary history and how they have adapted to the constant environmental pressures of the rainforest.

In conclusion, while mitochondrial genomics has significantly contributed to our understanding of species evolution, many taxa and ecosystems remain underexplored. Expanding mitochondrial genome studies to include a broader range of species from understudied ecosystems such as mangroves, coral reefs, and the deep sea will provide valuable insights into how species adapt to extreme environments. As research technologies advance, particularly with the use of metagenomics and eDNA, the field of mitochondrial genomics will play an increasingly important role in uncovering hidden biodiversity and understanding the complex evolutionary processes that shape life on Earth.

6. Conclusions

Mitochondrial DNA (mtDNA) possesses unique structural characteristics—including maternal inheritance, a high mutation rate, and the absence of recombination—making it an indispensable tool for studying biological evolution, phylogeny, and species classification. Through the efficient use of mtDNA, researchers have been able to trace the evolutionary history of species, elucidate relationships between taxa, and estimate the timing and mechanisms of species divergence. Furthermore, mtDNA barcoding has revolutionized species identification, offering rapid and accurate classification across diverse biological groups. Despite its numerous advantages, mtDNA applications face notable challenges, such as gene mixing, hybridization, and incomplete lineage sorting, which can lead to misinterpretations of species relationships. Additionally, the presence of nuclear mitochondrial DNA segments (NUMTs) further complicates analyses by mimicking genuine mitochondrial sequences. To address these limitations, combining mitochondrial and nuclear genome data has emerged as a robust strategy for achieving more accurate phylogenetic

and biodiversity assessments. Future research should emphasize multi-genome integration to enhance the resolution of phylogenetic studies and facilitate the identification of Evolutionarily Significant Units (ESUs) critical for conservation strategies. Furthermore, a deeper investigation into the role of mitochondrial genomes in species adaptation, particularly under varying environmental pressures, will offer valuable insights into evolutionary mechanisms. Expanding mitochondrial genomics to include understudied taxa and ecosystems, such as mangroves, coral reefs, and deep-sea environments, is crucial for uncovering hidden biodiversity and understanding species adaptations to extreme conditions. Incorporating advanced methodologies, including mitochondrial metagenomics and environmental DNA (eDNA) analysis, will further strengthen the field's ability to assess ecological adaptations and evolutionary processes. Overall, mitochondrial genome research not only enhances our understanding of biodiversity and its conservation but also provides a foundational framework for managing ecosystems and mitigating the impacts of environmental changes.

CRediT authorship contribution statement

Bingpeng Xing: Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization. **Liangyu Lin:** Validation, Supervision, Formal analysis. **Qiong Wu:** Writing – review & editing.

Financial support

This work was supported by the Science & Technology Fundamental Resources Investigation Program (grant number 2023FY100804); the Scientific Research Foundation of Third Institute of Oceanography, MNR (grant number 2020017); Hainan Province's Key Research and Development Project (grant number ZDYF2023SHFZ172), Natural Science Foundation of Fujian Province, China (grant number 2023J011373).

Declaration of competing interest

The authors have no conflict of interest to declare.

Supplementary material

https://doi.org/10.1016/j.ejbt.2025.04.001.

Data availability

No data was used for the research described in the article.

