Mitochondrial mutations and sterility in the interspecific hybrids of the hermaphroditic Argopecten scallops

Tieying Yu[1], Xia Lu[2], Junhao Ning[1], Min Chen[3], Yuan Wang[3], Guilong Liu[4], Quanchao Wang[1], Xin Xu[4], and Chunde Wang[1]

[1]Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, Shandong, 264003, China
[2]Yantai Institute of Coastal Zone Research
[3]Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences
[4]Yantai Spring-Sea AquaSeed, Ltd., Yantai, 264006, China

August 19, 2022

Abstract

The sterility of hybrids from interspecific hybridization limits speciation, which widely exists in interspecific hybrids of hermaphroditic plants and also been found in the F1 hybrids of hermaphroditic Argopecten scallops. However, the underlying mechanism of sterility remained unexplored in hermaphroditic animals. In this study, we firstly investigated the mechanism of sterility in the interspecific F1 hybrids of Argopecten scallops by examining the mutations in mitochondrial genes and expressions of nuclear genes. Our results showed that the ATP content in gonads of F1 hybrids was significantly lower than their parents, indicating that energy deficiency may be an immediate cause of sterility in F1 hybrids. The SNP variation types of transition in mitochondrial genes (CYTB, ND4, ND2, ATP6, and COX2) could change their hydrophobicity of amino acids and protein structures, which might contribute to sterility in F1 hybrids. The mutations and rearrangements of mitochondrial genes and abnormal expression of nuclear genes were found in F1 hybrids. Abnormal interaction between the mitochondrial and nuclear genes might contribute to sterility of the F1 hybrids through the following pathways: (1) inhibition of oocyte maturation by enhanced expression of Mfn2; (2) cell cycle arrest in G1 phase of oocytes by inhibited expression of CDK2; (3) cell apoptosis induced by mitochondrial apoptosis; (4) insufficient energy supply from abnormal mitochondria, and (5) mitochondrial dysfunction resulted from abnormal expression of other nuclear genes. The mechanism of sterility in interspecific hybrids of hermaphroditic animals would provide more information for understanding the reproductive isolation and exploitation of heterosis.

Mitochondrial mutations and sterility in the interspecific hybrids of the hermaphroditic *Argopecten* scallops

Tieying Yu[a,c], Xia Lu[a,*], Junhao Ning[a], Min Chen[a], Yuan Wang[a,c], Guilong Liu[d], Quanchao Wang[a], Xin Xu[d], Chunde Wang[a,b,c,*]

[a] Research and Development Center for Efficient Utilization of Coastal Bioresources, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, Shandong, 264003, China

[b] College of Marine Science and Engineering, Qingdao Agricultural University, Qingdao, Shandong, 266109, China

[c]University of Chinese Academy of Sciences, Beijing, 100049, China

[d] Yantai Spring-Sea AquaSeed, Ltd., Yantai, 264006, China

[*] Corresponding authors:Dr. Xia Lu & Dr. Chunde Wang

17 Chunhui Road, Yantai, 264003, China

E-mail: xialu@yic.ac.cn; chundewang2007@163.com

Abstract

The sterility of hybrids from interspecific hybridization limits speciation, which widely exists in interspecific hybrids of hermaphroditic plants and also been found in the F_1hybrids of hermaphroditic *Argopecten* scallops. However, the underlying mechanism of sterility remained unexplored in hermaphroditic animals. In this study, we firstly investigated the mechanism of sterility in the interspecific F_1 hybrids of*Argopecten* scallops by examining the mutations in mitochondrial genes and expressions of nuclear genes. Our results showed that the ATP content in gonads of F_1 hybrids was significantly lower than their parents, indicating that energy deficiency may be an immediate cause of sterility in F_1 hybrids. The SNP variation types of transition in mitochondrial genes (*CYTB* ,*ND4* , *ND2* , *ATP6* , and *COX2*) could change their hydrophobicity of amino acids and protein structures, which might contribute to sterility in F_1 hybrids. The mutations and rearrangements of mitochondrial genes and abnormal expression of nuclear genes were found in F_1 hybrids. Abnormal interaction between the mitochondrial and nuclear genes might contribute to sterility of the F_1 hybrids through the following pathways: (1) inhibition of oocyte maturation by enhanced expression of *Mfn2* ; (2) cell cycle arrest in G1 phase of oocytes by inhibited expression of *CDK2* ; (3) cell apoptosis induced by mitochondrial apoptosis; (4) insufficient energy supply from abnormal mitochondria, and (5) mitochondrial dysfunction resulted from abnormal expression of other nuclear genes. The mechanism of sterility in interspecific hybrids of hermaphroditic animals would provide more information for understanding the reproductive isolation and exploitation of heterosis.

Keywords: Hermaphroditic animals, hybridization and speciation, sterility, reproductive isolation, mtDNA mutation, energy deficiency

1 INTRODUCTION

The sterility of hybrids from interspecific hybridization limits speciation, which is mainly caused by reproductive isolation. Sterility widely exists in interspecific hybrids of hermaphroditic plants and dioecious animals (Sawamura *et al.* , 2004; Steiner and Ryder, 2013; Kim and Zhang, 2018; Kurhanewicz *et al.* , 2020; Dong*et al.* , 2021; Yu *et al.* , 2021; Zhao *et al.* , 2021). Reproductive isolation makes it impossible to hybridize between closely related groups or to produce no hybrids and sterile hybrids after hybridization. The interspecific hybrids between *indica* and*japonica* subspecies had decreased fertility and low seed setting rate (Shen *et al.* , 2017). Chromosomal abnormalities in the interspecific hybrids between horses and donkeys resulted in sterility (Steiner and Ryder, 2013). The fertilization rate of interspecific hybrids between *Haliotis fulgens* and *Haliotis discus hannai* was low and the survival rate of larvae was also low (Song, 2018). Analyzing the sterile mechanism of interspecific hybrids will help exploitation of heterosis, supplement the new understanding of reproductive isolation and promote the generation of new species. However, sterility has not been reported in hermaphroditic invertebrates such as *Argopecten* scallops.

Argopecten scallops such as the bay scallop (*A. irradians irradians*) and the Peruvian scallop (*A. purpuratus*), are typical hermaphroditic animals that possess both female and male acini in the same gonads. Bay scallops are naturally distributed along the Atlantic coast of the United States and Peruvian scallops are naturally distributed in the South Pacific. Geographic isolation is formed between them, and reproductive isolation is an important condition and result of speciation. Wang *et al* . (2011) successfully hybridized the bay scallop with the Peruvian scallop and obtained F_1hybrids with extremely significant heterosis in growth. It is interesting to note that most F_1 hybrid scallops were found to be both male and female sterile; their male part of the gonads either did not develop or was occupied by female acini. In very rare cases, the male part of gonads may partially develop but the spawned sperm were immobile and incapable of fertilization. The female part of the gonads were often well-developed and able to spawn a large quantity of eggs, but most of the eggs could not be fertilized by sperm of the bay scallops or Peruvian scallops (Feng *et al.* , 2012; Wang*et al.* , 2017). This presumably belongs to postzygotic isolation in reproductive isolation. Despite of the great potential of sterility in breeding of hermaphroditic scallops, however, the mechanism underlying

sterility in the F_1 hybrid scallops has not been explored.

In the previous studies of hermaphroditic plants and dioecious animals, several causes have been reported to contribute to sterility, among which mitochondrial abnormality was one of the most important factors. Hao *et al* . (2021) found that aberrant rearrangement of mitochondrial genes resulted in a new reading frame '*orf279* ' that was responsible for male sterility in wheats. Eslamieh *et al* . (2022) reported that in *Drosophila* , any mutation in the mitochondria could cause sterility. For instance, a SNP mutation in the cytochrome B gene in respiratory complex III resulted in male sterility (Wolff *et al.* , 2017). The mutations in mitochondria were often adverse to male offspring, showing early death, reduced survival rate or male sterility (Agren *et al.* , 2019). There are approximately 22-72 mitochondria in the mitochondrial sheath of a single sperm tail, which provide a large amount of energy for sperm production, motility, and sperm-egg binding (Frank and Hurst, 1996; Piomboni *et al.* , 2012; Heidari *et al.* , 2016). Therefore, mutations and rearrangement of mitochondrial DNA (mtDNA) may cause insufficient ATP synthesis and defective mitochondrial function, resulting in cytoplasmic male sterility and nucleus-cytoplasmic male sterility. Luo *et al* . (2013) found that in rice, rearrangement of mitochondrial genes produced a new gene which interacted with nuclear gene *COX11* to generate a large amount of reactive oxygen species (ROS), leading to apoptosis and degradation of tapetum cells and eventually male sterility. Also, the formation of new species usually underwent chromosomal mutation, gene mutation and gene recombination. In most cases, the functions of mitochondrial genes required the participation of nuclear genes, especially those genes involved in the respiratory chain and electron transport of mitochondria. Similarly, sterility may also be induced through the regulation of nuclear genes involved in critical pathways such as cell death and apoptosis by mitochondrial genes (Butow and Avadhani, 2004; Lam, 2004; Ouyang *et al.* , 2010; Chen and Liu, 2014; Grosser *et al.* , 2021). Thus, mitochondria may play an important role in sterility of interspecific F_1 hybrid scallops.

In most organisms, mitochondria follow the law of maternal inheritance, that is, the mitochondria of the offspring always come from their mother (Vaught and Dowling, 2018). However, the mitochondrial genome sequence and structure of molluscs are complex and variable. Doubly uniparental inheritance (DUI) of mitochondria was discovered in some molluscs (Degletagne *et al.* , 2021; Stewart *et al.* , 2021). There are two mitochondrial lineages in DUI species, containing one from the maternal parent and another from the paternal parent. In DUI species, both sexes inherited mitochondria from their mother (F-type), while the paternal mitochondria were only transmitted to male offspring (M-type) (Breton *et al.* , 2007). The M-type mitochondrial genomes in gonads of male offspring were quite different from the F-type mitochondrial genomes, which could directly determine sex and male fertility in offspring (Zouros and Rodakis, 2019). The inherited pattern of mitochondria in the interspecific F_1 hybrids of hermaphroditic scallops is unclear, and whether it is related to sterility is also not known.

In previous studies, we have obtained a new variety with normal male and female fertility, the 'Bohai Red', from a few fertile F_1hybrids and subsequently continuous selection (Wang *et al.* , 2017). The availability of sterile F_1 hybrid scallops and the fertile hybrid scallops ('Bohai Red') renders us a unique opportunity to explore the mechanisms of sterility in interspecific hybrids of hermaphroditic animals. Therefore, in this study, we aimed to uncover the mechanisms underlying sterility regulated by mitochondria and nuclear genes. We analyzed the inheritance pattern and rearrangements and mutations of mitochondrial DNA in interspecific F_1 hybrid scallops by whole genome re-sequencing and compared the differences in genotypes in the mitochondrial genes. ATP content in the gonads between sterile hybrid scallops and fertile scallops was also detected. We then examined the expression profile of nuclear genes that may participate in regulation of mitochondrial functions by transcriptomic analysis. The results may help to fill the gap in determination mechanism of sterility in interspecific F_1 hybrids of hermaphroditic animals, and provide more information for understanding reproductive isolation and speciation.

2 MATERIALS AND METHODS

2.1 Animals

The bay scallops (*Ai*), Peruvian scallops (*Ap*) and the scallop 'Baohai Red', a new variety selected from

a F_1IP hybrid scallop population (bay scallops × Peruvian scallops) were cultured in open sea in Yangma Island area of Yantai, Shandong Province, China. They were brought into the scallop hatchery of Yantai Spring-Sea AquaSeed Co., Ltd. located at Laizhou, Yantai, Shandong Province of China in early spring and conditioned to mature. They were then induced to spawn individually in 1-L beakers after exposing to air for 30 min. The spawning scallops were watched carefully to collect eggs and sperms separately. Eggs or sperm from more than 100 individuals of the same species were pooled together to produce the populations of bay scallop and Peruvian scallop, respectively. Simultaneously, the eggs of bay scallops were mixed with the sperm of Peruvian scallops to produce IP F_1 hybrid population, and the eggs of a bay scallop were mixed with the sperm of a Peruvian scallop to produce an IP F_1 hybrid family. Inversely, the eggs of Peruvian scallops were mixed with the sperm of bay scallops to produce PI F_1 hybrid population. About 10 days after fertilization, when about 50% larvae developed eyespots, they were set on plastic collectors. After metamorphosis, the juveniles were moved to a shrimp pond for nursery for a period of one month and then to the open sea for another month before they were dispersed into lantern nets for grow out. The bay scallops, Peruvian scallops, 'Baohai Red' hybrid scallops, PI F_1 hybrids, and IP F_1 hybrids were partially used for subsequent experiments, including high-throughput whole genome re-sequencing, genotyping for SNPs of mitochondrial genes, transcriptomic analysis, gonadal histological analysis, and ATP content determination.

2.2 Gonad morphological and histological observation of fertile scallops and sterile F_1 hybrids

To observe histological differences in gonad between sterile and fertile scallops, fresh intact gonadal tissues of fertile scallops (bay scallop, Peruvian scallop and 'Bohai Red') and sterile scallops (IP F_1 hybrids and PI F_1 hybrids) were fixed with 4% paraformaldehyde in 15-ml enzyme-free centrifuge tubes for Hematoxylin-Eosin (HE) staining when their gonads were mature. After 24h, the fixed gonadal tissues were subjected to gradient alcohol dehydration, xylene transparent, paraffin-embedded, and transverse section (3-5 μm thick) on a microtome (Leica RM2016, Shanghai, China). The sections were then dewaxed with xylene, hydrated with gradient ethanol, stained with hematoxylin, counterstained with eosin, dehydrated with ethanol, cleared with xylene, mounted with neutral gum, and finally observed under a microscope (Leica CIC XSP-C204, Shanghai, China). Each kind of the scallops was performed with three replicates.

