Current **A**pplied **S**cience and **T**echnology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. ….., Vol... (No...), e0270583

Research article

Complete Mitochondrial Genomes of *Magallana saidii* (Wong & Sigwart, 2021) from Malaysia and Phylogenetic Insight

Mohamad Qamarul Abidin Mohd Zawawi[1], Han Ming Gan[2], Nur Sabrina Badrulhisham[1], Zaidi Mohd Noh[3] and Kamarul Rahim Kamarudin[1]*

[1]International Institute of Biodiversity Technology and Sustainability, Universiti Tun Hussein Onn Malaysia (UTHM), Pagoh Branch Campus, Johor, Malaysia
[2]Patriot Biotech Sdn. Bhd., Bandar Sunway, Subang Jaya, Selangor, Malaysia
[3]Asam Pedas Pak Ndak, Tiram Muar Oyster, Lorong Tepi (Surau Parit Tiram) Off Jalan Salleh, Muar, Johor, Malaysia

Received: 4 February 2026, Revised: 14 April 2026, Accepted: 27 May 2026, Published: 13 August 2026

Abstract

Magallana saidii (Wong & Sigwart, 2021), locally known as the white oyster, is an oyster species endemic to the Muar River, Johor, Malaysia. Although long recognized and exploited by local fishers, it has not been reported elsewhere, making it of conservation concern due to its restricted distribution. Despite its ecological and economic importance, *M. saidii* has received limited molecular attention since its recent taxonomic description. In this study, two complete mitochondrial genomes (mitogenomes) of *M. saidii* from Muar, Johor are presented. Whole-genome sequencing was performed using the Illumina NovaSeq6000 platform, and the mitochondrial contigs were assembled using a *de novo* approach. The mitogenomes measured 21,499 bp (PV711311) and 21,505 bp (PV711312) in length. Each mitogenome comprised 38 genes, including 13 protein-coding genes, 23 transfer RNAs, and two ribosomal RNAs, with largely conserved gene arrangement. Notably, the *atp8* gene, which is often unresolved in oyster mitogenomes, was clearly identified. The mitogenome exhibited a strong adenine and thymine bias (A+T content of 66.3%), with nucleotide compositions of 37.3% thymine, 29.0% adenine, 20.6% guanine and 13.0% cytosine. Maximum likelihood phylogenetic analysis based on 13 concatenated protein-coding genes recovered *M. saidii* as a well-supported clade with another *M. saidii* mitogenome from GenBank (accession PV564049), and as sister to a clade comprising *M. nippona*, *M. hongkongensis*, and *M. ariakensis*. The complete mitogenome of *M. saidii* provides a valuable genetic reference to support future phylogenetic studies and conservation management of oysters in Malaysia.

Keywords: *Magallana sai*dii; mitochondrial genome; phylogeny; oyster; Malaysia

*Corresponding author: E-mail: kamarulr@uthm.edu.my
https://doi.org/10.55003/cast.2026.270583

1. Introduction

Oysters (family Ostreidae) are ecologically and economically important bivalves, playing roles in marine and estuarine ecosystems, including water filtration, nutrient cycling, and habitat formation (Xu et al., 2021). They also support coastal livelihoods and aquaculture industries worldwide, with China as the largest producer, accounting for 86% of global output by 2016 (Botta et al., 2020). Although oyster aquaculture remains relatively specialized, continuous efforts to increase productivity and to enhance sustainability are emphasized (Admodisastro et al., 2022; Doinsing et al., 2024). Despite extensive studies on oyster biology, development and productivity (Tan et al., 2014), detailed genomic and phylogenetic research remains limited. This lack of genetic information is particularly concerning given the high morphological plasticity of oysters, which is strongly influenced by environmental factors such as salinity, substrate type, and tidal exposure, often leading to taxonomic confusion.

In recent years, mitochondrial DNA (mtDNA) has been widely used as a molecular tool in taxonomy, population genetics, and phylogenetics due to its relatively small size, conserved gene content, maternal inheritance, and fast mutation rate compared with nuclear DNA (Ren et al., 2010; Arif et al., 2011). Typically, metazoan mtDNA is a circular molecule, except for some cnidarians (Bridge et al., 1992). It encodes 37 genes, including 13 protein-coding genes (PCGs) [cytochrome c oxidase subunit I-III (*cox1-cox3*), apocytochrome b (*cob*), ATP synthase subunits 6 and 8 (*atp6* and *atp8*), NADH dehydrogenase subunits 1-6 and 4L (*nad1-6, nad4L*)], 22 transfer RNAs and 2 ribosomal RNAs. Its rapid evolutionary rate and lack of recombination make mtDNA a sensitive marker for genetic variation and phylogenetic inference.

Recent phylogenetic studies on oysters increasingly rely on genomic data to resolve taxonomic ambiguities, uncover cryptic diversity, and clarify evolutionary relationships (Guo et al., 2018; McDougall et al., 2024). To overcome the limitations of shell morphology, researchers often used mitochondrial markers such as COI and 16S, alongside nuclear markers like ITS2, integrating them into multi-locus and sequence-structure models to improve phylogenetic resolution (Guo et al., 2018; Salvi & Mariottini, 2021; McDougall et al., 2024).

The genus *Magallana* has been shown to be genetically distinct from *Crassostrea*, based on deep divergence (~80-85 Mya), mitogenomic rearrangements, gene duplications and karyological differences (Salvi & Mariottini, 2021; Salvi et al., 2021; Sigwart et al., 2021). Mitogenomic and phylogenomic studies have clarified the positions of Asian *Magallana*, revealed cryptic species, identified lineage-specific genomic rearrangements, and highlighted the need to combine morphological and molecular evidence for accurate taxonomy (Salvi & Mariottini, 2021; Salvi et al., 2021; Sigwart et al., 2021; Santhi et al., 2025). However, some geographic regions, especially Southeast Asia, remain under-sampled, limiting the resolution of species boundaries and their placement in phylogenetic trees (Li et al., 2021a; McDougall et al., 2024). In particular, molecular information on *Magallana saidii* is limited. While it has been included in multi-locus phylogenetic reconstruction (Sigwart et al., 2021), comprehensive mitogenomic data remain limited, leaving its evolutionary position insufficiently characterized.