References

[1] DeSalle R, Schierwater B, Hadrys H. MtDNA: The small workhorse of evolutionary studies. Front Biosci-Landmark 2017;22(5):873–87. https://doi.org/10.2741/4522. PMid: 27814652.
[2] Elyasigorji Z, Izadpanah M, Hadi F, et al. Mitochondrial genes as strong molecular markers for species identification. Nucleus 2023;66(1):81–93. https://doi.org/10.1007/s13237-022-00393-4.
[3] Hwang U-W, Kim W. General properties and phylogenetic utilities of nuclear ribosomal DNA and mitochondrial DNA commonly used in molecular systematics. Korean J Parasitol 1999;37(4):215–28. https://doi.org/10.3347/kjp.1999.37.4.215. PMid: 10634037.
[4] White DJ, Wolff JN, Pierson M, et al. Revealing the hidden complexities of mtDNA inheritance. Mol Ecol 2008;17(23):4925–42. https://doi.org/10.1111/j.1365-294X.2008.03982.x. PMid: 19120984.
[5] Ladoukakis ED, Zouros E. Evolution and inheritance of animal mitochondrial DNA: Rules and exceptions. J Biol Res Thessaloniki 2017;24:2. https://doi.org/10.1186/s40709-017-0060-4. PMid: 28164041.
[6] Rubinoff D, Holland BS. Between two extremes: Mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Syst Biol 2005;54(6):952–61. https://doi.org/10.1080/10635150500234674.
[7] Ernster L, Schatz G. Mitochondria: A historical review. J Cell Biol 1981;91(3):227s–55s. https://doi.org/10.1083/jcb.91.3.227s. PMid: 7033239.
[8] Patwardhan A, Ray S, Roy A. Molecular markers in phylogenetic studies-a review. J Phylogenetics Evol Biol 2014;2(2):131.
[9] Simon C, Buckley TR, Frati F, et al. Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. Annu Rev Ecol Evol Syst 2006;37(1):545–79. https://doi.org/10.1146/annurev.ecolsys.37.091305.110018.
[10] Antoniou A, Magoulas A. Application of mitochondrial DNA in stock identification. In: Cadrin SX, Kerr LA, Mariani S, editors, second ed., Academic Press; 2014. p. 257–95. https://doi.org/10.1016/B978-0-12-397003-9.00013-8.
[11] Li M, Schönberg A, Schaefer M, et al. Detecting heteroplasmy from high-throughput sequencing of complete human mitochondrial DNA genomes. Am J Hum Genet 2010;87(2):237–49. https://doi.org/10.1016/j.ajhg.2010.07.014 PMid 20696290.
[12] Dong Z, Wang Y, Li C, et al. Mitochondrial DNA as a molecular marker in insect ecology: Current status and future prospects. Ann Entomol Soc Am 2021;114(4):470–6. https://doi.org/10.1093/aesa/saab020.
[13] Lang BF, Gray MW, Burger G. Mitochondrial genome evolution and the origin of eukaryotes. Annu Rev Genet 1999;33(1):351–97. https://doi.org/10.1146/annurev.genet.33.1.351. PMid: 10690412.
[14] Virgilio M, Backeljau T, Nevado B, et al. Comparative performances of DNA barcoding across insect orders. BMC Bioinf 2010;11:206. https://doi.org/10.1186/1471-2105-11-206. PMid: 20420717.
[15] Hebert PDN, Stoeckle MY, Zemlak TS, et al. Identification of birds through DNA barcodes. PLoS Biol 2004;2(10):e312. https://doi.org/10.1371/journal.pbio.0020312. PMid: 15455034.
[16] Muhammad TH, Akhtar S. Services of DNA barcoding in different fields. Mitochondrial DNA Part A 2016;27(6):4463–74. https://doi.org/10.3109/19401736.2015.1089572. PMid: 26470942.