2.3 Inherited pattern of mitochondrial DNA in F_1hybrid scallops

2.3.1 Re-sequencing of mitochondrial genomes with BGISEQ-500 platform

To find out the inherited pattern of mitochondria in F_1hybrids of *Argopecten* scallops, the mitochondria genomes of F_1 hybrids and their parents were sequenced on BGISEQ-500 platform and assembled. Adductor muscles of 13 offspring and their maternal (bay scallop) and paternal (Peruvian scallop) parents of the IP F_1 hybrid family, as well as from 10 individuals of the PI F_1 hybrid population were placed in enzyme-free cryopreservation tubes and quickly frozen in liquid nitrogen, and then stored in -80 °C. In brief, the mtDNA of them were performed whole genome re-sequencing with BGISEQ-500 (MGI Tech Co., Ltd) sequencing platform. The integrity and purity of genomic DNA were detected by agarose gel electrophoresis (concentration of agarose gel: 1%; voltage: 150 V; electrophoresis time: 40 min). About 1 μg genomic DNA were fragmented using Covaris (LE220), and the fragmented genomic DNA was selected by Agencourt AMPure XP-Medium kit to a mean size of 200-400 bp. Fragments were end-repaired and then 3'-adenylated, and adaptors were ligated to the ends of these 3'-adenylated fragments. PCR products were purified by the Agencourt AMPure XP-Medium kit. The double-stranded PCR products were heat denatured and circularized by the splint oligo sequence. The single-strand circle DNA was formatted as the final library. The qualified libraries were sequenced by BGISEQ-500: the single-strand circle DNA molecule formed a DNA nanoball (DNB) containing >300 copies through a rolling-cycle replication. The DNBs were loaded into the patterned nanoarray by using high-density DNA nanochip technology. Finally, pair-end 100-bp reads were obtained by combinatorial probe-anchor synthesis. Raw reads were filtered to remove adaptor sequences, contamination, and low-quality reads.

2.3.2 Assemble and annotation of mitochondrial genomes

The clean reads were mapped to the reference mitochondrial genome sequence by the software bwa v0.7.17.

Then, the mapped reads were extracted and assembled with GetOrganelle v1.7.5. Next, collinearity comparison of assembled sequences with reference genome sequences was performed using software mummer v4.0.0rc1. The reference genomes of bay scallop and Peruvian scallop were published in National Center for Biotechnology Information (NCBI) with accession numbers of NC_012977.1 and NC_027943.1, respectively. Finally, according to the results of collinearity comparison, the 5' and 3' ends of each assembled sequence were determined, and the final mitochondrial sequences were obtained. The mitochondria genome sequences were annotated with the MITOS web server (http://mitos2.bioinf.uni-leipzig.de/index.py) and the GeSeq web server (https://chlorobox.mpimp-golm.mpg.de/geseq.html). The mitochondria circle maps were drawn with the CGView web server (https://cgview.ca/maps) and the OGDRAW web server (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html). Then multiple sequence alignment was performed for the mtDNA of IP and PI F_1 hybrids with bay scallop and Peruvian scallop using DNAMAN and BioEdit software (default parameters) for analysis of inherited pattern of mitochondrial DNA in F_1 hybrid scallops.

2.3.3 Polymerase chain reaction (PCR) amplification of mitochondrial genes

To verify the inherited pattern of mitochondria DNA in F_1 hybrid scallops, two mitochondrial genes (*NAD1*and *ATP6*) that were significantly different in their sequences between bay scallops and Peruvian scallops were further used to exam in F_1 hybrids and their parents. The mtDNA genome of 10 bay scallops, Peruvian scallops, IP F_1 hybrid, and PI F_1 hybrids were separately extracted from their adductor muscles using the Column Animal $mtDNA_{OUT}$ kit (PCR grade, TIANDZ, China) according to the manufacturer's protocols, respectively. The purity and concentration of the DNA were determined by a Nanodrop 2000 spectrophotometer (Nano Drop Technologies, USA), and DNA integrity was detected by electrophoresis on a 1.5% agarose gel. Basing on the mitochondrial genome sequences of bay scallop and Peruvian scallop, primers of the *NAD1* and *ATP6* genes were designed using "Primer-Blast" (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome) in NCBI (Table 1). PCR amplification was performed in a total volume of 25-μL, containing 20-ng of DNA as a template, 1-μL of each primer, 12.5-μL of Premix Taq (TaKaRa, Japan), and 9.5-μL of ddH_2O. All samples were denatured for 3 min at 94 °C, followed by 35 cycles of 94 °C for 30 s, annealing for 15 s, and 72 °C for 1 min, with a final extension at 72 °C for 5 min. The annealing temperature was shown in Table 1.

The PCR products were separately electrophoresed on a 1% agarose gel, stained with Gel Green nucleic acid dye (Biomed, China), and observed with a gel electrophoresis imaging system. The target bands were purified using a D2500 Gel Extraction Kit (Omega, America). The purified DNA was then ligated into pTOTO-TA vector (BLS, China) and transformed into *Trelief* ^{TM} 5α (Tsingke, China), which were randomly selected for PCR and sequenced with ABI 3730XL sequencer by Sangon Biotech Co., Ltd. (Qingdao, China). Then multiple sequence alignment was performed for the *NAD1* and *ATP6* sequences of bay scallops, Peruvian scallops, IP F_1 hybrids, and PI F_1 hybrids for analysis of inherited pattern of mitochondrial DNA in F_1 hybrid scallops.

2.4 Mitochondrial mutations in the interspecific F_1 hybrids

2.4.1 Effects of nucleotide mutations on protein structures in PI F_1 hybrids

According to the alignment results for the mitochondrial genomes of Peruvian scallop and PI F_1 hybrids, the sequences of*CYTB* , *ND4* , *ND2* , *ATP6* , *COX2* and*trnD* were mutated in F_1 hybrids. To investigate the nucleotide mutations in these genes on their amino acid sequences and protein structures, their amino acid sequences in Peruvian scallop and PI F_1 hybrids were predicted by DNAMAN, and their secondary and tertiary protein structures were predicted by the SOPMA web server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) and the Swiss-Model web server (https://swissmodel.expasy.org/interactive).

2.4.2 Genotyping of mitochondrial SNPs in fertile populations and sterile F_1 hybrids

To explore the relationship between single nucleotide polymorphism (SNP) and haplotype mutations in mitochondrial genes and sterility of F_1 hybrid, the 29 SNPs in the above genes of*CYTB* , *ND4* , *ND2* , *trnD*

, *ATP6* and*COX2* were genotyped in 60 individuals of sterile PI F_1 hybrids and fertile populations with 8 individuals of Peruvian scallop and 30 individuals of 'Bohai Red'. The mtDNA was separately extracted from the mature gonads of Peruvian scallops, 'Bohai Red' and PI F_1 hybrids using the Column Animal $mtDNA_{OUT}$ kit (PCR grade, TIANDZ, China) according to the manufacturer's protocol. Basing on the multiple sequence alignment results between PI F_1 hybrids and Peruvian scallop, the sequences of the mtDNA genes that contained SNP mutations were divided into 16 regions and primers were designed using them with "Primer-Blast" (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome) in NCBI (Table 1). The genotyping of the selected SNPs was performed using PCR amplification and direct sequencing with the protocol described above. To identify and verify sterility-associated SNPs, the changes of allele frequency were assessed between the sterile PI F_1 hybrids and fertile populations of Peruvian scallop and 'Bohai Red' population, respectively, using chi-square test analysis with the software SPSS at the level of $P < 0.05$.

Table 1 The primers used in the present study.

Application	Primer Name	Sequence of primers (5'-3')	Length of target fragment (bp)	Annealing temperature and time
PCR amplification for mtDNA genes	*Ai-ATP6*	F: GTTTGC-TAAGGGT-GTTTGGG R: GCGACTATTGGGCATCAAAG	370	57 for 30s
	Ap-ATP6	F: TTAGTTGC-TACTGCTGCTG-TAGGA R: CTAAGAACTAAAAGACCGACTAGCAG	186	56 for 20s
	Ai-NAD1	F: GGGGTTTTTCAGC-CCTTTGC R: GCTCGGTTTCTCTCGGCTAA	461	55 for 30s
	Ap-NAD1	F: TAAGGGTTG-GTGTGTACGGG R: TGAAACAAGCTCTGACTCCCC	302	56 for 30s
PCR amplification for genotyping of mitochondrial SNPs	Region 1	F:TGATCCGGTTTTGCTTTCAGCA R:TGGATAATCAGGGATGCGGC	655	55 for 45s
	Region 2	F:AGATCGCCTGCTAGCAAAGTGG R:AAGCCAAATCCTCATGGGCTG	816	56 for 55s
	Region 3	F:GGATTTAGTCGGGATCTGTGG R:AAGACGCACATCGCCTCTTC	471	57 for 52s
	Region 4	F:GGAGGTATCAGGTTGGGGTTG R:TGCCTCCGAGCATAAACCAC	570	57 for 45s
	Region 5	F:GTCGTGTGCAGCCACTGGAAT R:TAGCCCCCAACACGGCATA	167	56 for 20s
	Region 6	F:GGTGGCAGTGGACTCTTTAGG R:GAGAATCCCGCACCCACAAA	153	56 for 20s
	Region 7	F:GGCTCTCTAGAGGACTCATGTGT R:CTACAATGGCACAACCCCACA	421	57 for 30s
	Region 8	F:GGAAGGTTTTCGAACTGCCCT R:CGCGGTCGAACAATCGAATC	501	56 for 40s

Application	Primer Name	Sequence of primers (5'-3')	Length of target fragment (bp)	Annealing temperature and time
	Region 9	F:TGGAGGTTGGCTTGTTGTATT R:AGTCCCAAACCCAATGTACCA	716	54 for 50s
	Region 10	F:CGCGCTTAAGTTCGTTGGG R:GTCCCCAAGACGGTTCGTT	329	56 for 30s
	Region 11	F:CCTAAACGCGTGTTGTGGAGT R:CAAAAACCTCGCCCCCAAAC	220	56 for 20s
	Region 12	F:AGTGGGTCTTCTAGGAAAAGGC R:ACAGGCATAACCCTAACACCC	861	56 for 55s
	Region 13	F:TAAAGGCGTGTGGGTTAAGGGG R:ACCAAGAAGCAATCACCCTG	586	55 for 40s
	Region 14	F:CCTGACATGTGGGTAGTGT R:TAGTGAGTGTAAGCCACCCG	109	56 for 20s
	Region 15	F:AAGGGGAGTCAGAGGCTTGTT R:GATGCAAAAGGTACGAGAACCC	509	55 for 45s
	Region 16	F:CGAAATACCTTTCTCGCTGAC R:CTAATTTTGCCCCCTTGCTGC	717	56 for 50s

2.5 Transcriptomic analysis of Peruvian scallop and PI F_1 hybrids

The mitochondrial genes and nuclear genes interact to regulate the biological function, so the mutated mitochondrial genes in F_1 hybrids might affect the expression of nuclear genes. To identify the candidate nuclear genes that might contribute to sterility of F_1 hybrid scallops, RNA-Seq was performed for PI F_1 hybrids and Peruvian scallops. The haplotype-resolved genomes that were completely phased at the chromosome level for maternal (bay scallop) and paternal (Peruvian scallop) assembling from their inter-specific F_1 hybrid scallops were used as reference genome for transcriptomic analysis (data not published).

2.5.1 RNA extraction and high-throughput sequencing

Gonadal tissues in mature stage of Peruvian scallops and PI F_1 hybrids with three replicates were placed in enzyme-free cryopreservation tubes and quickly frozen in liquid nitrogen, and then stored in -80 °C. The total mRNA of them was extracted using Trizol reagent kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. RNA quality was assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and checked using RNase free agarose gel electrophoresis. The mRNA was enriched by Oligo (dT) beads, and then the enriched mRNA was fragmented into short fragments using fragmentation buffer and reverse transcripted into cDNA with random primers. Second-strand cDNA were synthesized by DNA polymerase I, RNase H, dNTP and buffer. Then the cDNA fragments were purified with QiaQuick PCR extraction kit (Qiagen, Venlo, The Netherlands), end repaired, poly (A) added, and ligated to Illumina sequencing adapters. The ligation products were size selected by agarose gel electrophoresis, PCR amplified, and sequenced using Illumina HiSeq2500 by Gene Denovo Biotechnology Co. (GuangPIou, China).

2.5.2 Raw reads processing, alignment and differential expression gene analysis

Raw reads were filtered with fastp (version 0.18.0) to obtain high quality clean reads. The parameters were as follows: (1) removing reads containing adapters; (2) removing reads containing more than 10% of unknown nucleotides (N); (3) removing low quality reads containing more than 50% of low quality (Q value[?] 20) bases. Then short reads alignment tool Bowtie2 was used for mapping reads to ribosome RNA (rRNA) database. The rRNA mapped reads then will be removed. The remaining clean reads were further used in assembly and gene abundance calculation. An index of the reference genome was built, and paired-end clean reads were mapped to the reference genome using HISAT 2. 2.4 with "-rna-strandness RF" and other

parameters set as a default. The mapped reads of each sample were assembled by using StringTie v1.3.1 in a reference-based approach. For each transcription region, a FPKM (fragment per kilo base of transcript per million mapped reads) value was calculated to quantify its expression abundance and variations, using StringTie software. RNAs differential expression analysis was performed by DESeq2 software between two different groups.

2.6 ATP content in gonadal tissues of fertile scallops and sterile F_1 hybrids

Mitochondria play a vital role in energy supply by synthesizing ATP, which benefits the development and motility of sperm. To exam the effects of mitochondrial gene mutations and rearrangements on ATP synthesis in F_1 hybrid scallops, the ATP content in the mature fresh gonadal tissues of bay scallops, Peruvian scallops, 'Bohai Red', PI F_1 hybrids and IP F_1 hybrids were tested with three individuals, respectively. The ATP content was detected using the ATP content determination kit (Geruisi, China) according to the instructions with a microplate reader (TECAN Infinite$^{(r)}$ 200, Switzerland) and measured at 340 nm. Data was analyzed by one-way ANOVA using the software Prism 7.00 at the level of P <0.05.

3 RESULTS

3.1 Morphological and histological difference in gonads between the fertile scallops and sterile F_1 hybrids

At mature stage, the gonads of fertile scallops (bay scallop, Peruvian scallop and the 'Bohai Red' scallop) were all well-developed with distinct white male part and orange female part, whereas most F_1 hybrids (IP and PI F_1 hybrids) only had well developed female part, with either a transparent male part or the male part was occupied by female part (Figure 1). Furthermore, histological observation showed that in fertile scallops (bay scallop, Peruvian scallop and the Scallop Bohai Red), acini containing oogonia, developing oocytes and mature oocytes were seen in the female part and acini containing spermatogonia, spermatocytes and spermatids were seen in the male part of the gonads. However, in the F_1hybrids, only acini containing oogonia, developing oocytes and large mature oocytes, but no acini containing spermatogonia, spermatocytes, or spermatids were seen in the gonads. In very rare cases, acini containing only spermatogonium and spermatocyte, but not spermatid were seen in the testis of F_1 hybrids (Figure 2). Therefore, no functional sperm were identified in IP and PI F_1hybrids.