In this study, we report two complete mitochondrial genomes of *M. saidii* from Malaysia and provide a detailed analysis of its genomic features, nucleotide composition, and strand bias. Using concatenated mitochondrial PCGs, we reconstructed phylogenetic relationships to clarify the evolutionary placement of *M. saidii* within Ostreidae. The mitogenome generated in this study expands the available molecular resources for this

endemic species and provides a valuable reference for future taxonomic, phylogenetic, conservation, and environmental studies.

2. Materials and Methods

2.1 Specimen collection and DNA extraction

Specimens of *Magallana saidii* (BioSample numbers: SAMN48632525 and SAMN4863252) were collected from the Muar River, Muar District, Johor, Malaysia, located on the southwestern coast of Peninsular Malaysia (Figure 1). Sampling was conducted at Pengkalan Nelayan Parit Tiram during low tide on 10 November 2024 at 0919 h. The geographic coordinates of the sampling site were 2.060222° N, 102.571861°E (2°03'36.8" N, 102°34'18.7" E).

Mantle tissue from the specimens was excised, and total genomic DNA (gDNA) was isolated using PrimeWay Food DNA Extraction Kit (1st BASE, Malaysia) following the manufacturer's instructions. DNA quantity and quality were determined using a Nanophotometer®, N50 Touch (IMPLEN, Germany) and 2% (weight/volume) agarose horizontal gel electrophoresis (BIO-RAD) to confirm integrity and suitability for downstream sequencing.

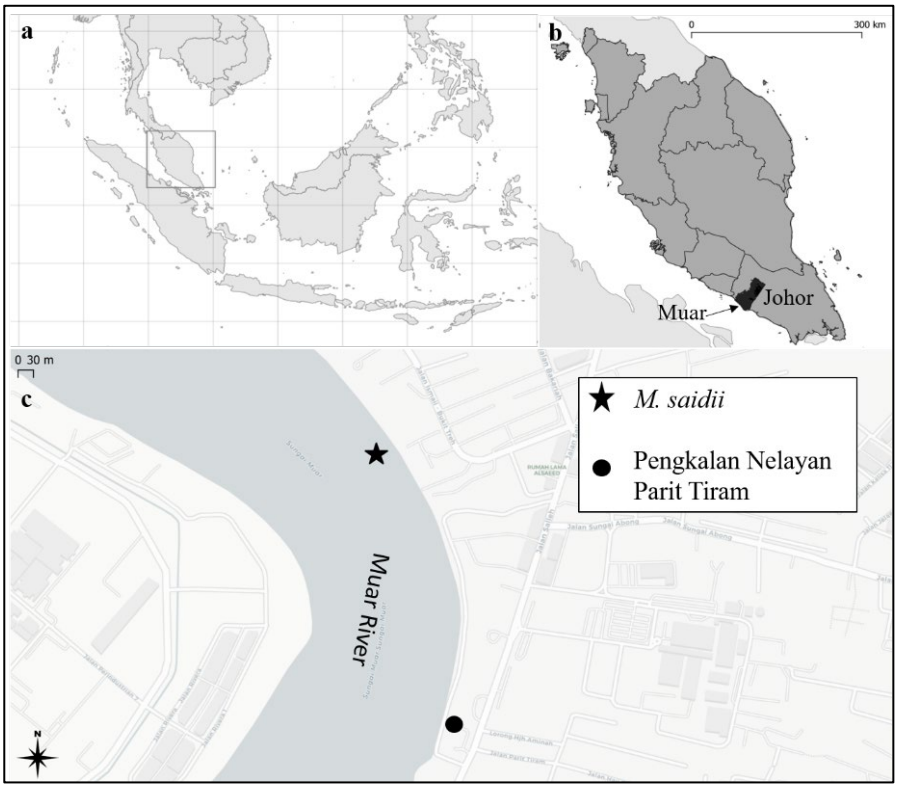

Figure 1. (a) Regional distribution map of Southeast Asia. (b) Map of Peninsular Malaysia highlighting the location of Muar District in Johor. (c) Sampling location of *Magallana saidii* within the Muar River, Johor, Malaysia.

2.2 Library preparation and mitogenome assembly

Genomic DNA (100 ng per sample), quantified using the Qubit High Sensitivity dsDNA Assay (Thermo Fisher Scientific, Waltham, MA), was fragmented to an average size of 300 bp using the Bioruptor sonicator (Diagenode, Denville, NJ). Libraries were prepared with the NEBUltra II Illumina Library Preparation Kit (NEB, Ipswich, MA) following the manufacturer's instructions. The constructed library was analyzed using a Fragment Analyzer (Agilent, Santa Clara, CA) to estimate its concentration and fragment size distribution. Whole-genome sequencing was performed on an Illumina NovaSeq 6000 platform (San Diego, CA) with 2 x 150 bp configuration, yielding approximately 1 GB of raw data per sample. Paired-end adapters and low-quality reads were trimmed and filtered using fastp v0.21 (Chen et al., 2018). Then, the mitochondrial genome was assembled from whole-genome sequencing data using GetOrganelle v1.7.7.0 (Jin et al., 2020), which extracts organelle-like reads with Bowtie2 v2.3.5.1 and performs iterative k-mer-based extensions to enrich mitochondrial sequences. Assembly was conducted with SPAdes v3.13.0, and the resulting contigs were simplified and circularized to reconstruct the complete mitogenome. The final circular mitogenome was manually reoriented to start at a conserved reference gene commonly used in oysters.

2.3 Mitogenome annotation and nucleotide analysis

Mitochondrial genome annotation was performed using MITOS2 v2.0.8 (Donath et al., 2019), with the RefSeq89 Metazoa reference database. RNA genes were annotated using Infernal v1.1.1 (Nawrocki & Eddy, 2013). Circular genome visualization was generated using MitoZ v3.5 (Meng et al., 2019), which employs Circos (Krzywinski et al., 2009) to illustrate gene arrangement.