[17] Xing B, Zhang Z, Sun R, et al. Mini-DNA barcoding for the identification of commercial fish sold in the markets along the Taiwan Strait. Food Control 2020;112:107143. https://doi.org/10.1016/j.foodcont.2020.107143.
[18] Xing B, Chen X, Wu Q, et al. Species authentication and conservation challenges in Chinese fish maw market using mini-DNA barcoding. Food Control 2024;167:110779. https://doi.org/10.1016/j.foodcont.2024.110779.
[19] James JE, Piganeau G, Eyre-Walker A. The rate of adaptive evolution in animal mitochondria. Mol Ecol 2016;25(1):67–78. https://doi.org/10.1111/mec.13475. PMid: 26578312.
[20] Gendron EM, Qing X, Sevigny JL, et al. Comparative mitochondrial genomics in Nematoda reveal astonishing variation in compositional biases and substitution rates indicative of multi-level selection. BMC Genomics 2024;25(1):615. https://doi.org/10.1186/s12864-024-10500-1.
[21] Gendron EM, Sevigny JL, Byiringiro I, et al. Nematode mitochondrial metagenomics: A new tool for biodiversity analysis. Mol Ecol Resour 2023;23(5):975–89. https://doi.org/10.1111/1755-0998.13761.
[22] Pacheco-Sierra G, Amavet PS. In: Zucoloto RB, Amavet PS, Verdade LM, et al., editors. Hybridization and speciation among new-world crocodilian species. Cham: Conservation Genetics of New World Crocodilians. Springer; 2021. p. 171–83. https://doi.org/10.1007/978-3-030-56383-7_7. PMid: 38102727.
[23] Cameron SL. Insect mitochondrial genomics: Implications for evolution and phylogeny. Annu Rev Entomol 2014;59(1):95–117. https://doi.org/10.1146/annurev-ento-011613-162007.
[24] Hahn A, Zuryn S. Mitochondrial genome (mtDNA) mutations that generate reactive oxygen species. Antioxidants 2019;8(9):392. https://doi.org/10.3390/antiox8090392. PMid: 31514455.
[25] Nosek J, Tomáška Ľ. Mitochondrial genome diversity: Evolution of the molecular architecture and replication strategy. Curr Genet 2003;44:73–84. https://doi.org/10.1007/s00294-003-0426-z. PMid: 12898180.
[26] Boore JL. Animal mitochondrial genomes. Nucleic Acids Res 1999;27(8):1767–80. https://doi.org/10.1093/nar/27.8.1767. PMid: 10101183.
[27] Roger AJ, Muñoz-Gómez SA, Kamikawa R. The origin and diversification of mitochondria. Curr Biol 2017;27(21):R1177–92. https://doi.org/10.1016/j.cub.2017.09.015.
[28] D'Souza AR, Minczuk M. Mitochondrial transcription and translation: Overview. Essays Biochem 2018;62(3):309–20. https://doi.org/10.1042/ebc20170102. PMid: 30030363.
[29] Zardoya R. Recent advances in understanding mitochondrial genome diversity. F1000Research 2020;9:270. https://doi.org/10.12688/f1000research.21490.1.
[30] Pastukh VM, Gorodnya OM, Gillespie MN, et al. Regulation of mitochondrial genome replication by hypoxia: The role of DNA oxidation in D-loop region. Free Radic Biol Med 2016;96:78–88. https://doi.org/10.1016/j.freeradbiomed.2016.04.011.
[31] Clayton DA. Transcription and replication of mitochondrial DNA. Hum Reprod 2000;15(suppl_2):11–7. https://doi.org/10.1093/humrep/15.suppl_2.11. PMid: 11041509.
[32] Sumana SL, Wang P, Zhang C, et al. Genetic diversity of the common carp black strain population based on mtDNA (D-loop and cytb). Heliyon 2024;10