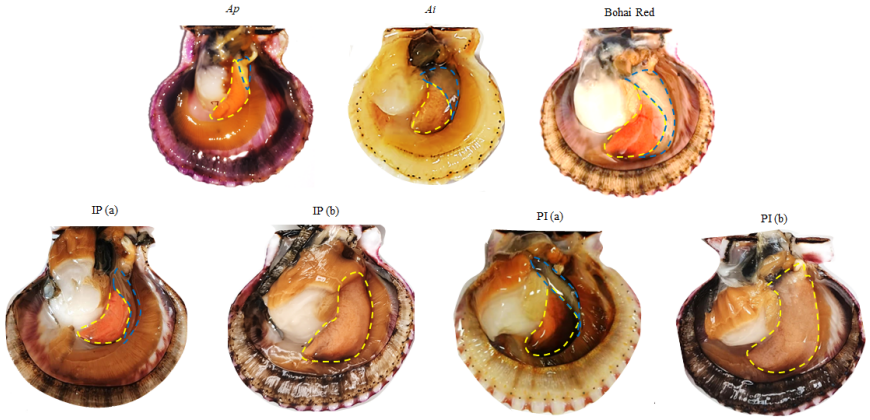

Figure 1 Morphological observation of the gonads of bay scallop (Ai), Peruvian scallop (Ap), 'Bohai Red', IP (bay scallop × Peruvian scallop) and PI (Peruvian scallop x bay scallop) F_1 hybrids. Note that the F_1 had gonads with transparent male parts and well-developed female parts in IP (a) and PI (a) or only fully-developed female part that occupied the whole gonads in IP (b) and PI (b). The yellow coil indicates the female parts and the blue coil indicates the male parts.

3.2 Maternal inheritance of mtDNA in the interspecific F_1 hybrids

Based on the multiple sequence alignment of whole mitochondrial genomes of 13 IP F_1 hybrids (bay scallop x Peruvian scallop) from the same family and their maternal parent (bay scallop) and paternal parent (Peruvian scallop), we found that except for the control region, the mtDNA sequences of hybrids were the same as their maternal parent but different from their paternal parent (Figure S1 A). Similarly, the multiple sequence alignment of whole mitochondrial genomes of 10 F_1 hybrids from a PI population (Peruvian scallop x bay scallop) showed that the mtDNA sequences of F_1 hybrids were the same as that of Peruvian scallops but different from that of bay scallops (Figure S1 B). Rich variations were found in the non-coding control regions among different individuals. In summary, the mtDNA of F_1 hybrid scallops followed maternal inheritance at whole genome level.

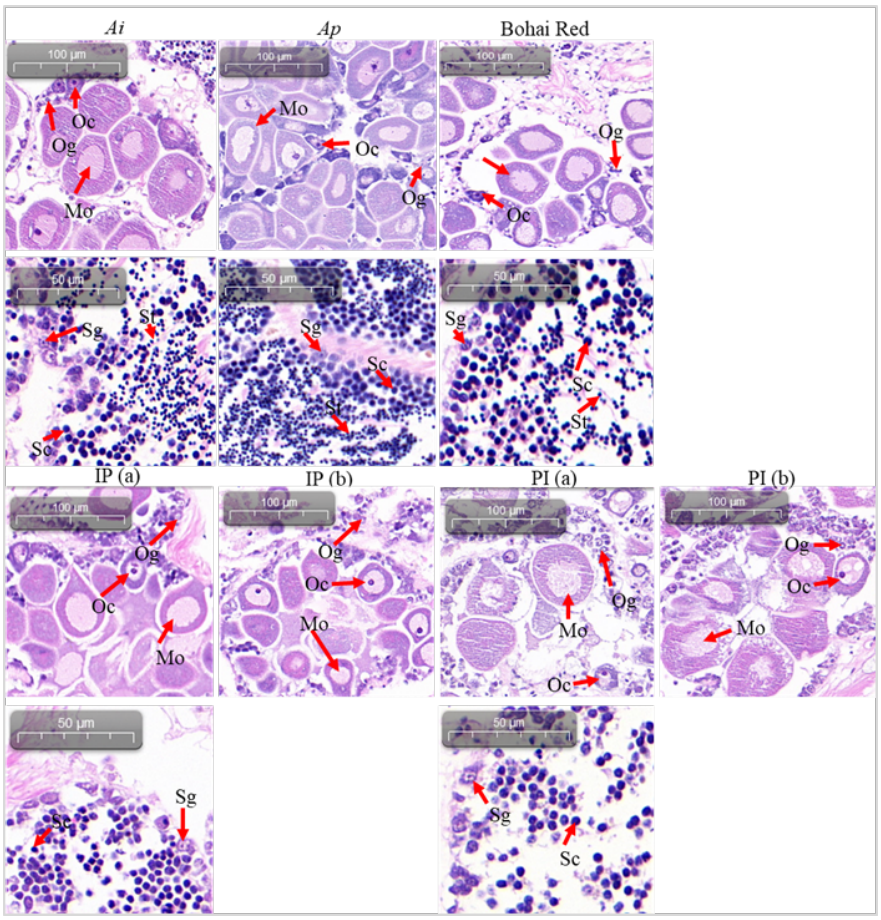

Figure 2 Histological observation of the gonads of the Peruvian scallop, bay scallop, Scallop Bohai Red, and the F_1 hybrids (bay scallop × Peruvian scallop , IP, and Peruvian scallop x bay scallop , PI). Female part on the top and male part on the bottom. Og, oogonium; Oc, oocyte; Mo, mature oocyte; Sg, spermatogonium; Sc, spermatocyte; St, spermatid.

We then selected two mitochondrial genes, *ATP6* and *NAD1* , that had significant difference in sequence between the bay scallop (*AiATP6* and *AiNAD1*) and the Peruvian scallop to verify the maternal inheritance of mtDNA in the interspecific F_1hybrid scallops by PCR. The results showed that the *AiATP6* and*AiNAD1* could only be amplified in bay scallop and IP F_1 hybrids whose maternal parents were bay scallop, and the *ApATP6* and *ApNAD1* could only be amplified in Peruvian scallop and PI F_1 hybrids whose maternal parents were Peruvian scallop (Figure 3). In addition, multiple sequence alignment of the two genes also confirmed that the sequences of IP F_1hybrids were identical with those of the bay scallops while those of

the PI F_1 hybrids and the Peruvian scallops were consistent (Figure 4), indicating that the mtDNA of F_1 hybrids also followed maternal inheritance at gene level.

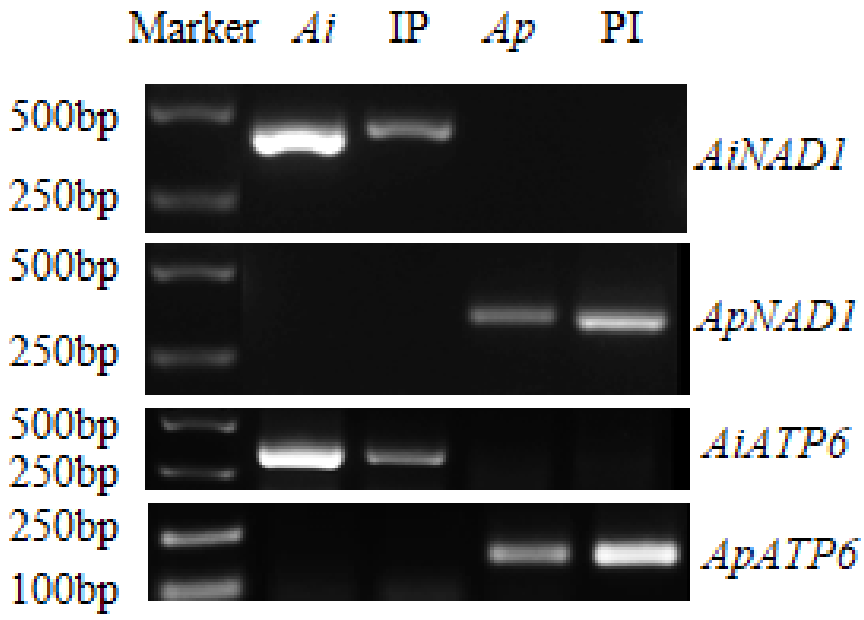

Figure 3 PCR amplification of mitochondrial genes *NAD1* and *ATP6* in bay scallops (*Ai*), Peruvian scallops (*Ap*), and IP (bay scallop × Peruvian scallop) and PI (Peruvian scallop x bay scallop) F_1 hybrids.

Figure 4 Multiple sequence alignment of *NAD1* and *ATP6* genes between IP (bay scallop × Peruvian scallop) F_1hybrids and their maternal parent bay scallop (*Ai*), and between PI (Peruvian scallop x bay scallop) F_1 hybrids and their maternal parent Peruvian scallop (*Ap*).

3.3 Gene rearrangements and genome mutations in mtDNA of F_1 hybrids

Assembly and annotation results indicated that both the bay scallop and the Peruvian scallop mitochondrial genome consisted of 37 genes, including 13 protein-coding genes, 2 rRNAs and 22 tRNAs (Figure S2). However, mitochondrial genome of IP F_1 hybrids consisted of only 35 genes, with *trnS1* and *trnS2* missing compared with their maternal parent (bay scallop). In the contrary, mitochondrial genome of PI F_1 hybrids consisted of 41 genes, with the*ATP8* gene missing but 5 extra genes (*trnG* , *trnF* ,*trnK* , *trnE* and *trnL*) and translocated *12S*and *16S* genes compared with their maternal parent (Peruvian scallop) (Figure 5 A). Furthermore, a control region with rich variations existed between the *ND4* and*ND2* genes in the mitochondrial genomes of the bay scallop, Peruvian scallop, and IP and PI hybrids. Compared with their maternal parent, there were more mutations in the *ND4* and *ND2* genes that located in the upstream and downstream of the control region of F_1 hybrids, respectively (Figure 5 B).

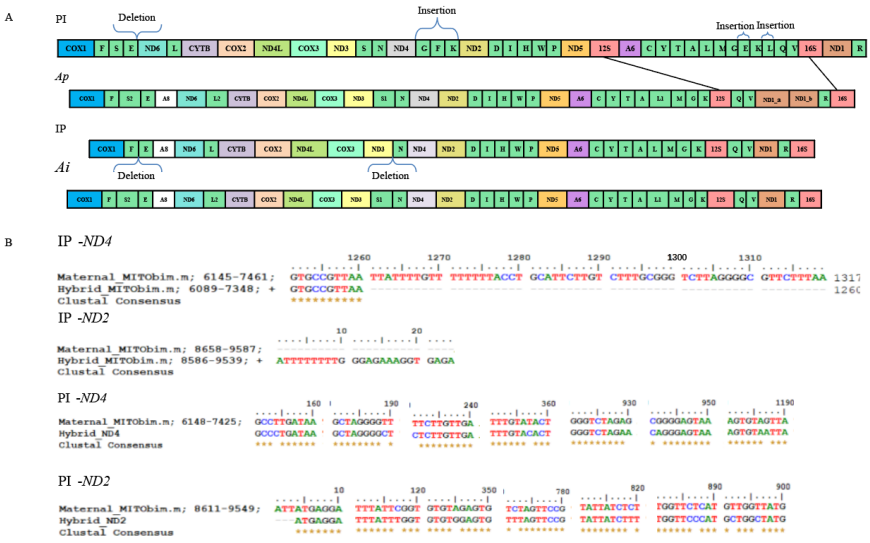

Figure 5 Gene rearrangements (A) and gene mutations in the upstream and downstream of the control region (B) in the interspecific F_1 hybrids compared with their maternal parents. (A) Translocations (represented by lines) and insertions and deletions (represented by brackets) in the mitochondrial genomes. (B) The mutations of *ND4* and *ND2* genes of IP (bay scallop × Peruvian scallop) and PI (Peruvian scallop x bay scallop) F_1 hybrids compared with their maternal parent.*Ai* , bay scallop, *Ap* , Peruvian scallop.

3.4 SNP mutations in mitochondrial genes of interspecific F_1 hybrids

The IP F_1 hybrids that were used to assemble mitochondrial genomes were from a family, so their mtDNA were almost identical to their maternal parent. Twenty-nine SNPs were detected in the genes of *CYTB* , *ND4* , *ND2* , *trnD* ,*ATP6* , and *COX2* in the mitochondrial genomes of PI F_1 hybrids from a population compared with their maternal parent. As predicted, the percentage of alpha helix, extended strand, beta turn and random coil in secondary structure of these proteins were different between PI F_1 hybrids and their maternal parent (Peruvian scallop) (Table 2). Significant changes have also occurred in their tertiary structures for that the winding and folding modes of peptide chains were different (Figure 6).

Table 2 Changes in secondary structure of proteins in PI F_1hybrids compared with Peruvian scallops (*Ap*).

Protein	*Ap*	*Ap*	*Ap*	*Ap*	PI	PI	PI	PI
	Alpha helix	Extended strand	Beta turn	Random coil	Alpha helix	Extended strand	Beta turn	Random coil
CYTB	44.62%	17.06%	3.15%	35.17%	44.79%	17.71%	3.91%	33.59%
ND4	53.65%	16.71%	6.59%	23.06%	50.71%	21.80%	7.11%	20.38%
ND2	53.21%	23.08%	6.09%	17.63%	53.40%	22.98%	6.15%	17.48%
ATP6	58.30%	12.56%	4.04%	25.11%	51.98%	17.46%	5.16%	25.40%
COX2	37.50%	23.21%	5.80%	33.48%	30.17%	23.28%	5.60%	40.95%

To determine whether the SNPs and specific haplotypes in the genes of*CYTB* , *ND4* , *ND2* , *trnD* , *ATP6* , and*COX2* were related to the sterility of F_1 hybrid scallops, these genes were further genotyped in more fertile scallops (Peruvian scallops and 'Bohai Red') and sterile hybrids (PI F_1 hybrids). The results showed that the allele frequencies of 13 SNP loci in PI F_1 hybrids were significantly different from 'Bohai Red' and Peruvian scallops (Table 3). In comparison, there was only one allele for each SNP locus in Peruvian

scallops, which was the dominant allele in 'Bohai Red' population whose maternal parents were bay scallops. Instead, the newly emerged allele was dominant in the PI F_1 hybrid scallops. Among these 13 SNPs in PI F_1 hybrids, 9 were synonymous and 4 non-synonymous with those of Peruvian scallops and 'Bohai Red', and 92.3% of them was transition type that could change the hydrophobicity of amino acids (Table 3).