The base composition of the whole mitogenome, including protein-coding genes, tRNAs, and rRNAs, was calculated using MEGA 11 (Tamura et al., 2021). Strand bias was calculated using the formulas: AT skew = (A – T) / (A + T) and GC skew = (G – C) / (G + C), where A, T, G, and C represent the number of respective nucleotides.

2.4 Phylogenetic analysis

A maximum likelihood phylogenetic tree with 1,000 bootstrap replicates was constructed using MEGA 11 (Tamura et al., 2021) based on concatenated sequences of 13 mitochondrial protein-coding genes (PCGs) from 17 mitogenomes representing 14 oyster species across four genera (Table 1). Sequences were obtained from GenBank (NCBI) (https://www.ncbi.nlm.nih.gov/) and included two *Magallana saidii* mitogenomes generated in this study (accession numbers: PV711311 and PV711312). The deep-sea mussel, *Bathymodiolus septemdierum* (accession number: AP014562), was used as the outgroup. Among 17 mitogenomes, only five, including the three *M. saidii* mitogenomes, *Talonostrea talonata* and *B. septemdierum*, contained a complete set of 13 PCGs, whereas the remaining mitogenomes lacked the atp8 gene. Nevertheless, all 13 PCGs were retained in the concatenated dataset to preserve the completeness and uniqueness of the *M. saidii* mitogenome.

The 13 PCGs were individually extracted from the mitogenome and aligned separately using a codon-based alignment method in MEGA 11 (Tamura et al., 2021), with the MUSCLE algorithm (Edgar, 2004), to preserve reading frames. No manual gap trimming was performed to prevent frame shifts and ensure consistent alignment lengths

across all taxa. PCGs were concatenated into a single matrix using Microsoft Excel for subsequent phylogenetic analysis. The General Time Reversible model (Tavaré, 1986; Nei & Kumar, 2000) with Gamma distribution and invariant sites (GTR + G + I) was selected as the best-fit evolutionary model for the maximum likelihood phylogenetic tree based on the lowest Bayesian Information Criterion (BIC) score.

Table 1. Mitogenomes used in the phylogenetic analysis

Present Study Mitochondrial Genome			
Family	**Species**	**Length (bp)**	**Accession No.**
Ostreidae	*Magallana saidii*	21,499	PV711311
Ostreidae	*Magallana saidii*	21,505	PV711312
GenBank Mitochondrial Genome			
Family	**Species**	**Length (bp)**	**Accession No.**
Ostreidae	*Magallana saidii*	21,500	PV564049
Ostreidae	*Magallana hongkongensis*	18,622	NC011518
Ostreidae	*Magallana gigas*	18,224	NC001276
Ostreidae	*Magallana angulata*	18,225	NC012648
Ostreidae	*Magallana bilineata*	22,420	MT985154
Ostreidae	*Magallana ariakensis*	18,414	NC012650
Ostreidae	*Magallana nippona*	20,030	NC015248
Ostreidae	*Magallana belcheri*	21,020	MH051332
Ostreidae	*Magallana sikamea*	18,243	NC012649
Ostreidae	*Crassostrea iredalei*	22,446	NC013997
Ostreidae	*Talonostrea talonata*	20,552	NC077458
Ostreidae	*Alectryonella plicatula*	18,225	NC056354
Ostreidae	*Saccostrea cucullata*	16,396	NC027724
Ostreidae	*Saccostrea mordax*	16,512	KP769562
Modiolidae	*Bathymodiolus septemdierum*	17,069	AP014562

3. Results and Discussion

3.1 *Magallana saidii* morphology

The specimens of *Magallana saidii* (Figure 2) exhibited morphological traits consistent with the diagnostic features described by Sigwart et al. (2021), supporting the identification of the collected specimens as members of this species. The shells were generally compressed, relatively thick, and ovately elongated, with a slightly asymmetrical shape that curved posteriorly at the dorsal end. The outer surface shows thin, rough, pigmented brownish-layered ridges and some whitish areas on the eroded part. The left valve was more convex and heavier compared to the right valve, which was compressed and thinner. The hinge area was broad and aligned parallel to sagittal plane of the valve, curving sharply towards the posterior end.

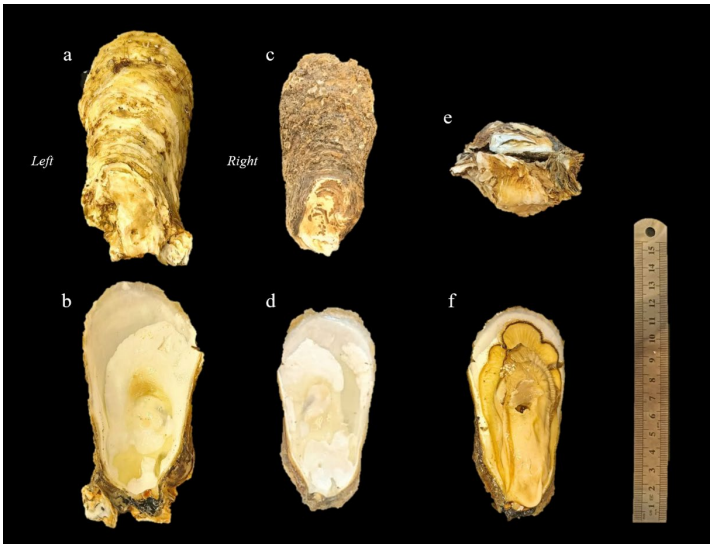

Figure 2. External and internal shell morphology of *Magallana saidii* showing (a) left valve, (b) inner surface of left valve, (c) right valve, (d) inner surface of right valve, (e) dorsal view, and (f) soft parts on the right valve.

Internally, the valve surface was chalky white and partially pearly, but not fully nacreous. The flesh and adductor muscle were cream to light brown in color, contrasting the pearly inner shell surface. The posterior adductor muscle scar was shaped like a kidney and located closer to the ventral margin than to the hinge. The scar was pale to whitish, often with a faint golden tinge across the valve. There was a narrow black marginal line that was visible along the ventral mantle.