(10):e30307. https://doi.org/10.1016/j.heliyon.2024.e30307. PMID: 38774331.
[33] Archibald JM. Endosymbiosis and eukaryotic cell evolution. Curr Biol 2015;25 (19):R911–21. https://doi.org/10.1016/j.cub.2015.07.055.
[34] Kutschera U. In: Delisle R, editor. Symbiogenesis and cell evolution: An anti-Darwinian research agenda?. Cham: The Darwinian Tradition in Context. Springer; 2017. p. 309–31. https://doi.org/10.1007/978-3-319-69123-7_14.
[35] Atlante A, Valenti D. Mitochondria have made a long evolutionary path from ancient bacteria immigrants within eukaryotic cells to essential cellular hosts and key players in human health and disease. Curr Issues Mol Biol 2023;45 (5):4451–79. https://doi.org/10.3390/cimb45050283.
[36] Martin WF, Garg S, Zimorski V. Endosymbiotic theories for eukaryote origin. Philos Trans R Soc B: Biol Sci 2015;370(1678):20140330. https://doi.org/10.1098/rstb.2014.0330.
[37] Kelly S. The economics of organellar gene loss and endosymbiotic gene transfer. Genome Biol 2021;22:345. https://doi.org/10.1186/s13059-021-02567-w.
[38] Wang Z, Wu M. An integrated phylogenomic approach toward pinpointing the origin of mitochondria. Sci Rep 2015;5(1):7949. https://doi.org/10.1038/srep07949.
[39] Degli EM. Bioenergetic evolution in proteobacteria and mitochondria. Genome Biol Evol 2014;6(12):3238–51. https://doi.org/10.1093/gbe/evu257.
[40] Giannotti D, Boscaro V, Husnik F, et al. The "other" Rickettsiales: An overview of the family "*Candidatus* Midichloriaceae". Appl Environ Microbiol 2022;88 (6):e02432–e2521. https://doi.org/10.1128/aem.02432-21.
[41] Mallo N, Fellows J, Johnson C, et al. Protein import into the endosymbiotic organelles of apicomplexan parasites. Genes 2018;9(8):412. https://doi.org/10.3390/genes9080412.
[42] Martin WF, Tielens AG, Mentel M, et al. The physiology of phagocytosis in the context of mitochondrial origin. Microbiol Mol Biol Rev 2017;81(3): e00008–e17. https://doi.org/10.1128/MMBR.00008-17.
[43] Suomalainen A, Nunnari J. Mitochondria at the crossroads of health and disease. Cell 2024;187(11):2601–27. https://doi.org/10.1016/j.cell.2024.04.037.
[44] Gentil J, Hempel F, Moog D, et al. Origin of complex algae by secondary endosymbiosis: A journey through time. Protoplasma 2017;254:1835–43. https://doi.org/10.1007/s00709-017-1098-8. PMid: 28290059.
[45] Zammit G, Zammit MG, Buttigieg KG. Emerging technologies for the discovery of novel diversity in cyanobacteria and algae and the elucidation of their valuable metabolites. Diversity 2023;15(11):1142. https://doi.org/10.3390/d15111142.
[46] Stairs CW, Leger MM, Roger AJ. Diversity and origins of anaerobic metabolism in mitochondria and related organelles. Philos Trans R Soc B: Biol Sci 2015;370(1678):20140326. https://doi.org/10.1098/rstb.2014.0326. PMid: 26323757.
[47] Sato M, Sato K. Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. Biochimica et Biophysica Acta (BBA)-Mol Cell Res 2013;1833(8):1979–84. https://doi.org/10.1016/j.bbamcr.2013.03.010. PMid: 23524114.
[48] Allio R, Donega S, Galtier N, et al. Large variation in the ratio of mitochondrial to nuclear mutation rate across animals: Implications for genetic diversity and the use of mitochondrial DNA as a molecular marker. Mol Biol Evol 2017;34(11):2762–72. https://doi.org/10.1093/molbev/msx197. PMid: 28981721.
[49] Wallace DC. Mitochondrial DNA mutations in disease and aging. Environ Mol Mutagen 2010;51(5):440–50. https://doi.org/10.1002/em.20586. PMid: 20544884.
[50] Sloan DB, Havird JC, Sharbrough J. The on-again, off-again relationship between mitochondrial genomes and species boundaries. Mol Ecol 2017;26 (8):2212–36. https://doi.org/10.1111/mec.13959. PMid: 27997046.