Table 3 Significantly different allele frequency of SNPs in the six mitochondrial genes between PI F_1 hybrids and 'Bohai Red' and Peruvian scallop (*Ap*).

Gene	Site	*Ap*	Bohai Red	PI	Amino acid	Amino acid	Amino acid	*P* value	*P* value
					Ap	Bohai Red	PI	*Ap* vs PI	Bohai Red vs PI
CYTB	1	0T, 8C	13T,16C	44T, 14C	N	N	N	2.0ˆe-5	4.0ˆe-3
	2	0T, 8C	13T,16C	46T,14C	I	I	I	1.3ˆe-5	3.0ˆe-3
ND4	1	8T, 0C	20T, 4C	19T, 38C	L	L	L	3.4ˆe-4	4.0ˆe-5
	2	8T, 0C	20T, 4C	7T, 50C	F	F	F	3.5ˆe-8	6.0ˆe-10
	3	8T, 0C	19T, 5C	19T, 38C	Y	Y	Y	3.4ˆe-4	1.6ˆe-4
	4	8G, 0A	27G, 0A	22G, 17A	**S**	**S**	**N**	1.9ˆe-2	6.8ˆe-5
ND2	1	8T, 0C	30T, 0C	17T, 43C	L	L	L	7.8ˆe-5	1.4ˆe-10
	2	8T, 0C	30T, 0C	17T, 43C	**V**	**V**	**A**	7.8ˆe-5	1.4ˆe-10
trnD	1	8T, 0C	30T, 0C	17T, 43C	/	/	/	7.8ˆe-5	1.4ˆe-10
ATP6	1	8T, 0C	26T, 4C	22T, 38C	L	L	L	7.0ˆe-4	7.4ˆe-6
	2	0A, 8G	3A, 27G	43A, 17G	**M**	**M**	**I**	7.8ˆe-5	3.0ˆe-8
COX2	1	8G, 0A	30G, 0A	22G, 36A	**M**	**M**	**I**	9.5ˆe-4	2.0ˆe-8
	2	8T, 0G	30T, 0G	17T, 41G	A	A	A	1.1ˆe-4	3.0ˆe-10

Note: Letters in bold represent amino acid changes caused by missense mutations.

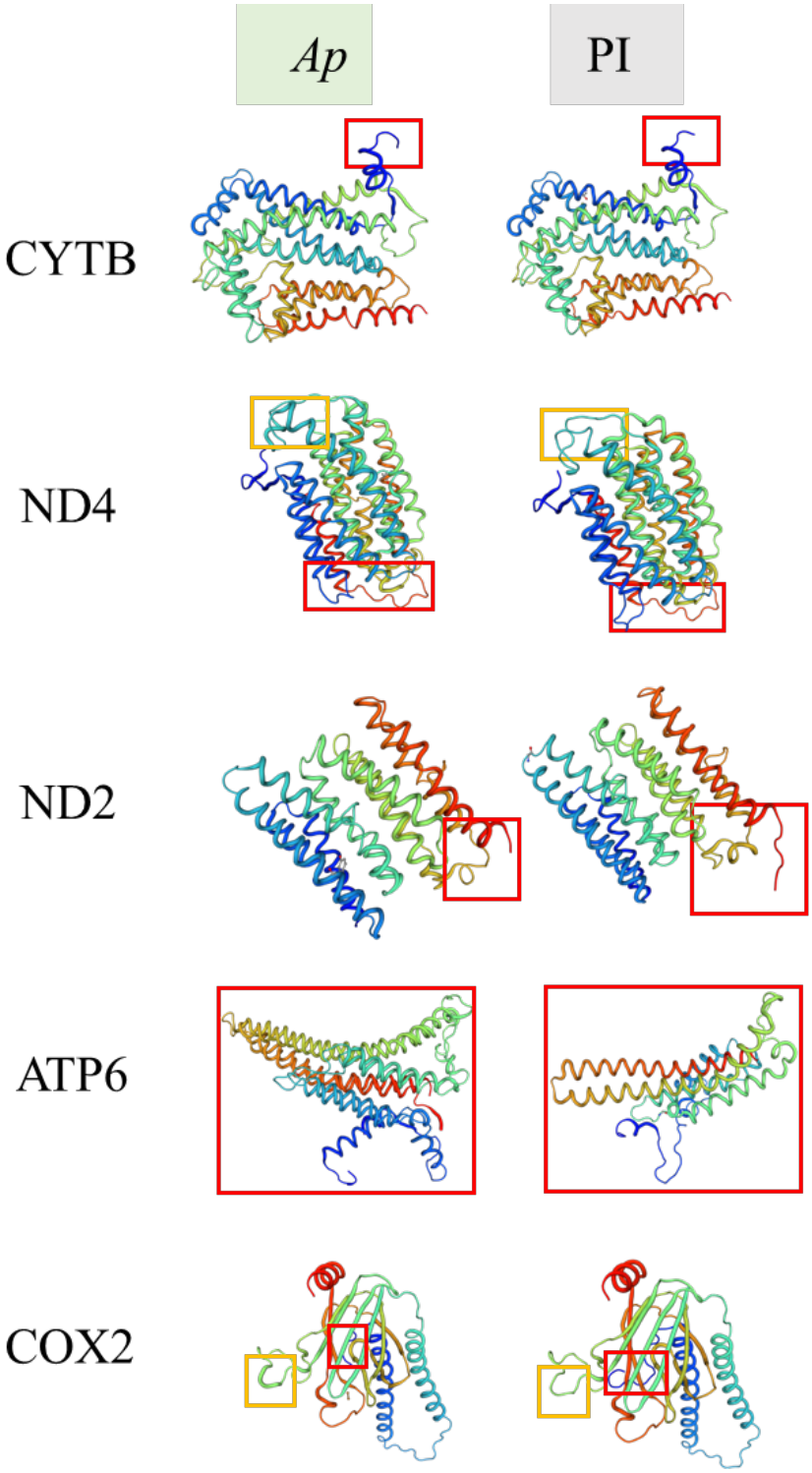

Figure 6 The tertiary structure of five proteins with SNP mutations in PI (Peruvian scallop × bay scallop

) F_1 hybrids compared with Peruvian scallop (*Ap*). The red and yellow boxes represent the differences between Peruvian scallop and PI F_1hybrids.

Compared with Peruvian scallop, there were four new SNP haplotypes in *COX2* gene in PI F_1 hybrids, among which GAG was the dominant haplotype. Meanwhile, three new SNP haplotypes were found in *ND4* , with CCCA and CCC- as the dominant haplotypes; two new SNP haplotypes were found in *ND2* , with TCC as the dominant haplotype; and six new SNP haplotypes were found in *ATP6* , with CCCA as the dominant haplotype (Table 4). Among the SNPs in the five new dominant haplotypes, 80% was transition type, which was predicted to result in changes in amino acid sequence (Figure 7).

Table 4 Significantly different SNP haplotypes in four genes between PI F_1 hybrids and 'Bohai Red' and Peruvian scallop (*Ap*).

Scallop species Gene name	*Ap*	Bohai Red	PI
COX2	8 AGT	30 TGT	**36 GAG** 7 GGT 3 TGT 7 AGT 5 GGG
ND4	8 TTTG	18 TTTG 2 CCCG 2 CCC- 1 TTCG 1 TTT-	**20 CCC- 17 CCCA** 13 TCTG 7 TTTG
ND2	8 CTT	30 CTT	**38 TCC** 5 CCC 17 CTT
ATP6	8 TTTG	17 CTTG 1 CTCG 3 CTCA 1 CCTG	**24 CCCA** 5 TTTG 9 TCTG 2 TTTA 11 CTCA 2 TCTA 2 CTTG

Note: Letters in bold represent the specific dominant SNP haplotypes in PI F_1 hybrids.

Figure 7 Amino acid sequences of four genes with SNP haplotypes that were different between PI F_1 hybrids (Peruvian scallop × bay scallop) and Peruvian scallop (*Ap*). The letters in red represent the different amino acids.

3.5 Comparative transcriptomic analyses between the PI F_1 hybrids and Peruvian scallops

Transcriptomic analyses between the PI F_1 hybrids and Peruvian scallops revealed 260 differentially expressed nuclear genes with 199 up-regulated and 61 down-regulated. Among them, 16 nuclear genes (*SCO1* , *SDHB* , *ATPAF2* , *NDUFS3* ,*NDUFS4* , *NDUFS7* , *ATPsynB* , *ATPsynF* ,*ATPsyngamma* , *ATP5F1B* , *ATP5MC2* , *ATPsynO* ,*ATPsynCF6* , *atp23* , *ATP5F1A* and *ATP5f1d*) involved in mitochondrial ATP synthesis and mitochondrial respiratory chain enzyme complex synthesis were down-regulated in F_1 hybrids. In addition, among 6 nuclear genes related to mitochondria regulation, five (*dGK* ,*TK2* , *COX11* , *POLG* and *POLG2*) were significantly down-regulated and one (*MTCH2*) was up-regulated in F_1 hybrids. Among 7 nuclear genes that were involved in mitochondrial apoptosis, one (*CDK2*) was significantly down-regulated while 6 (*VDAC2* , *BAX* , *AIFM3* ,*ENDOG* , *Mfn2* and *PARP1*) were up-regulated in F_1 hybrids (Table 5).

Table 5 Differentially expressed genes in PI F_1 hybrids (Peruvian scallop x bay scallop).

Function	Gene	Description
mitochondrial respiratory chain enzyme complex synthesis	*SCO1*	protein SCO1 homolog
	SDHB	succinate dehydrogenase iron-sulfur subunit
	ATPAF2	ATP synthase mitochondrial F_1 complex ass
	NDUFS3	NADH dehydrogenase iron-sulfur protein 3
	NDUFS4	NADH dehydrogenase iron-sulfur protein 4
	NDUFS7	NADH dehydrogenase iron-sulfur protein 7
mitochondrial ATP synthesis	*ATPsynB*	ATP synthase subunit
	ATPsynF	putative ATP synthase subunit f
	ATPsyngamma	ATP synthase subunit gamma
	ATP5F1B	ATP synthase subunit beta
	ATP5MC2	ATP synthase lipid-binding protein
	ATPsynO	ATP synthase subunit O
	ATPsynCF6	ATP synthase-coupling factor 6
	atp23	mitochondrial inner membrane protease ATP
	ATP5F1A	ATP synthase subunit alpha
	ATP5f1d	ATP synthase subunit delta
mitochondria regulation	*dGK*	NADH dehydrogenase 1 alpha subcomplex su
	TK2	thymidine kinase 2
	COX11	cytochrome c oxidase assembly protein COX
	POLG	DNA polymerase subunit γ
	POLG2	DNA polymerase subunit gamma-2
	MTCH2	mitochondrial carrier homolog
Apoptosis	*VDAC2*	voltage-dependent anion-selective channel pr
	BAX	Bcl-2 protein
	Mfn2	mitofusin2
	PARP1	poly ADP-Ribose polymerase 1
	CDK2	cyclin dependent kinase 2
	AIFM3	apoptosis-inducing factor 3-like
	ENDOG	endonuclease G, mitochondrial-like

3.6 ATP contents in gonads of fertile scallops and sterile F_1 hybrids

ATP synthesized by mitochondrial genes plays important roles in sperm production and motility. To elucidate the involvement of ATP production in determination of fertility, ATP content was examined in the gonads of fertile scallops (bay scallops, Peruvian scallops and 'Bohai Red') and sterile hybrids (IP and PI F_1 hybrids) at mature stage. The results showed that the ATP contents in the gonads of sterile hybrids (PI and IP F_1 hybrids) were significantly lower than that in the fertile scallops (bay scallops, Peruvian scallops and 'Bohai Red') ($P < 0.001$). The ATP content in 'Bohai Red' scallops was significantly higher than that in bay scallops and Peruvian scallops ($P < 0.0001$) (Figure 8). There were no difference between bay scallop and Peruvian scallop ($P > 0.05$), and no difference between PI and IP F_1 hybrids ($P > 0.05$).

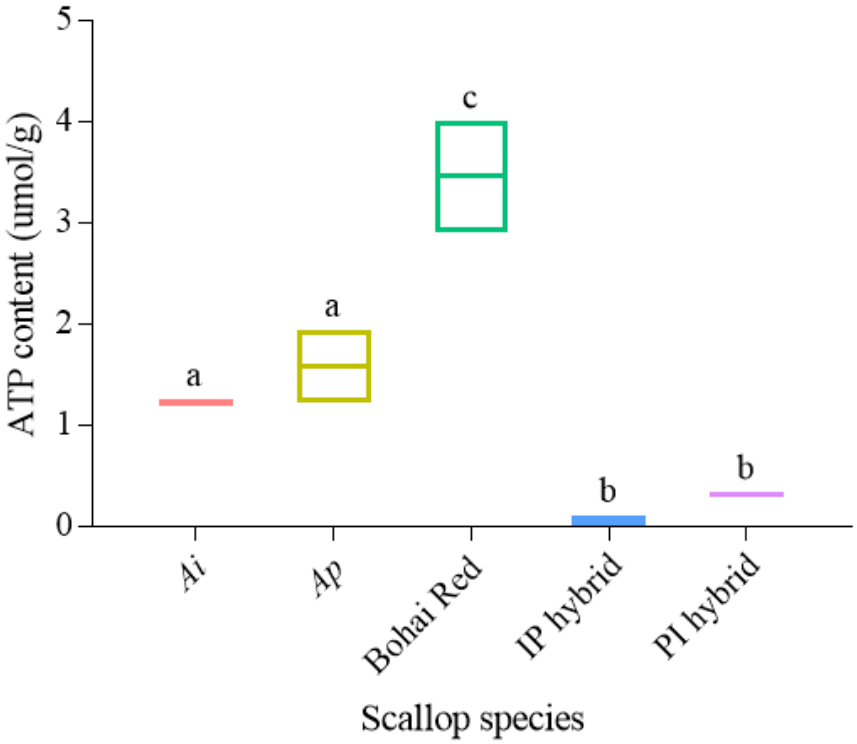

Figure 8 ATP content in mature gonads of bay scallops (*Ai*), Peruvian scallops (*Ap*), 'Bohai Red', IP (bay scallop × Peruvian scallop) and PI (Peruvian scallop x bay scallop) F_1hybrids. Different letters over any two bars indicate significant difference between them.