3.2 *Magallana saidii* mitochondrial genome

A total of 1.8 and 2.0 GB of data were generated for the *Magallana saidii* samples. The raw reads were deposited in NCBI under the BioProject ID PRJNA1263161. The complete mitochondrial genomes of *Magallana saidii* generated in this study were 21,499 bp (accession no. PV711311) (Figure 3) and 21,505 bp (accession no. PV711312) in length, containing 13 protein-coding genes (PCGs), 23 tRNA genes, and two rRNA genes. The differences in length may reflect genuine biological variation, sequencing or assembly artefacts, or minor differences in annotation, particularly in non-coding regions such as the control region. Notably, *M. saidii* possessed the *atp8* gene, which has been reported as missing in some bivalve species. However, increasing evidence suggests that this apparent absence may be from annotation challenges, as the *atp8* gene is often highly divergent and varies significantly in length (Zhao et al., 2022). Despite the overall conservation of gene order on the heavy strand, characteristic of all marine bivalves (Ghiselli et al., 2021), both assemblies exhibited repetitive tracts and evidence of gene duplication and inactivation, which complicated the assembly. These features resulted in several unannotated regions that were suspected to include control elements and pseudogenic fragments of *rrn* and *nad2*. To address this, annotation was performed on MITOS, where only the highest BLAST-scoring copy from each duplicated gene set was retained.

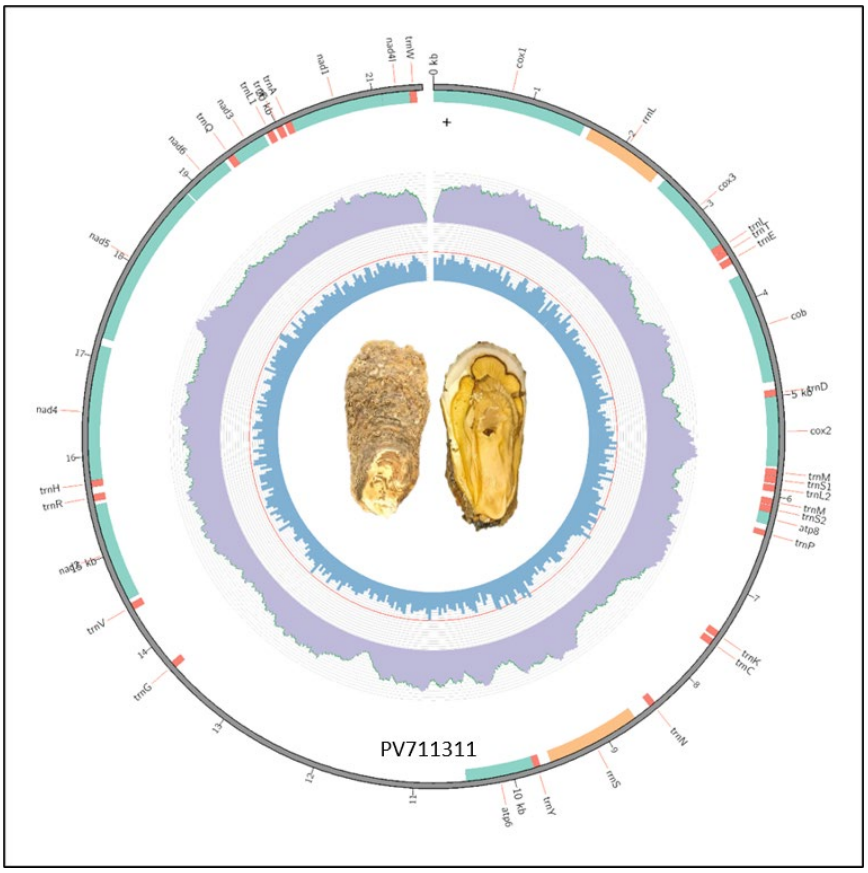

Figure 3. Circular map of mitochondrial genome of *M. saidii* (PV711311; 21,499 bp) visualized using MitoZ. The genes encoded on the inner side show forward strand. Green color indicates PCGs, orange color indicates tRNAs, and yellow color indicates rRNAs.

3.3 Start and stop codon usage

Among the protein-coding genes (PCGs) of *Magallana saidii*, 12 genes (*cox3*, *cob*, *cox2*, *atp8*, *atp6*, *nad2*, *nad4*, *nad5*, *nad6*, *nad3*, *nad1*, and *nad4l*) initiated with the typical mitochondrial start codon of ATG (methionine) (Quek et al., 2021) consistent with mitochondrial gene expression in marine bivalves (Bisbal-Pardo et al., 2014). The remaining gene, *cox1*, initiated with ATT, an alternative start codon in some bivalve mitochondrial genes, including those of oysters (Bisbal-Pardo et al., 2014). The prevalence of ATT in *cox1* aligned with recent findings that non-ATG start codons may arise from AT-skewed mitogenomes or relaxed selection pressure in marine invertebrates (Zhu et al., 2021).

Stop codons exhibited variability, with eight genes (*cox1*, *cox3*, *cox2*, *atp8*, *nad2*, *nad4*, *nad5*, and *nad1*) ending with TAA, while four genes (*cob*, *atp6*, *nad6*, and *nad3*) ended with TAG, which are the most common stop codons in marine bivalve mitochondrial genomes (Bisbal-Pardo et al., 2014). The *nad4l* terminated with an incomplete stop codon (T), presumed to be completed to TAA through post-transcriptional polyadenylation (3' A),

a mechanism widely observed in metazoan mitochondrial (Venton, 2014; Marková et al., 2015). All open reading frames were free of internal stop codons and translated into proteins of the expected length, confirming the accuracy of the annotations. The 13 PCGs of *M. saidii* are presented with the other 25 genes (23 tRNAs and two rRNAs) in Table 2 and Table 3.

Table 2. Mitogenome features of *M. saidii* (PV711311), where PCGs are represented in italic letters. All 38 genes are encoded on the forward strand, denoted by "+".