[51] Antil S, Abraham JS, Sripoorna S, et al. DNA barcoding, an effective tool for species identification: A review. Mol Biol Rep 2023;50(1):761–75. https://doi.org/10.1007/s11033-022-08015-7. PMid: 36308581.
[52] Andújar C, Arribas P, Yu DW, et al. Why the COI barcode should be the community DNA metabarcode for the metazoan. Mol Ecol 2018;27 (20):3968–75. https://doi.org/10.1111/mec.14844. PMid: 30129071.
[53] Thomsen PF, Willerslev E. Environmental DNA–An emerging tool in conservation for monitoring past and present biodiversity. Biol Conserv 2015;183:4–18. https://doi.org/10.1016/j.biocon.2014.11.019.
[54] Wanga C, Chenc S, Chenc G, et al. The complete mitochondrial genome of *Littoraria ardouiniana* (Heude, 1885) (Gastropoda, Littorininae): Sequence, structure, and phylogenetic analyses. Russ J Genet 2024;60(1):100–8. https://doi.org/10.1134/S1022795424010113.
[55] Zhang K, Sun J, Xu T, et al. Phylogenetic relationships and adaptation in deep-sea mussels: Insights from mitochondrial genomes. Int J Mol Sci 2021;22 (4):1900. https://doi.org/10.3390/ijms22041900. PMid: 33672964.
[56] Boore JL, Macey JR, Medina M. Sequencing and comparing whole mitochondrial genomes of animals. Methods Enzymol 2005;395:311–48. https://doi.org/10.1016/S0076-6879(05)95019-2. PMid: 15865975.
[57] Xing B, Chen X, Wang Y, et al. The complete mitochondrial genome of *Capitulum mitella* with characterization and phylogenetic implications. Russ J Genet 2023;59(10):1032–43. https://doi.org/10.1134/S1022795423100149.
[58] Satoh TP, Miya M, Mabuchi K, et al. Structure and variation of the mitochondrial genome of fishes. BMC Genomics 2016;17:719. https://doi.org/10.1186/s12864-016-3054-y. PMid: 27604148.
[59] Tobler M, Barts N, Greenway R. Mitochondria and the origin of species: Bridging genetic and ecological perspectives on speciation processes. Integr Comp Biol 2019;59(4):900–11. https://doi.org/10.1093/icb/icz025. PMid: 31004483.
[60] Soucy SM, Huang J, Gogarten JP. Horizontal gene transfer: Building the web of life. Nat Rev Genet 2015;16(8):472–82. https://doi.org/10.1038/nrg3962. PMid: 26184597.
[61] Dobrogojski J, Adamiec M, Luciński R. The chloroplast genome: A review. Acta Physiol Plant 2020;42(6):98. https://doi.org/10.1007/s11738-020-03089-x.
[62] Youle RJ. Mitochondria—Striking a balance between host and endosymbiont. Science 2019;365(6454):eaaw9855. https://doi.org/10.1126/science.aaw9855. PMid: 31416937.
[63] Allen JF. Why chloroplasts and mitochondria retain their own genomes and genetic systems: Colocation for redox regulation of gene expression. Proc Natl Acad Sci 2015;112(33):10231–8. https://doi.org/10.1073/pnas.1500012112. PMid: 26286985.
[64] Puertas MJ, González-Sánchez M. Insertions of mitochondrial DNA into the nucleus—effects and role in cell evolution. Genome 2020;63(8):365–74. https://doi.org/10.1139/gen-2019-0151. PMid: 32396758.
[65] Michalovová M, Vyskot B, Kejnovsky E. Analysis of plastid and mitochondrial DNA insertions in the nucleus (NUPTs and NUMTs) of six plant species: Size, relative age and chromosomal localization. Heredity 2013;111(4):314–20. https://doi.org/10.1038/hdy.2013.51.
[66] Havird JC, Sloan DB. The roles of mutation, selection, and expression in determining relative rates of evolution in mitochondrial versus nuclear genomes. Mol Biol Evol 2016;33(12):3042–53. https://doi.org/10.1093/molbev/msw185. PMid: 27563053.
[67] Pinard D, Myburg AA, Mizrachi E. The plastid and mitochondrial genomes of *Eucalyptus grandis*. BMC Genomics 2019;20:132. https://doi.org/10.1186/s12864-019-5444-4. PMid: 30760198.