4 DISCUSSION

Production of sterile F_1 hybrids from the two hermaphroditic scallops provided precious genetic resources for stock improvement in scallops and promoted the speciation. From these sterile F_1 hybrids, at least three new varieties, including 'Bohai Red', 'QN-2' and 'QN Orange' were obtained (Wang *et al.* , 2017; Xu *et al.* , 2019; Chen *et al.* , 2020). It is also possible to establish the first three-line breeding systems (male sterile line, maintainer line and restorer line) in hermaphroditic animals, like those obtained in hermaphroditic plants such as rice and soybean (Wang and Deng, 2018; Chen *et al.* , 2021). However, little information is available on the determination mechanism of sterility in the hermaphroditic animals. The present study firstly explored the potential roles of mitochondrial and nuclear genes in determination of sterility in the interspecific hybrids of hermaphroditic animals. Our results suggested that rearrangements and mutations of mitochondrial genes and their interaction with nuclear genes may be responsible for the energy deficiency which is directly involved in male sterility in F_1 hybrids of *Argopecten* scallops.

4.1 Inhibited energy production in F_1 hybrids caused by mutations and rearrangements in mtDNA

Our results showed that the ATP content in the sterile hybrids was significantly lower than that in the fertile scallops and transcriptomic analysis showed that some nuclear genes related to mitochondrial ATP synthesis were also down-regulated in F_1 hybrids (Table 5), suggesting that the sterility might be a direct consequence of reduced energy supply in the hybrids. Mitochondrial gene deletions, insertions and translocations caused by gene rearrangements in both IP and PI F_1 hybrid scallops might contribute to the reduction in energy production. *ATP8* gene played an important role in oxidative phosphorylation and was involved in sperm motility. Mutations in *ATP8* could reduce the motility of sperm and cause oligodysmoospermia

leading to male sterility (Ni, 2017). In PI F_1 hybrid scallops, the deletion of *ATP8* might cause reduction in energy production in mitochondria and affect sperm motility. Insertions of *trnG* , *trnF* , *trnK* ,*trnE* and *trnL* genes in PI F_1 hybrid scallops might improve the abundance of tRNA, which affected the synthesis of mitochondrial proteins (Kane, 2019). Mutations in*trnS* have been reported to reduce the synthesis of mitochondrial proteins and the activity of respiratory chain, resulting in decreased motility of sperm (Lou *et al.* , 2013). In IP F_1hybrid scallops, the deletion of *trnS1* and *trnS2* might affect protein synthesis by limiting the transportation of serine. In PI F_1 hybrid scallops, the translocations of *12S* and*16S* genes may also alter expression of mitochondrial genes by changing the sequences of mitochondrial genes. Besides, the *ND4*and *ND2* genes located in the upstream and downstream of the control regions were rich in mutations. Mutations in *DN4* were found to be an important cause of decreased sperm motility in patients with oligoasthenozoospermia (Sawyer *et al.* , 2003). The mutations in *ND2* gene in asthenospermia patients had a high frequency of mutations might cause changes in amino acids and result in abnormal energy metabolism in mitochondria (Zheng *et al.* , 2010).

In PI F_1 hybrids, 29 SNPs were detected in the mitochondrial genes of *CYTB* , *ND4* , *ND2* , *ATP6* ,*COX2* and *trnD* , which were known to be involved in electron transport chain and oxidative phosphorylation process. Mutations in these genes have been reported to induce cytoplasmic male sterility by limiting energy supply from mitochondria (Akagi *et al.* , 1994; Spiropoulos *et al.* , 2002; Wei *et al.* , 2005; Wei *et al.* , 2005; Wu *et al.* , 2012; Wolff *et al.* , 2016; Wu*et al.* , 2019; Dahadhah *et al.* , 2021). TRNA with mutations might fail to bind mRNA normally, affect the entire translation mechanism and resulted in a reduced synthesis rate of respiratory chain subunit (Florentz *et al.* , 2003). Sperm motility and maturation may thus be affected by changes in energy supply from mitochondria caused by mutations in these genes (Zhang *et al.* , 2013; Zhao*et al.* , 2021). Furthermore, we found that the allele frequencies of 13 SNPs and 5 new SNP haplotypes in these genes were significantly different between fertile scallops and sterile F_1hybrids, and the type of these SNP mutations was mainly transition that have been previously proved to be related to asthenozoospermia (Nakada*et al.* , 2006; Kucharczyk *et al.* , 2009; Copeland, 2012; Baklouti-Gargouri *et al.* , 2014).

In summary, the variations resulted from rearrangements and mutations in mitochondria might affect the cellular respiration intensity and ATP synthesis, leading to insufficiency of energy supply (Dzudzor *et al.* , 2021; Li *et al.* , 2021).

4.2 Apoptosis and cell cycle arrest in F_1hybrids

Once the normal intracellular respiratory pathway is blocked, the structure and function of the mitochondrial respiratory chain would be inevitably affected and resulted in production of intracellular ROS and cell death as well as the formation of gamete cells in sterile hybrid scallops (Balk and Leaver, 2001; Hauser *et al.* , 2006). Transcriptomic analysis showed that the interaction between nuclear genes and mitochondrial genes might also alter fertility of hybrid scallops by apoptosis pathway. The pro-apoptotic protein *Mfn2* and*BAX* , apoptotic factors *CysC* , *AIFM3* , and*EndoG* were all highly expressed in F_1 hybrids compared with Peruvian scallops. The increased expression of *Mfn2*could up-regulate the expression of *BAX* , which might promote release of pro-apoptotic factors *CytC* , *AIF* and*EndoG* into cytoplasm, further activate the cell apoptosis induced by mitochondria apoptosis, decrease mitochondrial membrane potential and increase of mitochondrial membrane permeability (Cande *et al.* , 2004; Green and Kroemer, 2004; Schafer *et al.* , 2004; van Delft and Huang, 2006; Lin *et al.* , 2007). Increased expression of *Mfn2* gene could also inhibit the expression of *CDK2* gene, which induced the cell cycle arrest in G1 phase and inhibition of oocyte maturation (Chen *et al.* , 2003; Miele *et al.* , 2005; Wang *et al.* , 2012). Down-regulation of*Mfn2* and *CDK2* might be involved in sterility of F_1 hybrids by inhibiting the development of oocytes and spermatids.

4.3 Mitochondrial dysfunction induced by altered nuclear genes expression

Besides the apoptosis pathway, expression of some nuclear genes related to mitochondria regulation, including *MTCH2* , *dGK* ,*TK2* , *COX11* , *POLG* , *PARP1* and *VDAC2* , were also affected in sterile hybrid scallops. Enhanced expression of*MTCH2* in sterile F_1 hybrids could inhibit mitochondrial metabolism (Kraja *et al.* , 2019). Down regulation of*dGK* and *TK2* might lead to depletion of mtDNA in sterile F_1 hybrids

(Spinazzola and Zeviani, 2007). The down-regulation of *COX11* reduced the production of mitochondrial ATP and inhibited the mitochondrial respiratory chain complex IV from assembling into functional protein complexes (Tzagoloff *et al.* , 1990; Olsson *et al.* , 2011). Besides, sixteen nuclear genes that involved in mitochondrial ATP synthesis and mitochondrial respiratory chain enzyme complex synthesis were down-regulated in F_1hybrids, which reduced the production of ATP. This was consistent with our finding that the ATP content in the sterile hybrids was significantly lower than that in the fertile scallops. The functional defects of ATP synthesis in the sterile hybrids might result in production of intracellular ROS and cell death (Balk and Leaver, 2001; Hauser *et al.* , 2006), affecting the formation of gamete cells in these sterile hybrid scallops. As the only DNA polymerase in mitochondria, the inhibited expression of *POLG* in sterile PI F_1 hybrids might cause mitochondrial dysfunction (Spinazzola and Zeviani, 2007; Kraja *et al.* , 2019). Up-regulated expression of *PARP1* might enhance the synthesis of PAR polymers and promote the binding and releasing of *AIF* , which might cause DNA fragmentation in F_1 hybrids (Wang *et al.* , 2011; Lee *et al.* , 2013). *VDAC2* on the mitochondrial membrane recruited and interacted with *BAX* to promote apoptosis (Green and Kroemer, 2004) and increased expression of *VDAC2* has been reported to be associated with asthenozoospermia in human(Liu*et al.* , 2010). It is possible that the up-regulation of*VDAC2* inhibits spermatogenesis in F_1 hybrids.

In summary, the abnormal expression of these nuclear genes was closely related to mitochondrial function regulation, suggesting that they might cause abnormal mitochondrial metabolism, mtDNA depletion, abnormal synthesis of respiratory complexes, and insufficient energy supply from mitochondrial in sterile F_1 hybrids. Therefore, the mutations and rearrangements of mtDNA might cause changes in the expression of both mitochondrial and nuclear genes, as well as their interaction, and eventually lead to sterility in F_1hybrids.

5 CONCLUSION

Reproductive isolation is a product in the evolution of species and sterility is a complex biological phenomenon widely existing in hermaphroditic plants and some dioecious animals. In this study, we found sterility in the interspecific F_1 hybrids between hermaphroditic bay scallops and Peruvian scallops for the first time. We further revealed that mutations and rearrangements of mitochondrial genome DNA might cause the abnormal expression of nuclear genes, and abnormal interaction between the mitochondria and nuclear genes might result in five potential pathways contributing to sterility of the F_1 hybrids: (1) higher expression of *Mfn2*inhibiting oocyte maturation, (2) down expression of *CDK2* leading to cell cycle arrest in G1 phase of oocyte, (3) cell apoptosis induced by mitochondrial apoptosis, (4) insufficient energy supply from abnormal mitochondria, and (5) mitochondrial dysfunction resulted from abnormal expression of other nuclear genes (Figure 9). These results might provide new insights into understanding of reproductive isolation and speciation in molluscs.

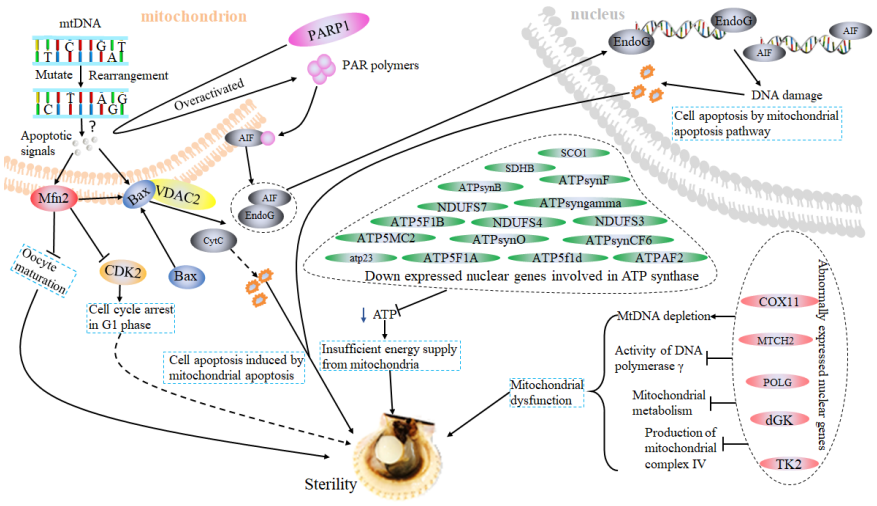

Figure 9 Potential regulatory network on sterility of F_1 hybrid scallops. The solid lines represent the direct effect, the dotted lines represent the indirect effect, arrows represent the accelerating effect and blunt arrows represent the inhibiting effect.

ACKNOWLEDGMENTS

This project was financially supported by the National Natural Science Foundation of China (No. 31972791); the Earmarked Fund for Agriculture Seed Improvement Project of Shandong Province (No. 2020LZGC016); the Scientific and Technological Project of Yantai, Shandong Province (No. 2022XCZX083); the Earmarked Fund for Shandong Modern Agro-Industry Technology Research System (No. SDAIT-14).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

X.L. and C.W. designed the study. T.Y. performed the experiments and analyzed the results. T.Y. and X.L.wrote the manuscript. N.J., C.M., W.Y., L.G., W.Q., and X.X. provided materials. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The raw data for RNA-seq had been deposited in the NCBI Sequence Read Archive (SRA) database with accession no. PRJNA869841.

REFERENCES

Agren, J. A., M. Munasinghe and A. G. Clark (2019). Sexual conflict through mother's curse and father's curse. Theor Popul Biol 129: 9-17.

Akagi, H., M. Sakamoto, C. Shinjyo, H. Shimada and T. Fujimura (1994). A unique sequence located downstream from the rice mitochondrial atp6 may cause male sterility. Curr Genet 25(1): 52-58.

Baklouti-Gargouri, S., M. Ghorbel, A. Ben Mahmoud, E. Mkaouar-Rebai, M. Cherif, N. Chakroun, A. Sellami, F. Fakhfakh and L. Ammar-Keskes (2014). Identification of a novel m.9588G > a missense mutation in the mitochondrial COIII gene in asthenozoospermic Tunisian infertile men. J Assist Reprod Genet 31(5): 595-600.

Balk, J. and C. J. Leaver (2001). The PET1-CMS mitochondrial mutation in sunflower is associated with premature programmed cell death and cytochrome c release. Plant Cell 13(8): 1803-1818.

Breton, S., H. D. Beaupre, D. T. Stewart, W. R. Hoeh and P. U. Blier (2007). The unusual system of doubly uniparental inheritance of mtDNA: isn't one enough? Trends Genet 23(9): 465-474.

Butow, R. A. and N. G. Avadhani (2004). Mitochondrial signaling: the retrograde response. Mol Cell 14(1): 1-15.

Cande, C., N. Vahsen, C. Garrido and G. Kroemer (2004). Apoptosis-inducing factor (AIF): caspase-independent after all. Cell Death Differ 11(6): 591-595.

Chen, H., S. A. Detmer, A. J. Ewald, E. E. Griffin, S. E. Fraser and D. C. Chan (2003). Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. J Cell Biol 160(2): 189-200.

Chen, L. and Y. G. Liu (2014). Male sterility and fertility restoration in crops. Annu Rev Plant Biol 65: 579-606.

Chen, M., B. Liu, B. Ma, G. Liu and C. J. A. Wang (2020). Selection of a carotenoid-rich scallop strain, QN Orange, from the inter-specific hybrids between the bay scallop and the Peruvian scallop. 528: 735513.