Gene (codon)	Location	Size (bp)	Start Codon	Stop Codon	Direction
cox1	1-1560	1560	ATT	TAA	+
rrnL	1632-2431	800			+
cox3	2528-3373	846	ATG	TAA	+
trnI (gat)	3374-3441	68			+
trnT (tgt)	3446-3506	61			+
trnE (ttc)	3536-3601	66			+
cob	3736-4848	1113	ATG	TAG	+
trnD (gtc)	4932-5000	69			+
cox2	5003-5701	699	ATG	TAA	+
trnM (cat)	5729-5794	66			+
trnS1 (tct)	5800-5869	70			+
trnL2 (taa)	5888-5954	67			+
trnM (cat)	6023-6087	65			+
trnS2 (tga)	6096-6165	70			+
atp8	6167-6286	120	ATG	TAA	+
trnP (tgg)	6348-6402	55			+
trnK (ttt)	7457-7525	69			+
trnC (gca)	7554-7621	68			+
trnN (gtt)	8409-8475	67			+
rrnS	8663-9608	946			+
trnY (gta)	9722-9786	65			+
atp6	9792-10475	684	ATG	TAG	+
trnG (tcc)	13696-13765	70			+
trnV (tac)	14405-14475	71			+
nad2	14520-15518	999	ATG	TAA	+
trnR (tcg)	15559-15625	67			+
trnH (gtg)	15702-15767	66			+
nad4	15770-17119	1350	ATG	TAA	+
nad5	17201-18874	1674	ATG	TAA	+
nad6	18890-19369	480	ATG	TAG	+
trnQ (ttg)	19413-19483	71			+
nad3	19488-19838-	351	ATG	TAG	+
trnL1 (tag)	19875-19940	66			+
trnF (gaa)	19980-20047	68			+
trnA (tgc)	20077-20143	67			+
nad1	20149-21084	936	ATG	TAA	+
nad4l	21088-21367	280	ATG	T	+
trnW (tca)	21368-21436	69			+

Table 3. Mitogenome features of *M. saidii* (PV711312), where PCGs are represented in italic letters. All 38 genes are encoded on the forward strand, denoted by "+".

Gene (codon)	Location	Size (bp)	Start Codon	Stop Codon	Direction
cox1	1-1560	1560	ATT	TAA	+
rrnL	1632-2431	800			+
cox3	2528-3373	846	ATG	TAA	+
trnI (gat)	3374-3441	68			+
trnT (tgt)	3446-3506	61			+
trnE (ttc)	3536-3601	66			+
cob	3736-4848	1113	ATG	TAG	+
trnD (gtc)	4932-5000	69			+
cox2	5003-5701	699	ATG	TAA	+
trnM (cat)	5729-5794	66			+
trnS1 (tct)	5800-5869	70			+
trnL2 (taa)	5888-5954	67			+
trnM (cat)	6023-6087	65			+
trnS2 (tga)	6096-6165	70			+
atp8	6167-6286	120	ATG	TAA	+
trnP (tgg)	6348-6402	55			+
trnK (ttt)	7457-7525	69			+
trnC (gca)	7554-7621	68			+
trnN (gtt)	8409-8475	67			+
rrnS	8663-9608	946			+
trnY (gta)	9722-9786	65			+
atp6	9792-10475	684	ATG	TAG	+
trnG (tcc)	13701-13770	70			+
trnV (tac)	14411-14481	71			+
nad2	14526-15524	999	ATG	TAA	+
trnR (tcg)	15565-15631	67			+
trnH (gtg)	15708-15773	66			+
nad4	15776-17125	1350	ATG	TAA	+
nad5	17207-18880	1674	ATG	TAA	+
nad6	18896-19375	480	ATG	TAG	+
trnQ (ttg)	19419-19489	71			+
nad3	19494-198344	351	ATG	TAG	+
trnL1 (tag)	19881-19946	66			+
trnF (gaa)	19986-20053	68			+
trnA (tgc)	20083-20149	67			+
nad1	20155-21090	936	ATG	TAA	+
nad4l	21094-21373	280	ATG	T	+
trnW (tca)	21374-21442	69			+

3.4 Nucleotide composition and strand bias

The mitochondrial genome of *Magallana saidii* is AT-rich, with nucleotide frequencies of 37.2% T, 29.0% A, 20.6% G, and 13.0% C, resulting in an overall AT content of 66.3%. This AT bias was consistent with the mitochondrial genomes of other bivalves and molluscan mitochondrial genomes and may be associated with replication processes and

evolutionary pressures (Liao et al., 2020; Sun et al., 2021). Strand asymmetry analyses revealed a negative AT-skew (-0.12519) and a positive GC-skew (0.22619) for the complete mitogenome, indicating an excess of T over A and G over C. At the gene level, AT- and GC-skew values varied among loci, with *nad2* exhibiting the strongest strand bias. Similar compositional patterns have been reported in other bivalve mitogenomes and are generally attributed to strand-specific mutational pressures during mitochondrial replication (Yu & Li, 2011). Overall, the nucleotide composition and strand bias of *M. saidii* conformed to conserved mitochondrial features in Ostreidae, including AT-rich composition and characteristic strand-skew patterns (Li et al., 2021b; Mu, 2022). Detailed nucleotide composition and skew values are provided in Table 4.

Table 4. Percentage frequency of nucleotides, AT-skewness, and GC-skewness of *M. saidii* from this study

Region	A%	T%	C%	G%	AT%	CG%	AT-skew	GC-skew
Mitogenome *M. saidii*	37.3	13.0	29.0	20.6	66.3	33.6	-0.12519	0.22619
13 PCGs *M. saidii*	39.8	13.7	25.5	21.0	65.3	34.7	-0.21899	0.21037
cox1	39.6	16.0	23.9	20.4	63.5	36.4	-0.24724	0.12088
cox3	41.1	14.1	25.1	19.7	66.2	33.8	-0.24169	0.16568
cob	37.3	14.2	27.8	20.8	65.1	35.0	-0.14593	0.18857
cox2	38.6	13.2	24.6	23.6	63.2	36.8	-0.22152	0.28261
atp8	37.5	13.3	28.3	20.8	65.8	34.1	-0.13982	0.21994
atp6	38.9	12.7	30.6	17.8	69.5	30.5	-0.11942	0.16721
nad2	41.3	12.7	23.3	22.6	64.6	35.3	-0.27864	0.28045
nad4	41.7	11.7	24.9	21.7	66.6	33.4	-0.25225	0.29940
nad5	38.1	14.3	26.5	21.1	64.6	35.4	-0.17957	0.19209
nad6	41.5	14.0	26.5	18.1	68.0	32.1	-0.22059	0.12773
nad3	41.9	12.0	24.2	21.9	66.1	33.9	-0.26778	0.29204
nad1	42.0	13.0	23.6	21.4	65.6	34.4	-0.28049	0.24419
nad4l	37.7	14.0	24.3	24.0	62.0	38.0	-0.21613	0.26316
tRNAs	33.9	13.6	31.7	20.8	65.6	34.4	-0.03354	0.20930
rrnL	31.3	16.0	31.9	20.9	63.2	36.9	0.00949	0.13279
rrnS	27.8	16.0	31.2	25.1	59.0	41.1	0.05763	0.22141