[68] Zhang G-J, Dong R, Lan L-N, et al. Nuclear integrants of organellar DNA contribute to genome structure and evolution in plants. Int J Mol Sci 2020;21 (3):707. https://doi.org/10.3390/ijms21030707. PMid: 31973163.
[69] Shapiro JA. Living organisms author their read-write genomes in evolution. Biology 2017;6(4):42. https://doi.org/10.3390/biology6040042. PMid: 29211049.
[70] Kleine T, Maier UG, Leister D. DNA transfer from organelles to the nucleus: The idiosyncratic genetics of endosymbiosis. Annu Rev Plant Biol 2009;60 (1):115–38. https://doi.org/10.1146/annurev.arplant.043008.092119. PMid: 19014347.
[71] Arnold ML. Evolution through genetic exchange. Oxford University Press 2007. https://doi.org/10.1093/acprof:oso/9780199229031.001.0001.
[72] Montanari SR, Hobbs JPA, Pratchett MS, et al. The importance of ecological and behavioural data in studies of hybridisation among marine fishes. Rev Fish Biol Fish 2016;26:181–98. https://doi.org/10.1007/s11160-016-9420-7.
[73] Harrison RG, Larson EL. Hybridization, introgression, and the nature of species boundaries. J Hered 2014;105(S1):795–809. https://doi.org/10.1093/jhered/esu033.
[74] Glover KA, Solberg MF, McGinnity P, et al. Half a century of genetic interaction between farmed and wild Atlantic salmon: Status of knowledge and unanswered questions. Fish Fish 2017;18(5):890–927. https://doi.org/10.1111/faf.12214.
[75] Foltz DW. Hybridization frequency is negatively correlated with divergence time of mitochondrial DNA haplotypes in a sea star (*Leptasterias* spp.) species complex. Evolution 1997;51(1):283–8. https://doi.org/10.1111/j.1558-5646.1997.tb02410.x. PMid: 28568776.
[76] Dufresnes C, Litvinchuk SN, Rozenblut-Kościsty B, et al. Hybridization and introgression between toads with different sex chromosome systems. Evol Lett 2020;4(5):444–56. https://doi.org/10.1002/evl3.191. PMid: 33014420.
[77] Maddison WP, Knowles LL. Inferring phylogeny despite incomplete lineage sorting. Syst Biol 2006;55(1):21–30. https://doi.org/10.1080/10635150500354928. PMid: 16507521.
[78] Pahad G, Montgelard C, Jansen van Vuuren B. Phylogeography and niche modelling: Reciprocal enlightenment. Mammalia 2019;84(1):10–25. https://doi.org/10.1515/mammalia-2018-0191.
[79] Moum T, Árnason E. Genetic diversity and population history of two related seabird species based on mitochondrial DNA control region sequences. Mol Ecol 2001;10(10):2463–78. https://doi.org/10.1046/j.0962-1083.2001.01375.x. PMid: 11703652.
[80] Moore WS. Inferring phylogenies from mtDNA variation: Mitochondrial-gene trees versus nuclear-gene trees. Evolution 1995;49(4):718–26. https://doi.org/10.1111/j.1558-5646.1995.tb02308.x. PMid: 28565131.
[81] Dexter KG, Pennington TD, Cunningham CW. Using DNA to assess erors in tropical tree identifications: How often are ecologists wrong and when does it matter? Ecol Monogr 2010;80(2):267–86. https://doi.org/10.1890/09-0267.1.
[82] Liu Y, Cox CJ, Wang W, et al. Mitochondrial phylogenomics of early land plants: Mitigating the effects of saturation, compositional heterogeneity, and codon-usage bias. Syst Biol 2014;63(6):862–78. https://doi.org/10.1093/sysbio/syu049. PMid: 25070972.
[83] Bingpeng X, Heshan L, Zhilan Z, et al. DNA barcoding for identification of fish species in the Taiwan Strait. PLoS One 2018;13(6):e0198109. https://doi.org/10.1371/journal.pone.0198109. PMid: 29856794.
[84] Hebert PD, Bock DG, Prosser SW. Interrogating 1000 insect genomes for NUMTs: A risk assessment for estimates of species richness. PLoS One 2023;18(6):e0286620. https://doi.org/10.1371/journal.pone.0286620. PMid: 37289794.