Chen, X., S. Yang, Y. Zhang, X. Zhu, X. Yang, C. Zhang, H. Li and X. Feng (2021). Generation of male-sterile soybean lines with the CRISPR/Cas9 system. The Crop Journal. 9(6): 1270-1277.

Copeland, W. C. (2012). Defects in mitochondrial DNA replication and human disease. Crit Rev Biochem Mol Biol 47(1): 64-74.

Dahadhah, F. W., M. S. Jaweesh, M. S. Al Zoubi, M. I. A. Alarjah, M. E. Hammadeh and H. Amor (2021). Mitochondrial nicotinamide adenine dinucleotide hydride dehydrogenase (NADH) subunit 4 (MTND4) polymorphisms and their association with male infertility. J Assist Reprod Genet 38(8): 2021-2029.

Degletagne, C., D. Abele, G. Glockner, B. Alric, H. Gruber and C. Held (2021). Presence of male mitochondria in somatic tissues and their functional importance at the whole animal level in the marine bivalve Arctica islandica. Commun Biol 4(1): 1104.

Dong, W., D. Wu, C. Yan and D. Wu (2021). Mapping and analysis of a novel genic male sterility gene in watermelon (Citrullus lanatus). Front Plant Sci 12: 639431.

Dzudzor, B., B. Bimah, V. Amarh and A. Ocloo (2021). Sperm parameters and mitochondrial DNA sequence variants among patients at a fertility clinic in Ghana. PLoS One 16(6): e0252923.

Eslamieh, M., A. Mirsalehi, D. N. Markova and E. Betran (2022). COX4-like, a nuclear-Encoded mitochondrial gene duplicate, is essential for male fertility in Drosophila melanogaster. Genes (Basel) 13(3).

Feng, W., J. Li, D. Cai, C. Wang and L. Nan (2012). Comparison of development and growth of the F1 and F2 hybrids between the Peruvian and bay Scallop. Chinese Agricultural Science Bulletin 28(20): 118-124.

Florentz, C., B. Sohm, P. Tryoen-Toth, J. Putz and M. Sissler (2003). Human mitochondrial tRNAs in health and disease. Cell Mol Life Sci 60(7): 1356-1375.

Frank, S. A. and L. D. Hurst (1996). Mitochondria and male disease. Nature 383(6597): 224.

Green, D. R. and G. Kroemer (2004). The pathophysiology of mitochondrial cell death. Science 305(5684): 626-629.

Grosser, J. A., R. L. Fehrman, D. Keefe, M. Redmon and R. W. Nickells (2021). The effects of a mitochondrial targeted peptide (elamipretide/SS31) on BAX recruitment and activation during apoptosis. BMC Res Notes 14(1): 198.

Hao, M., W. Yang, W. Lu, L. Sun, M. Shoaib, J. Sun, D. Liu, X. Li and A. Zhang (2021). Characterization of the mitochondrial genome of a wheat AL-type male sterility line and the candidate CMS gene. Int J Mol Sci 22(12).

Hauser, B. A., K. Sun, D. G. Oppenheimer and T. L. Sage (2006). Changes in mitochondrial membrane potential and accumulation of reactive oxygen species precede ultrastructural changes during ovule abortion. Planta 223(3): 492-499.

Heidari, M. M., M. Khatami, A. Danafar, T. Dianat, G. Farahmand and A. R. Talebi (2016). Mitochondrial Genetic variation in iranian infertile men with varicocele. Int J Fertil Steril 10(3): 303-309.

Kane, J. F. (2019). Effects of rare codon clusters on high-level expression of heterologous proteins in escherichia coil. Curr Opin Biotechnol 6(5):494-500.

Kim, Y. J. and D. Zhang (2018). Molecular control of male fertility for crop hybrid breeding. Trends Plant Sci 23(1): 53-65.

Kraja, A. T., C. Liu, J. L. Fetterman, M. Graff, C. T. Have, C. Gu, L. R. Yanek, M. F. Feitosa, D. E. Arking, *et al.* (2019). Associations of mitochondrial and nuclear mitochondrial variants and genes with seven metabolic traits. Am J Hum Genet 104(1): 112-138.

Kucharczyk, R., B. Salin and J. P. di Rago (2009). Introducing the human Leigh syndrome mutation T9176G into Saccharomyces cerevisiae mitochondrial DNA leads to severe defects in the incorporation of Atp6p into the ATP synthase and in the mitochondrial morphology. Hum Mol Genet 18(15): 2889-2898.

Kurhanewicz, N. A., D. Dinwiddie, Z. D. Bush and D. E. Libuda (2020). Elevated temperatures cause transposon-associated DNA damage in C. elegans spermatocytes. Curr Biol 30(24): 5007-5017 e5004.

Lam, E. (2004). Controlled cell death, plant survival and development. Nat Rev Mol Cell Biol 5(4): 305-315.

Lee, Y., S. S. Karuppagounder, J. H. Shin, Y. I. Lee, H. S. Ko, D. Swing, H. Jiang, S. U. Kang, B. D. Lee, *et al.* (2013). Parthanatos mediates AIMP2-activated age-dependent dopaminergic neuronal loss. Nat Neurosci 16(10): 1392-1400.

Li, M., L. Chen, A. Khan, X. Kong, M. R. Khan, M. J. Rao, J. Wang, L. Wang and R. Zhou (2021). Transcriptome and MiRNAomics analyses identify genes associated with cytoplasmic male sterility in cotton (Gossypium hirsutum L.). Int J Mol Sci 22(9).

Lin, H. H., J. H. Chen, C. C. Huang and C. J. Wang (2007). Apoptotic effect of 3,4-dihydroxybenzoic acid on human gastric carcinoma cells involving JNK/p38 MAPK signaling activation. Int J Cancer 120(11): 2306-2316.

Liu, B., P. Wang, Z. Wang, Y. Jia, X. Niu, W. Wang and W. Zhang (2010). Analysis and difference of voltage-dependent anion channel mRNA in ejaculated spermatozoa from normozoospermic fertile donors and infertile patients with idiopathic asthenozoospermia. J Assist Reprod Genet 27(12): 719-724.

Lou, Z., Z. Yang, P. Li, J. Zheng, L. Zhang, X. Yang, X. Huang and L. Jin (2013). Mutation of mitochondrial $tRNA^{His}$, $tRNA^{Ser(AGY)}$ and $tRNA^{Leu(CUN)}$ gene in asthenospermia patients. Chinese Journal of Urology 34(06): 417-421 (Abstract in English).

Luo, D., H. Xu, Z. Liu, J. Guo, H. Li, L. Chen, C. Fang, Q. Zhang, M. Bai, *et al.* (2013). A detrimental mitochondrial-nuclear interaction causes cytoplasmic male sterility in rice. Nat Genet 45(5): 573-577.

Miele, A., C. D. Braastad, W. F. Holmes, P. Mitra, R. Medina, R. Xie, S. K. Zaidi, X. Ye, Y. Wei, *et al.* (2005). HiNF-P directly links the cyclin E/CDK2/p220NPAT pathway to histone H4 gene regulation at the G1/S phase cell cycle transition. Mol Cell Biol 25(14): 6140-6153.

Nakada, K., A. Sato and J. I. Hayashi (2006). Mitochondria-related male infertility. Neuromuscular disorders 16: S58-S58

Ni, F. (2017). Genetic study on male infertility and female unexplained recurrent spontaneous abortion in Chinese population. Ph.D, Anhui Medical University (Abstract in English).

Olsson, A. H., B. T. Yang, E. Hall, J. Taneera, A. Salehi, M. D. Nitert and C. Ling (2011). Decreased expression of genes involved in oxidative phosphorylation in human pancreatic islets from patients with type 2 diabetes. Eur J Endocrinol 165(4): 589-595.

Ouyang, Y., Y. G. Liu and Q. Zhang (2010). Hybrid sterility in plant: stories from rice. Curr Opin Plant Biol 13(2): 186-192.

Piomboni, P., R. Focarelli, A. Stendardi, A. Ferramosca and V. Zara (2012). The role of mitochondria in energy production for human sperm motility. Int J Androl 35(2): 109-124.

Sawamura, K., J. Roote, C. I. Wu and M. T. Yamamoto (2004). Genetic complexity underlying hybrid male sterility in Drosophila. Genetics 166(2): 789-796.

Sawyer, D. E., B. G. Mercer, A. M. Wiklendt and R. J. Aitken (2003). Quantitative analysis of gene-specific DNA damage in human spermatozoa. 529(1-2): 21-34.

Schafer, P., S. R. Scholz, O. Gimadutdinow, I. A. Cymerman, J. M. Bujnicki, A. Ruiz-Carrillo, A. Pingoud and G. Meiss (2004). Structural and functional characterization of mitochondrial EndoG, a sugar non-specific nuclease which plays an important role during apoptosis. J Mol Biol 338(2): 217-228.

Shen, R., L. Wang, X. Liu, J. Wu, W. Jin, X. Zhao, X. Xie, Q. Zhu, H. Tang*, et al.* (2017). Genomic structural variation-mediated allelic suppression causes hybrid male sterility in rice. Nat Commun 8(1): 1310.

Song, S. Y. (2018). Study on the influencing factors of fertilization rate of interspecific hybridization between Haliotis discus hannai and Haliotis fulgens. Master, Xiamen University (Abstract in English).

Spinazzola, A. and M. Zeviani (2007). Disorders of nuclear-mitochondrial intergenomic communication. Biosci Rep 27(1-3): 39-51.

Spiropoulos, J., D. M. Turnbull and P. F. Chinnery (2002). Can mitochondrial DNA mutations cause sperm dysfunction? Mol Hum Reprod 8(8): 719-721.

Steiner, C. C. and O. A. Ryder (2013). Characterization of Prdm9 in equids and sterility in mules. PLoS One 8(4): e61746.

Stewart, D. T., B. M. Robicheau, N. Youssef, M. A. Garrido-Ramos, E. E. Chase and S. Breton (2021). Expanding the Search for Sperm Transmission elements in the mitochondrial genomes of bivalve mollusks. Genes (Basel) 12(8).

Tzagoloff, A., N. Capitanio, M. P. Nobrega and D. Gatti (1990). Cytochrome oxidase assembly in yeast requires the product of COX11, a homolog of the P. denitrificans protein encoded by ORF3. EMBO J 9(9): 2759-2764.

van Delft, M. F. and D. C. Huang (2006). How the Bcl-2 family of proteins interact to regulate apoptosis. Cell Res 16(2): 203-213.

Vaught, R. C. and D. K. Dowling (2018). Maternal inheritance of mitochondria: implications for male fertility? Reproduction 155(4): R159-R168.

Wang, C., B. Liu, J. Li, S. Liu, J. Li, L. Hu, F. Xiao, H. Du and H. J. A. Fang (2011). Introduction of the Peruvian scallop and its hybridization with the bay scallop in China. 310(3-4): 380-387.

Wang, C., B. Liu, X. Liu, B. Ma, Y. Zhao, X. Zhao, F. Liu and G. Liu (2017). Selection of a new scallop strain, the Bohai Red, from the hybrid between the bay scallop and the Peruvian scallop. Aquaculture 479: 250-255.

Wang, H. and X. W. Deng (2018). Development of the "third-generation" hybrid rice in China. Genomics Proteomics Bioinformatics 16(6): 393-396.

Wang, W., J. Lu, F. Zhu, J. Wei, C. Jia, Y. Zhang, L. Zhou, H. Xie and S. Zheng (2012). Pro-apoptotic and anti-proliferative effects of mitofusin-2 via Bax signaling in hepatocellular carcinoma cells. Med Oncol 29(1): 70-76.

Wang, Y., N. S. Kim, J. F. Haince, H. C. Kang, K. K. David, S. A. Andrabi, G. G. Poirier, V. L. Dawson and T. M. Dawson (2011). Poly(ADP-ribose) (PAR) binding to apoptosis-inducing factor is critical for PAR polymerase-1-dependent cell death (parthanatos). Sci Signal 4(167): ra20.

Wei, W. L., H. Z. Wang and G. H. Liu (2005). Advanced biological studies on the cytoplasmic male sterility and its fertility restoration in plants. Yi Chuan 27(4): 651-658.

Wolff, J. N., M. F. Camus, D. J. Clancy and D. K. Dowling (2016). Complete mitochondrial genome sequences of thirteen globally sourced strains of fruit fly (Drosophila melanogaster) form a powerful model for mitochondrial research. Mitochondrial DNA A DNA Mapp Seq Anal 27(6): 4672-4674.

Wolff, J. N., N. J. Gemmell, D. M. Tompkins and D. K. Dowling (2017). Introduction of a male-harming mitochondrial haplotype via 'Trojan Females' achieves population suppression in fruit flies. Elife 6.

Wu, Z., J. Cheng, X. Tang, J. Cui and K. Hu (2012). Editing sites in transcript of mitochondrial gene in hot pepper. Journal of Nuclear Agricultural Sciences 26 (01): 49-53.

Wu, Z., K. Hu, M. Yan, L. Song, J. Wen, C. Ma, J. Shen, T. Fu, B. Yi, *et al.* (2019). Mitochondrial genome and transcriptome analysis of five alloplasmic male-sterile lines in Brassica juncea. BMC Genomics 20(1): 348.

Xu, D., C. Wang, B. Liu, M. Chen and J. J. A. R. Zhang (2019). Selection of a new scallop strain, QN-2, from the backcross of Peruvian scallop×bay scallop F1 hybrids with bay scallops.

Yu, J., G. Zhao, W. Li, Y. Zhang, P. Wang, A. Fu, L. Zhao, C. Zhang and M. Xu (2021). A single nucleotide polymorphism in an R2R3 MYB transcription factor gene triggers the male sterility in soybean ms6 (Ames1). Theor Appl Genet 134(11): 3661-3674.

Zhang, C., F. Li, X. Jia, Z. Li and Q. Feng (2013). Bioinformatics analysis of mitochondrial genes sdh3 and sdh4 of cytoplasmic male sterility in tobacco. Chinese Tobacco Science 34(3): 42-47.

Zhao, T., Y. Ma, Z. Zhang, J. Xian, X. Geng, F. Wang, J. Huang, Z. Yang, Y. Luo, *et al.* (2021). Young and early-onset dilated cardiomyopathy with malignant ventricular arrhythmia and sudden cardiac death induced by the heterozygous LDB3, MYH6, and SYNE1 missense mutations. Ann Noninvasive Electrocardiol 26(4): e12840.