3.5 Phylogenetic relationships

The identification of *Magallana saidii* was confirmed using both morphological and molecular data. Preliminary BLAST comparisons of mitochondrial PCGs showed 99.86% identity between the specimens in this study and the GenBank *M. saidii* sequence (accession no. PV564049), validating the assembly and species assignment. Maximum likelihood phylogenetic analysis (Figure 4) based on 13 concatenated protein-coding genes (PCGs) recovered all *M. saidii* sequences as a strongly supported monophyletic clade (bootstrap = 100%). Figure 4 depicts only the tree topology, while the corresponding tree showing branch lengths proportional to substitutions per site is provided in the following Newick tree format:

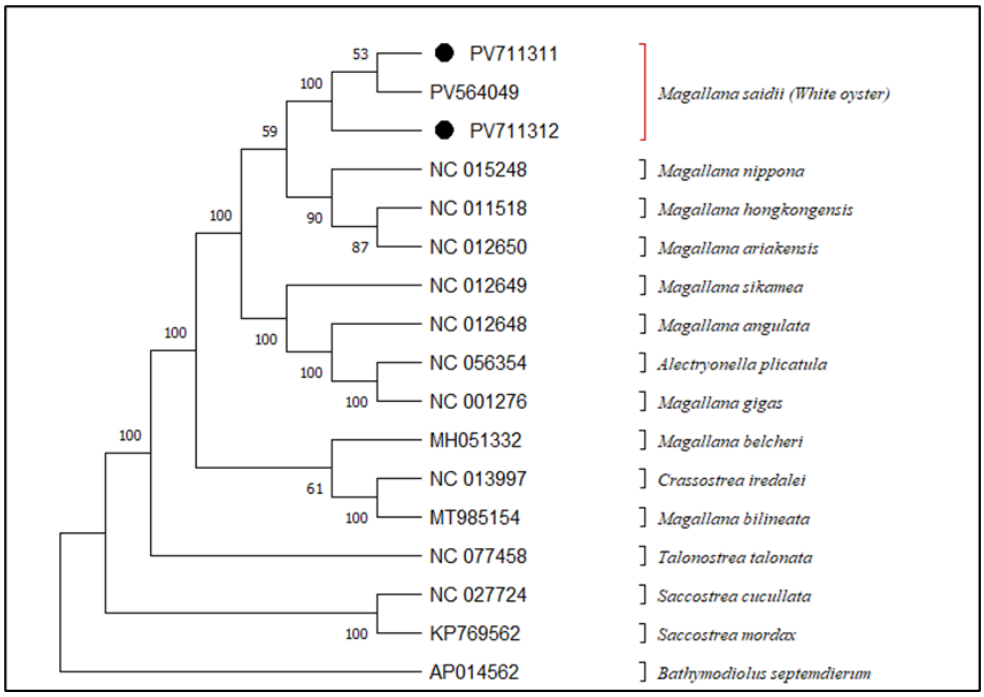

Figure 4. Maximum likelihood bootstrap consensus tree based on 13 mitochondrial PCGs from 16 oyster mitogenomes and one mussel mitogenome (outgroup), generated with 1000 bootstrap replicates. The positions of the *M. saidii* sequences from this study are indicated with solid circles. Node numbers represent bootstrap support values.

(((((((PV711311:0.00081984,PV564049:0.00010082)0.5380:0.00071598,PV711312:0.00000158)1.0000:0.23063201,(NC_015248:0.19725552,(NC_011518:0.09168104,NC_012650:0.18922007)0.8770:0.01770697)0.9000:0.01357038)0.5910:0.00884855,(NC_012649:0.08565022,(NC_012648:0.01795623,(NC_056354:0.00054625,NC_001276:0.00103819)1.0000:0.01408672)1.0000:0.11222460)1.0000:0.13338140)1.0000:0.08131096,(MH051332:0.21971075,(NC_013997:0.01426343,MT985154:0.00921674)1.0000:0.29500051)0.6150:0.02640801)1.0000:0.22964562,NC_077458:0.52194809)1.0000:0.19230475,(NC_027724:0.28892429,KP769562:0.25642816)1.0000:1.12833713,AP014562:99.43582345);

Whole mitogenome sequences were not used for tree reconstruction due to the high evolutionary rate of tRNAs, which is approximately seven to ten times higher than the genome-wide average, potentially disrupting phylogenetic inference (Sun et al., 2017; Thornlow et al., 2018).

Within the *M. saidii* clade, internal relationships had low bootstrap support (53%), reflecting low genetic divergence among conspecific individuals. In the broader topology, *M. bilineata* represented the basal lineage of *Magallana*, whereas *M. saidii* was nested within the more derived radiation, forming a sister group to a clade of *M. nippona*, *M. hongkongensis*, and *M. ariakensis*. This topology was consistent with previous multi-locus and mitochondrial studies (Sigwart et al., 2021), supporting the evolutionary placement of *M. saidii* within the genus.

Taxonomic reassessment of public sequences showed that NC056354, previously labelled as *Alectryonella plicatula*, corresponded to *M. gigas* (Salvi et al., 2021), while NC013997, formerly identified as *Crassostrea iredalei*, represented *M. bilineata* according to WoRMS (World Register of Marine Species, 2025). These corrected taxa are grouped within the broader *Magallana* assemblage. Species of *Talonostrea* and *Saccostrea* were placed distantly from *Magallana*, consistent with their taxonomic separation. The tree was rooted using *Bathymodiolus septemdierum* (Modiolidae), which effectively resolved lineage relationships within Ostreidae.