[85] Song H, Buhay JE, Whiting MF, et al. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. Proc Natl Acad Sci 2008;105(36):13486–91. https://doi.org/10.1073/pnas.0803076105. PMid: 18757756.
[86] Baeza JA, Fuentes MS. Exploring phylogenetic informativeness and nuclear copies of mitochondrial DNA (numts) in three commonly used mitochondrial genes: Mitochondrial phylogeny of peppermint, cleaner, and semi-terrestrial shrimps (Caridea: *Lysmata*, *Exhippolysmata*, and *Merguia*). Zool J Linn Soc 2013;168(4):699–722. https://doi.org/10.1111/zoj.12044.
[87] Ragan MA, McInerney JO, Lake JA. The network of life: Genome beginnings and evolution. Philos Trans R Soc B: Biol Sci 2009;364:2169–75. https://doi.org/10.1098/rstb.2009.0046. PMid: 19571237.
[88] Jiang J, Yu J, Li J, et al. Mitochondrial genome and nuclear markers provide new insight into the evolutionary history of macaques. PLoS One 2016;11(5):e0154665. https://doi.org/10.1371/journal.pone.0154665. PMid: 27135608.
[89] Wolf JB, Lindell J, Backström N. Speciation genetics: Current status and evolving approaches. Philos Trans R Soc B: Biol Sci 2010;365(1547):1717–33. https://doi.org/10.1098/rstb.2010.0023. PMid: 20439277.
[90] Harris EE, Disotell TR. Nuclear gene trees and the phylogenetic relationships of the mangabeys (Primates: Papionini). Mol Biol Evol 1998;15(7):892–900. https://doi.org/10.1093/oxfordjournals.molbev.a025993. PMid: 9656488.
[91] Barker FK. Monophyly and relationships of wrens (Aves: Troglodytidae): A congruence analysis of heterogeneous mitochondrial and nuclear DNA sequence data. Mol Phylogenet Evol 2004;31(2):486–504. https://doi.org/10.1016/j.ympev.2003.08.005. PMid: 15062790.
[92] Tetteh M, de Lima A, McEllin J, et al. Evolving multi-output digital circuits using multi-genome grammatical evolution. Algorithms 2023;16(8):365. https://doi.org/10.3390/a16080365.
[93] Hoelzel AR. Where to now with the evolutionarily significant unit? Trends Ecol Evol 2023;38(12):1134–42. https://doi.org/10.1016/j.tree.2023.07.005. PMid: 37596130.
[94] Garcia de Leaniz C, Fleming I, Einum S, et al. A critical review of adaptive genetic variation in Atlantic salmon: Implications for conservation. Biol Rev 2007;82(2):173–211. https://doi.org/10.1111/j.1469-185X.2006.00004.x. PMid: 17437557.
[95] Theodoridis S, Patsiou TS, Randin C, et al. Forecasting range shifts of a cold-adapted species under climate change: Are genomic and ecological diversity within species crucial for future resilience? Ecography 2018;41(8):1357–69. https://doi.org/10.1111/ecog.03346.
[96] El-Regal MA, Satheesh S. Biodiversity of Marine Ecosystems. In: Marine Ecosystems: A Unique Source of Valuable Bioactive Compounds. p. 1–42. https://doi.org/10.2174/9789815051995123030003.
[97] Kathiresan K, Bingham BL. Biology of mangroves and mangrove ecosystems. Adv Mar Biol 2001;40:81–251. https://doi.org/10.1016/S0065-2881(01)40003-4.
[98] Greenway R, Barts N, Henpita C, et al. Convergent evolution of conserved mitochondrial pathways underlies repeated adaptation to extreme environments. Proc Natl Acad Sci 2020;117(28):16424–30. https://doi.org/10.1073/pnas.2016076117. PMid: 32817514.
[99] Zhang B, Zhang YH, Wang X, et al. The mitochondrial genome of a sea anemone *Bolocera* sp. exhibits novel genetic structures potentially involved in adaptation to the deep-sea environment. Ecol Evol 2017;7(13):4951–62. https://doi.org/10.1002/ece3.3067. PMid: 28690821.
[100] Schiffer M, Kennington W, Hoffmann A, et al. Lack of genetic structure among ecologically adapted populations of an Australian rainforest *Drosophila* species as indicated by microsatellite markers and mitochondrial DNA sequences. Mol Ecol 2007;16(8):1687–700. https://doi.org/10.1111/j.1365-294X.2006.03200.x. PMid: 17402983.