Zhao, W., S. Ahmed, J. Liu, S. Ahmed, E. Quansah, T. H. Solangi, Y. Wu, Y. Yangliu, H. Wang, *et al.* (2021). Comparative iTRAQ proteomics identified proteins associated with sperm maturation between yak and cattleyak epididymis. BMC Vet Res 17(1): 255.

Zheng, J., X. Huang, Z. Yang, X. Yang, L. Zhang, J. Lv and L. Jin (2010). Analysis of the mtND2 gene polymorphisms associated with asthenospermia patients. Chinese Journal of Cell Biology 32(04): 546-554 (Abstract in English).

Zouros, E. and G. C. Rodakis (2019). Doubly uniparental inheritance of mtDNA: an unappreciated defiance of a general rule. Adv Anat Embryol Cell Biol 231: 25-49.

SUPPORTING INFORMATION

Figure S1 (A) Multiple sequence alignment of 13 IP F_1 hybrids (bay scallop x Peruvian scallop) and their parental mtDNAs.**Figure S1 (B)** Multiple sequence alignment of 10 PI F_1 hybrids (Peruvian scallop x bay scallop) and their parental mtDNAs. Note: significantly different loci are indicated by red boxes.**Figure S2** Mitochondrial circle maps of bay scallop, Peruvian scallop, IP (bay scallop x Peruvian scallop) and PI (Peruvian scallop x bay scallop) F_1 hybrids.

Figure legends

Figure 1 Morphological observation of the gonads of bay scallop (*Ai*), Peruvian scallop (*Ap*), 'Bohai Red', IP (bay scallop x Peruvian scallop) and PI (Peruvian scallop x bay scallop) F_1 hybrids. Note that the F_1 had gonads with transparent male parts and well-developed female parts in IP (a) and PI (a) or only fully-developed female part that occupied the whole gonads in IP (b) and PI (b). The yellow coil indicates the female parts and the blue coil indicates the male parts.

Figure 2 Histological observation of the gonads of the Peruvian scallop, bay scallop, Scallop Bohai Red, and the F_1hybrids (bay scallop x Peruvian scallop , IP, and Peruvian scallop x bay scallop , PI). Female part on the top and male part on the bottom. Og, oogonium; Oc, oocyte; Mo, mature oocyte; Sg, spermatogonium; Sc, spermatocyte; St, spermatid.

Figure 3 PCR amplification of mitochondrial genes *NAD1* and *ATP6* in bay scallops (*Ai*), Peruvian scallops (*Ap*), and IP (bay scallop x Peruvian scallop) and PI (Peruvian scallop x bay scallop) F_1 hybrids.

Figure 4 Multiple sequence alignment of *NAD1* and*ATP6* genes between IP (bay scallop x Peruvian scallop) F_1 hybrids and their maternal parent bay scallop (*Ai*), and between PI (Peruvian scallop x bay scallop) F_1 hybrids and their maternal parent Peruvian scallop (*Ap*).

Figure 5 Gene rearrangements (A) and gene mutations in the upstream and downstream of the control region (B) in the interspecific F_1 hybrids compared with their maternal parents. (A) Translocations (represented by lines) and insertions and deletions (represented by brackets) in the mitochondrial genomes. (B) The mutations of *ND4* and *ND2* genes of IP (bay scallop x Peruvian scallop) and PI (Peruvian scallop x bay scallop) F_1 hybrids compared with their maternal parent.*Ai* , bay scallop, *Ap* , Peruvian scallop.

Figure 6 The tertiary structure of five proteins with SNP mutations in PI (Peruvian scallop x bay scallop) F_1hybrids compared with Peruvian scallop (*Ap*). The red and yellow boxes represent the differences between Peruvian scallop and PI F_1 hybrids.

Figure 7 Amino acid sequences of four genes with SNP haplotypes that were different between PI F_1 hybrids (Peruvian scallop x bay scallop) and Peruvian scallop (*Ap*). The letters in red represent the different amino acids.

Figure 8 ATP content in mature gonads of bay scallops (*Ai*), Peruvian scallops (*Ap*), 'Bohai Red', IP (bay scallop x Peruvian scallop) and PI (Peruvian scallop x bay scallop) F_1 hybrids. Different letters over any two bars indicate significant difference between them.

Figure 9 Potential regulatory network on sterility of F_1 hybrid scallops. The solid lines represent the direct effect, the dotted lines represent the indirect effect, arrows represent the accelerating effect and blunt arrows represent the inhibiting effect.