4. Conclusions

This study presents two complete mitochondrial genomes of the endemic Malaysian oyster, *Magallana saidii*, and clarifies its evolutionary placement within Ostreidae using concatenated 13 protein-coding genes (PCGs). The mitogenomes exhibit a conserved AT-rich composition and strand-specific nucleotide bias, similar to other oysters in Ostreidae. Phylogenetic analyses based on concatenated 13 PCGs strongly support the monophyly of *M. saidii* and confirm its position within a derived lineage of the genus *Magallana*.

These findings provide molecular validation towards species identity and contribute to resolving phylogenetic relationships within a taxonomically complex oyster group. The mitogenome generated in this study serves as an important genomic resource for future comparative, phylogenetic, and evolutionary studies of oysters, especially for endemic and understudied species in Southeast Asia.

5. Acknowledgements

This research was supported by the International Grant (Code No. W028) and the Postgraduate Research Grant (GPPS, Code No. Q584) from Universiti Tun Hussein Onn Malaysia (UTHM). The authors thank the local fishermen at Pengkalan Nelayan Parit Tiram, Muar, Johor, Malaysia, for their assistance and valuable contributions.

6. Authors' Contributions

Mohamad Qamarul Abidin Mohd Zawawi: Conceptualization, Software, Formal analysis, Methodology, Investigation, Data curation, Writing – original draft, Visualization; Nur Sabrina Badrulhisham: Conceptualization, Software, Methodology, Investigation; Gan Han Ming: Conceptualization, Resources, Formal analysis, Software, Data curation, Methodology; Zaidi Mohd Noh: Resources, Supervision, and Kamarul Rahim Kamarudin: Funding acquisition, Conceptualization, Software, Investigation, Resources, Methodology, Writing – review & editing, Supervision.

ORCID

Han Ming Gan https://orcid.org/0000-0001-7987-738X

Kamarul Rahim Kamarudin https://orcid.org/0000-0001-5695-838X

7. Conflicts of Interest

The authors declare no conflict of interest.

References

Admodisastro, V. A., Ransangan, J., Ilias, N., & Tan, S. H. (2022). Oyster farming potential in Sabah, Malaysia. *International Journal of Aquatic Research and Environmental Studies*, *2*(1), 17-22. https://doi.org/10.70102/IJARES/V2I1/3

Arif, I. A., Khan, H. A., Bahkali, A. H., Al Homaidan, A. A., Al Farhan, A. H., Al Sadoon, M., & Shobrak, M. (2011). DNA marker technology for wildlife conservation. *Saudi Journal of Biological Sciences*, *18*(3), 219-225. https://doi.org/10.1016/j.sjbs.2011.03.002

Bisbal-Pardo, C. I., Río-Portilla, M. A. del, & Rocha-Olivares, A. (2014). Novel gene arrangement in the mitochondrial genome of the Cortés geoduck clam (*Panopea globosa*). *Mitochondrial DNA Part A*, *27*(3), 1957-1958. https://doi.org/10.3109/19401736.2014.971305

Botta, R., Asche, F., Borsum, J. S., & Camp, E. V. (2020). A review of global oyster aquaculture production and consumption. *Marine Policy*, *117*, Article 103952. https://doi.org/10.1016/j.marpol.2020.103952

Bridge, D., Cunningham, C. W., Schierwater, B., DeSalle, R., & Buss, L. W. (1992). Class-level relationships in the phylum Cnidaria: evidence from mitochondrial genome structure. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(18), 8750-8753. https://doi.org/10.1073/pnas.89.18.8750

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884-i890. https://doi.org/10.1093/bioinformatics/bty560

Doinsing, J. W., Al-Azad, S., & Ransangan, J. (2024). Reproductive biology and spawning pattern of oyster *Magallana bilineata* in Mengkabong Bay, Sabah, Malaysia. *Croatian Journal of Fisheries*, *82*(1), 19-32. https://doi.org/10.2478/cjf-2024-0003

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792-1797. https://doi.org/10.1093/nar/gkh340

Ghiselli, F., Iannello, M., Piccinini, G., & Milani, L. (2021). Bivalve molluscs as model systems for studying mitochondrial biology. *Integrative and Comparative Biology*, *61*(5), 1699-1714. https://doi.org/10.1093/icb/icab057

Guo, X., Li, C., Wang, H., & Xu, Z. (2018). Diversity and evolution of living oysters. *Journal of Shellfish Research*, *37*(4), 755-771. https://doi.org/10.2983/035.037.0407

Jin, J. J., Yu, W. B., Yang, J. B., Song, Y., dePamphilis, C. W., Yi, T. S., & Li, D. Z. (2020). GetOrganelle: A fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biology*, *21*, Article 241. https://doi.org/10.1186/s13059-020-02154-5

Krzywinski, M., Schein, J., Birol, İ., Connors, J., Gascoyne, R., Horsman, D., Jones, S. J., & Marra, M. A. (2009). Circos: An information aesthetic for comparative genomics. *Genome Research*, *19*(9), 1639-1645. https://doi.org/10.1101/gr.092759.109

Li, C., Kou, Q., Zhang, Z., Hu, L., Huang, W., Cui, Z., Liu, Y., Ma, P. & Wang, H. (2021a). Reconstruction of the evolutionary biogeography reveal the origins and diversification of oysters (Bivalvia: Ostreidae). *Molecular Phylogenetics and Evolution*, *164*, Article 107268. https://doi.org/10.1016/j.ympev.2021.107268

Li, Y., Xu, L., Wang, X., Huang, D., Wang, L., Ning, J., Liu, S & Du, F. (2021b). The complete mitochondrial genome of the tropical oyster *Saccostrea echinata* (Bivalvia: Ostreidae) from the South China Sea. *Mitochondrial DNA Part B*, *6*(2), 384-386. https://doi.org/10.1080/23802359.2020.1869610

Liao, X., Zhu, X., & Yang, M. (2020). Complete mitochondrial DNA genomes for *Geloina erosa* (Veneroida: Corbiculidae). *Mitochondrial DNA Part B*, *5*(2), 1590-1591. https://doi.org/10.1080/23802359.2020.1742616