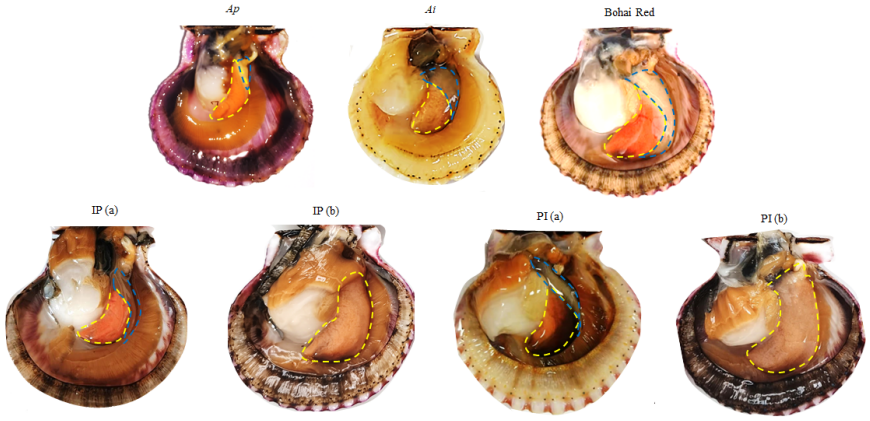

Figure 1

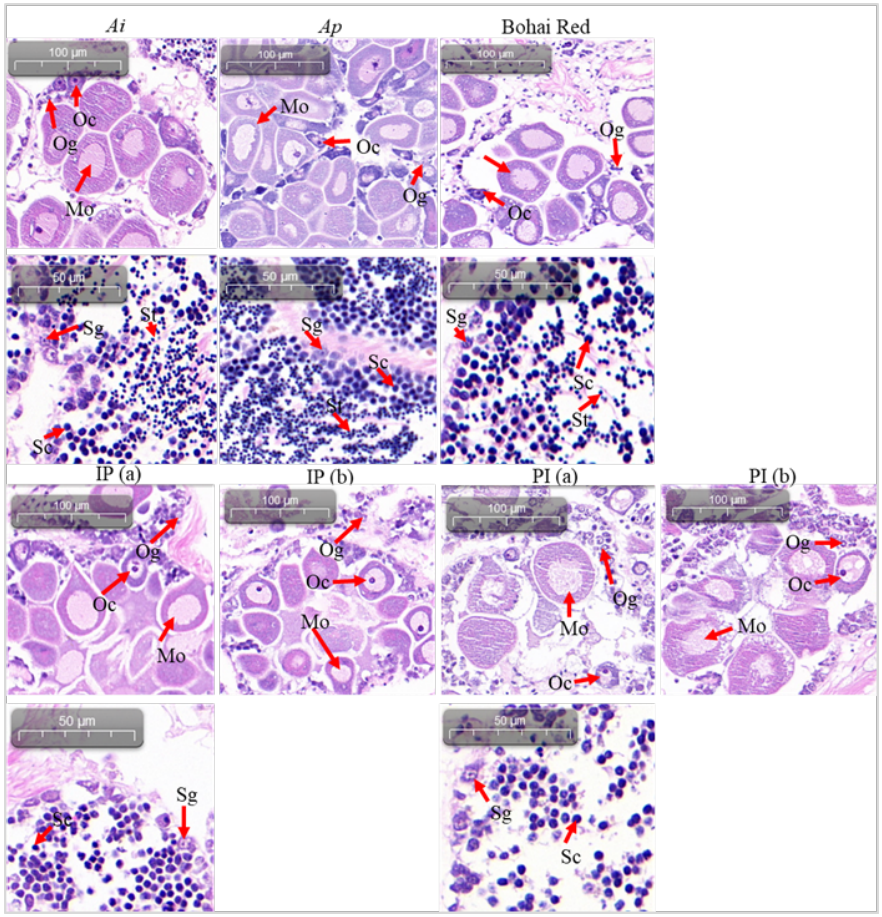

Figure 2

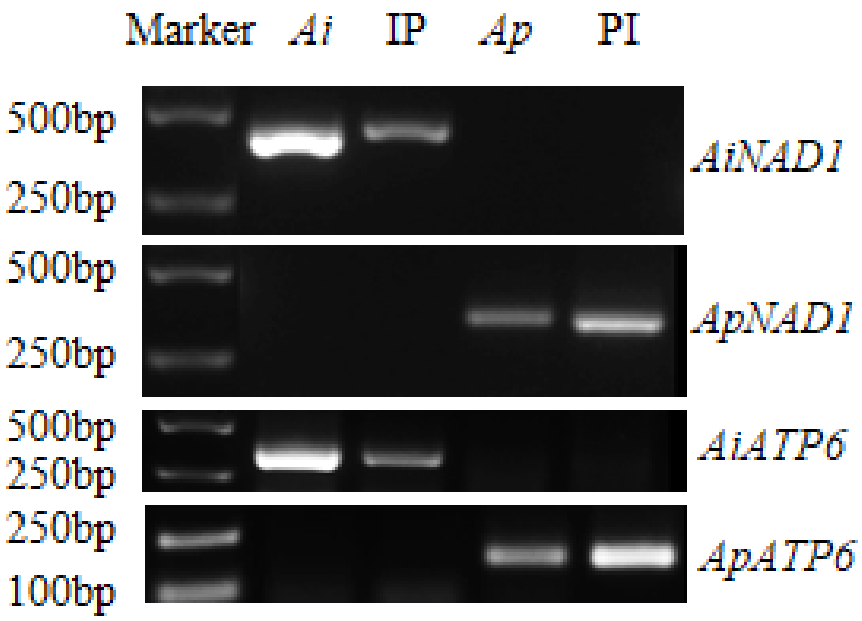

Figure 3

Figure 4

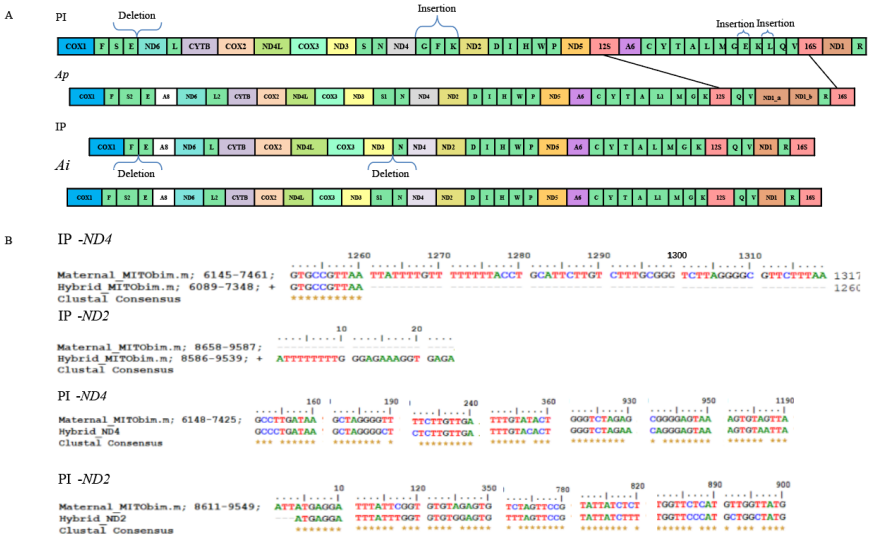

Figure 5

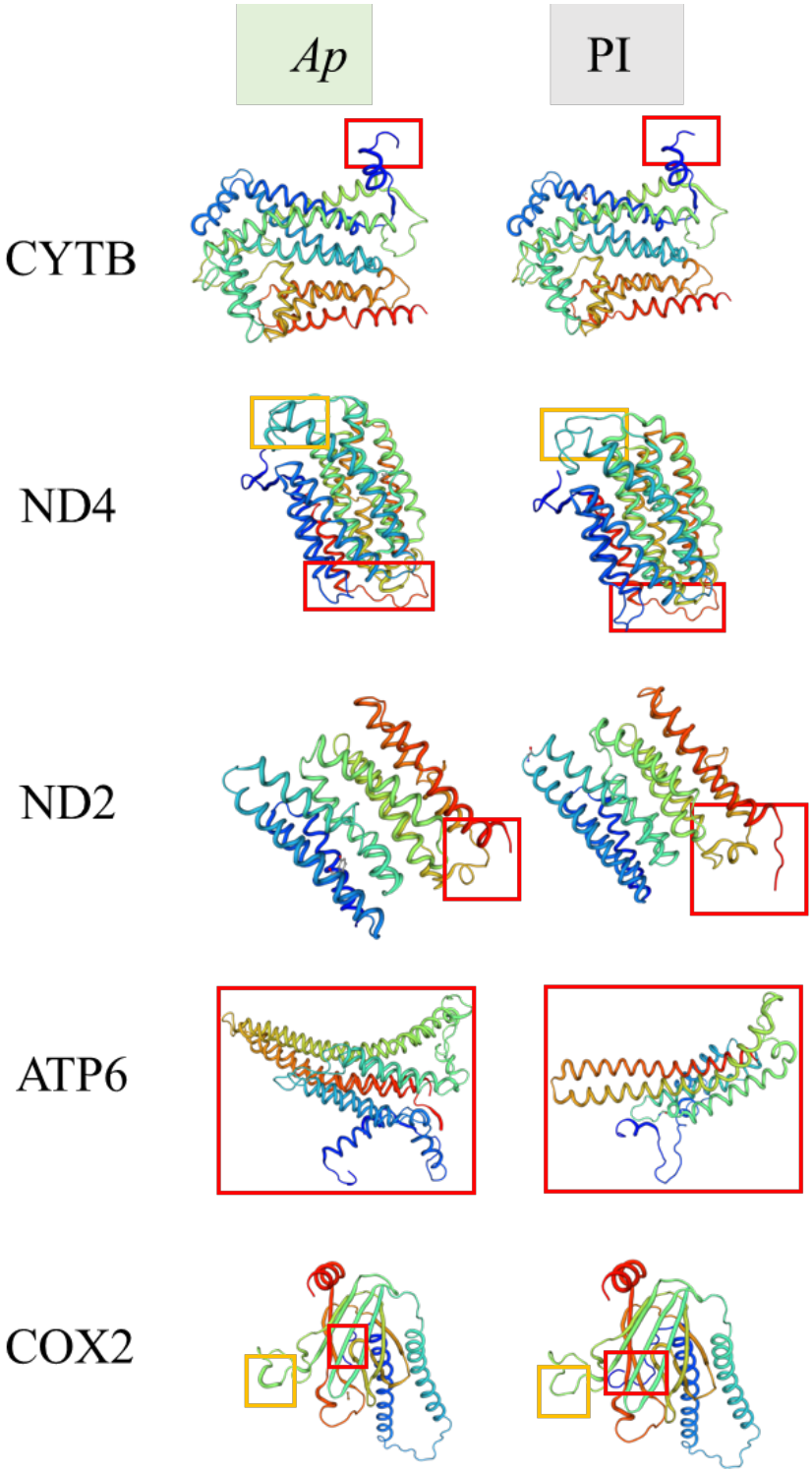

Figure 6

Figure 7

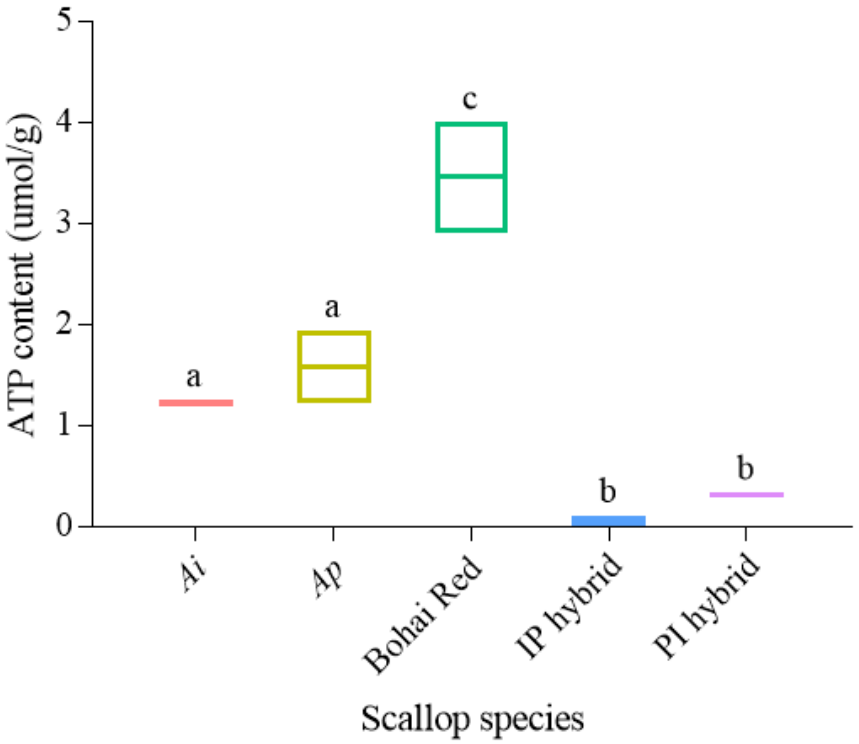

Figure 8

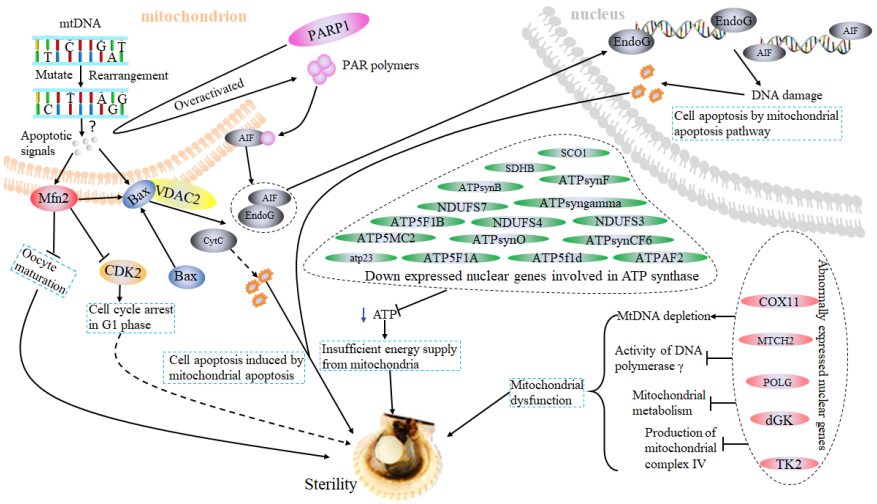

Figure 9

Table 1 The primers used in the present study.

Application	Primer Name	Sequence of primers (5'-3')	Length of target fragment (bp)	Annealing temperature and time
PCR amplification for mtDNA genes	*Ai-ATP6*	F: GTTTGC-TAAGGGT-GTTTGGG R: GCGACTATTGGGCATCAAAG	370	57 for 30s
	Ap-ATP6	F: TTAGTTGC-TACTGCTGCTG-TAGGA R: CTAAGAACTAAAAGACCGACTAGCAG	186	56 for 20s
	Ai-NAD1	F: GGGGTTTTTCAGC-CCTTTGC R: GCTCGGTTTCTCTCGGCTAA	461	55 for 30s
	Ap-NAD1	F: TAAGGGTTG-GTGTGTACGGG R: TGAAACAAGCTCTGACTCCCC	302	56 for 30s
PCR amplification for genotyping of mitochondrial SNPs	Region 1	F:TGATCCGGTTTTGTTTCAGCA R:TGGATAATCAGGGATGCGGC	655	55 for 45s
	Region 2	F:AGATCGCCTGCTAGCAAAGTGG R:AAGCCAAATCCTCATGGGCTG	816	56 for 55s
	Region 3	F:GGATTTAGTCGGCGTCTGTGG R:AAGACGCACATCGCCTCTTC	401	57 for 52s
	Region 4	F:GGAGGTATCAGGTTGGGGTTG R:TGCCTCCGAGCATAAACCAC	670	57 for 45s
	Region 5	F:GTCGTGTGCAGCCACCGAAT R:TAGCCCCCAACACGGCATA	167	56 for 20s

Application	Primer Name	Sequence of primers (5'-3')	Length of target fragment (bp)	Annealing temperature and time
	Region 6	F:GGTGGCAGTGGAC[illegible]TTTAGG R:GAGAATCCCGCACCCACAAA	[illegible]	56 for 20s
	Region 7	F:GGCTCTCTAGAG[illegible]TCATGTGT R:CTACAATGGCACAACCCCACA	42[illegible]	57 for 30s
	Region 8	F:GGAAGGTTTTCG[illegible]TGCCCT R:CGCGGTCGAACAATCGAATC	5[illegible]	56 for 40s
	Region 9	F:TGGAGGTTGGCT[illegible]TTGTATT R:AGTCCCAAACCCAATGTACCA	7[illegible]	54 for 50s
	Region 10	F:CGCGCTTAAGTTC[illegible]TTGGG R:GTCCCCAAGACGGTTCGTT	32[illegible]	56 for 30s
	Region 11	F:CCTAAACGCGTG[illegible]TGGAGT R:CAAAAACCTCGCCCCCAAAC	22[illegible]	56 for 20s
	Region 12	F:AGTGGGTCTTCTA[illegible]AAAAGGC R:ACAGGCATAACCCTAACACCC	3[illegible]	56 for 55s
	Region 13	F:TAAAGGCGTGTG[illegible]TAAGGGG R:ACCAAGAAGCAATCACCCTG	58[illegible]	55 for 40s
	Region 14	F:CCTGACATGTGG[illegible]AGTGT R:TAGTGAGTGTAAGCCACCCG	10[illegible]	56 for 20s
	Region 15	F:AAGGGGAGTCAG[illegible]CTTGTT R:GATGCAAAAGGTACGAGAACCC	10[illegible]	55 for 45s
	Region 16	F:CGAAATACCTTTC[illegible]CGCTGAC R:CTAATTTTGCCCCCTTGCTGC	7[illegible]	56 for 50s

Table 2 Changes in secondary structure of proteins in PI F_1 hybrids compared with Peruvian scallops (*Ap*).

Protein	*Ap*	*Ap*	*Ap*	*Ap*	PI	PI	PI	PI
	Alpha helix	Extended strand	Beta turn	Random coil	Alpha helix	Extended strand	Beta turn	Random coil
CYTB	44.62%	17.06%	3.15%	35.17%	44.79%	17.71%	3.91%	33.59%
ND4	53.65%	16.71%	6.59%	23.06%	50.71%	21.80%	7.11%	20.38%
ND2	53.21%	23.08%	6.09%	17.63%	53.40%	22.98%	6.15%	17.48%
ATP6	58.30%	12.56%	4.04%	25.11%	51.98%	17.46%	5.16%	25.40%
COX2	37.50%	23.21%	5.80%	33.48%	30.17%	23.28%	5.60%	40.95%

Table 3 Significantly different allele frequency of SNPs in the six mitochondrial genes between PI F_1 hybrids and 'Bohai Red' and Peruvian scallop (*Ap*).

Gene	Site	*Ap*	Bohai Red	PI	Amino acid	Amino acid	Amino acid	*P* value	*P* value
					Ap	Bohai Red	PI	*Ap* vs PI	Bohai Red vs PI
CYTB	1	0T, 8C	13T,16C	44T, 14C	N	N	N	2.0^e-5	4.0^e-3
	2	0T, 8C	13T,16C	46T,14C	I	I	I	1.3^e-5	3.0^e-3
ND4	1	8T, 0C	20T, 4C	19T, 38C	L	L	L	3.4^e-4	4.0^e-5
	2	8T, 0C	20T, 4C	7T, 50C	F	F	F	3.5^e-8	6.0^e-10
	3	8T, 0C	19T, 5C	19T, 38C	Y	Y	Y	3.4^e-4	1.6^e-4

Gene	Site	*Ap*	Bohai Red	PI	Amino acid	Amino acid	Amino acid	*P* value	*P* value
	4	8G, 0A	27G, 0A	22G, 17A	**S**	**S**	**N**	1.9ˆe-2	6.8ˆe-5
ND2	1	8T, 0C	30T, 0C	17T, 43C	L	L	L	7.8ˆe-5	1.4ˆe-10
	2	8T, 0C	30T, 0C	17T, 43C	**V**	**V**	**A**	7.8ˆe-5	1.4ˆe-10
trnD	1	8T, 0C	30T, 0C	17T, 43C	/	/	/	7.8ˆe-5	1.4ˆe-10
ATP6	1	8T, 0C	26T, 4C	22T, 38C	L	L	L	7.0ˆe-4	7.4ˆe-6
	2	0A, 8G	3A, 27G	43A, 17G	**M**	**M**	**I**	7.8ˆe-5	3.0ˆe-8
COX2	1	8G, 0A	30G, 0A	22G, 36A	**M**	**M**	**I**	9.5ˆe-4	2.0ˆe-8
	2	8T, 0G	30T, 0G	17T, 41G	A	A	A	1.1ˆe-4	3.0ˆe-10

Note: Letters in bold represent amino acid changes caused by missense mutations.

Table 4 Significantly different SNP haplotypes in four genes between PI F_1 hybrids and 'Bohai Red' and Peruvian scallop (*Ap*).

Scallop species Gene name	*Ap*	Bohai Red	PI
COX2	8 AGT	30 TGT	**36 GAG** 7 GGT 3 TGT 7 AGT 5 GGG
ND4	8 TTTG	18 TTTG 2 CCCG 2 CCC- 1 TTCG 1 TTT-	**20 CCC- 17 CCCA** 13 TCTG 7 TTTG
ND2	8 CTT	30 CTT	**38 TCC** 5 CCC 17 CTT
ATP6	8 TTTG	17 CTTG 1 CTCG 3 CTCA 1 CCTG	**24 CCCA** 5 TTTG 9 TCTG 2 TTTA 11 CTCA 2 TCTA 2 CTTG

Note: Letters in bold represent the specific dominant SNP haplotypes in PI F_1 hybrids.

Table 5 Differentially expressed genes in PI F_1hybrids (Peruvian scallop × bay scallop).

Function	Gene	Description
mitochondrial respiratory chain enzyme complex synthesis	*SCO1*	protein SCO1 homolog
	SDHB	succinate dehydrogenase iron-sulfur subunit
	ATPAF2	ATP synthase mitochondrial F_1 complex ass
	NDUFS3	NADH dehydrogenase iron-sulfur protein 3
	NDUFS4	NADH dehydrogenase iron-sulfur protein 4
	NDUFS7	NADH dehydrogenase iron-sulfur protein 7
mitochondrial ATP synthesis	*ATPsynB*	ATP synthase subunit
	ATPsynF	putative ATP synthase subunit f
	ATPsyngamma	ATP synthase subunit gamma
	ATP5F1B	ATP synthase subunit beta
	ATP5MC2	ATP synthase lipid-binding protein
	ATPsynO	ATP synthase subunit O
	ATPsynCF6	ATP synthase-coupling factor 6
	atp23	mitochondrial inner membrane protease ATP
	ATP5F1A	ATP synthase subunit alpha
	ATP5f1d	ATP synthase subunit delta
mitochondria regulation	*dGK*	NADH dehydrogenase 1 alpha subcomplex su

Function	Gene	Description
Apoptosis	*TK2*	thymidine kinase 2
	COX11	cytochrome c oxidase assembly protein COX
	POLG	DNA polymerase subunit γ
	POLG2	DNA polymerase subunit gamma-2
	MTCH2	mitochondrial carrier homolog
	VDAC2	voltage-dependent anion-selective channel pr
	BAX	Bcl-2 protein
	Mfn2	mitofusin2
	PARP1	poly ADP-Ribose polymerase 1
	CDK2	cyclin dependent kinase 2
	AIFM3	apoptosis-inducing factor 3-like
	ENDOG	endonuclease G, mitochondrial-like

Hosted file

`Figure.zip` available at https://authorea.com/users/502217/articles/582350-mitochondrial-mutations-and-sterility-in-the-interspecific-hybrids-of-the-hermaphroditic-argopecten-scallops

Hosted file

`Table.docx` available at https://authorea.com/users/502217/articles/582350-mitochondrial-mutations-and-sterility-in-the-interspecific-hybrids-of-the-hermaphroditic-argopecten-scallops