Marková, S., Filipi, K., Searle, J. B., & Kotlík, P. (2015). Mapping 3′ transcript ends in the bank vole (*Clethrionomys glareolus*) mitochondrial genome with RNA-Seq. *BMC Genomics*, *16*, Article 870. https://doi.org/10.1186/s12864-015-2103-2

McDougall, C., Nenadic, N., Richardson, M., & Healy, J. M. (2024). Molecular identification of intertidal rock oyster species in north-eastern Australia reveals new candidates for aquaculture. *Aquaculture*, *587*, Article 740838. https://doi.org/10.1016/j.aquaculture.2024.740838

Meng, G., Li, Y., Yang, C., & Liu, S. (2019). MitoZ: A toolkit for animal mitochondrial genome assembly, annotation and visualization. *Nucleic Acids Research*, *47*(11), Article e63. https://doi.org/10.1093/nar/gkz173

Mu, W. (2022). The complete mitochondrial genome of *Saccostrea malabonensis* (Ostreida: Ostreidae): Characterization and phylogenetic position. *Mitochondrial DNA Part B*, *7*(11), 1945-1947. https://doi.org/10.1080/23802359.2022.2139160

Nawrocki, E. P., & Eddy, S. R. (2013). Infernal 1.1: 100-fold faster RNA homology searches. *Bioinformatics*, *29*(22), 2933-2935. https://doi.org/10.1093/bioinformatics/btt509

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford University Press.

Quek, Z. B. R., Chang, J. J. M., Ip, Y. C. A., Chan, Y. K. S., & Huang, D. (2021). Mitogenomes reveal alternative initiation codons and lineage-specific gene order conservation in echinoderms. *Molecular Biology and Evolution*, *38*(3), 981-985. https://doi.org/10.1093/molbev/msaa262

Ren, J., Liu, X., Jiang, F., Guo, X., & Liu, B. (2010). Unusual conservation of mitochondrial gene order in *Crassostrea* oysters: Evidence for recent speciation in Asia. *BMC Evolutionary Biology*, *10*, Article 394. https://doi.org/10.1186/1471-2148-10-394

Salvi, D., & Mariottini, P. (2021). Revision shock in Pacific oyster taxonomy: The genus *Magallana* (formerly *Crassostrea* in part) is well-founded and necessary. *Zoological Journal of the Linnean Society*, *192*(1), 43-58. https://doi.org/10.1093/zoolinnean/zlaa112

Salvi, D., Berrilli, E., Garzia, M., & Mariottini, P. (2021). Yet another mitochondrial genome of the Pacific cupped oyster: The published mitogenome of *Alectryonella plicatula* (Ostreinae) is based on a misidentified *Magallana gigas* (Crassostreinae). *Frontiers in Marine Science*, *8*, Article 741455. https://doi.org/10.3389/fmars.2021.741455

Santhi, V. V., Chaube, S. K., & Antony, M. M. (2025). A novel mitochondrial gene rearrangement delineate *Magallana gryphoides* species from other *Magallana* crassostreine oysters. *Molecular Biology Reports*, *52*(1), Article 247. https://doi.org/10.1007/s11033-025-10335-3

Sigwart, J., Wong, N. L. W. S., & Esa, Y. (2021). Global controversy in oyster systematics and a newly described species from SE Asia (Bivalvia: Ostreidae: Crassostreinae). *Marine Biodiversity*, *51*, Article 83. https://doi.org/10.1007/s12526-021-01203-x

Sun, S., Li, Q., & Kong, L. (2021). Relaxation of selective constraint on the ultra-large mitochondrial genomes of Arcidae (Mollusca: Bivalvia). *Journal of Ocean University of China*, *20*(5), 1157-1166. https://doi.org/10.1007/s11802-021-4707-2

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022-3027. https://doi.org/10.1093/molbev/msab120

Tan, S.-H. A., Chang, G.-O., Yen, P. K., & Teh, C. P. (2014). Oyster culture in Malaysia: Opportunities and challenges. *Journal of Science and Technology in the Tropics*, *10*(2), 99-108.

Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. In *Lectures on mathematics in the life sciences* (Vol. 17, pp. 57-86). American Mathematical Society.

Thornlow, B. P., Hough, J., Roger, J. M., Gong, H., Lowe, T. M., & Corbett-Detig, R. B. (2018). Transfer RNA genes experience exceptionally elevated mutation rates. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(36), 8996-9001. https://doi.org/10.1073/pnas.1801240115

Venton, D. (2014). Highlight: Not like a textbook-Nuclear genes in *Blastocystis* use mRNA polyadenylation for stop codons. *Genome Biology and Evolution*, *6*(8), 1962-1963. https://doi.org/10.1093/gbe/evu167

World Register of Marine Species. (2025). *Magallana bilineata* (Röding, 1798). https://www.marinespecies.org/aphia.php?p=taxdetails&id=836036

Xu, M., Yang, X., Song, X., Xu, K., & Yang, L. (2021). Seasonal analysis of artificial oyster reef ecosystems: Implications for sustainable fisheries management. *Aquaculture International, 29*(1), 167-192. https://doi.org/10.1007/s10499-020-00617-x

Yu, H., & Li, Q. (2011). Mutation and selection on the wobble nucleotide in tRNA anticodons in marine bivalve mitochondrial genomes. *PLoS ONE*, *6*(1), Article e16147. https://doi.org/10.1371/journal.pone.0016147

Zhao, B., Gao, S., Zhao, M., Lv, H., Song, J., Wang, H., Zeng, Q., & Liu, J. (2022). Mitochondrial genomic analyses provide new insights into the “missing” atp8 and adaptive evolution of Mytilidae. *BMC Genomics*, *23*(1), Article 738. https://doi.org/10.1186/s12864-022-08940-8

Zhu, L., Zhu, Z., Zhu, L., Wang, D., Wang, J., & Lin, Q. (2021). The complete mitogenome of *Lysmata vittata* (Crustacea: Decapoda: Hippolytidae) with implication of phylogenomics and population genetics. *PLoS ONE*, *16*(11), Article e0255547. https://doi.org/10.1371/journal.pone.0255547