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Mitogenomic Analysis of Pterioidea (Bivalvia: Pteriomorphia): Insights into the Evolution of the Gene Rearrangements

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Abstract: The complete mitogenomes of *Pinctada albina* and *Pinctada margaritifera* were sequenced in this study, with sizes of 23,841 bp and 15,556 bp, respectively. The mitochondrial genome analysis of eight Pterioidea species indicated the existence of gene rearrangements within the superfamily. The *ATP8* gene was not detected in the two new mitogenomes, and *rrnS* was found to be duplicated in *P. albina*'s mitogenome. The reconstructed phylogeny based on mitogenomes strongly supported the monophyly of Pterioidea and provided robust statistical evidence of the phylogenetic relationships within Pteriomorphia. The analysis of the mitochondrial gene order revealed that of *P. margaritifera* to be the same as the ancestral order of Pterioidea. The gene orders of the Pterioidea species were mapped to the phylogenetic tree, and the gene rearrangement events were inferred. These results provide important insights that will support future research, such as studies extending the evolutionary patterns of the gene order from *P. margaritifera* to other species and determining the evolutionary status of Pterioidea within the infraclass Pteriomorphia.

Keywords: mitochondrial genome; pearl oyster; phylogeny; *Pinctada albina*; *Pinctada margaritifera*

Key Contribution: The gene rearrangement analysis of Pterioidea indicated the gene order of *P. margaritifera* as the most ancestral character. Different gene rearrangement events within Pterioidea were inferred.

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1. Introduction

The complete mitochondrial genome has been widely used as a reliable phylogenetic marker due to its abundance in animal tissues, the strict orthology of encoded genes [1,2], and the presence of genes and regions evolving at different rates [3,4]. Initial assumptions regarding uniparental inheritance and the absence of recombination have been overturned in some studies [5,6]. In some molluscan mitogenomes, the existence of doubly uniparental inheritance patterns [7–11], wide variations in gene size [12,13], radical genome rearrangements [14–16], and gene duplications and losses [17–19] have been found. Compared with nuclear genes, the substitution rates of mitochondrial genes are much higher and can provide more phylogenetic information [20–22]. In addition, mitochondrial genes have been widely used to analyze genetic diversity [23,24] and population genetic variability in bivalves [25–27]. Although the animal mitochondrial gene order remains relatively conserved during long periods of evolution [6,28,29], recent studies have revealed a large number of gene rearrangement events in mitogenomes belonging to different animal

groups [30–32]. The comparison of animal mitochondrial gene arrangements has become a very powerful tool for inferring ancient evolutionary relationships, as rearrangements appear to be unique, generally rare events that are unlikely to arise independently in separate evolutionary lineages [33,34].

Pterioidea is classified in the order Ostreida and infraclass Pteriomorphia [35]. The members of Pterioidea are mainly distributed in tropical and subtropical regions of the world [36]. The infraclass Pteriomorphia contains four orders (Ostreida, Arcida, Mytilida, and Pectinida) including 17,422 extant species, among which 818 species belong to Pterioidea (https://www.marinespecies.org (accessed on 22 August 2023)). To date, there are only seven complete mitochondrial genomes of Pterioidea available on GenBank. The limited molecular data have restricted the understanding of the mitogenome evolution and phylogenetic relationships of this superfamily. In addition, the phylogenetic position of Pterioidea within Pteriomorphia has been controversial [37–41].

In this study, we sequenced the complete mitogenomes of two pearl oysters, *Pinctada albina* and *Pinctada margaritifera*. Based on the published mitogenomes and the two newly determined ones, our aims were as follows: (1) to explore the gene rearrangements within Pterioidea, (2) to reconstruct their phylogenetic relationship, and (3) to determine the phylogenetic position of Pterioidea within Pteriomorphia.

2. Materials and Methods

2.1. Sample Collection and DNA Extraction

The specimen of *P. albina* was sampled from Wuzhizhou Island (18.3138° N, 109.7731° E) in December 2021. The specimen of *P. margaritifera* was collected in Changjiang, Hainan Province (19.5311° N, 108.9576° E), in April 2022. The adductor muscle of the specimens was fixed and preserved in 95% ethanol in the Laboratory of Economic Shellfish Genetic Breeding and Culture Technology (LESGBCT), Hainan University. The total genomic DNA was extracted from the adductor muscle using a TIANamp Marine Animals DNA Kit (Tiangen, Beijing, China) following the manufacturer's protocol. DNA quality was assessed via agarose gel electrophoresis.

2.2. Mitochondrial Genome Sequencing and Assembly

Qualified samples were submitted to Novogene (Beijing, China) for library construction and high-throughput sequencing. Sequencing libraries were obtained using the NEB Next Ultra™ DNA Library Prep Kit for Illumina (NEB, Ipswich, MA, USA) following the manufacturer's instructions, with average insert sizes of approximately 300 bp and sequenced as 150 bp paired-end runs on the Illumina NovaSeq 6000 platform. Finally, approximately 8 Gb of raw data were generated for each library. The clean data were obtained from each library after filtering and trimming using Trimmomatic [42] and then imported into Geneious Prime [43] software for mitogenome assembly.

2.3. Mitogenome Annotation and Sequence Analysis

The two mitogenomes were initially annotated with the MITOS webserver [44] using the invertebrate genetic code. The boundaries of the PCGs were further annotated using the ORF Finder (http://www.ncbi.nlm.nih.gov/orffinder (accessed on 15 May 2023)) by comparing them with orthologous genes of closely related species of Pterioidea using BLASTX against the non-redundant protein sequence database in GenBank. The secondary structure of tRNA genes was predicted via ARWEN [45] and tRNAscan-SE [46], while the ribosomal RNA genes (*rrnL* and *rrnS*) were edited through alignment with published homologous genes of closely related species. The nucleotide composition, codon usage, and relative synonymous codon usage (RSCU) of the mitochondrial genome were calculated in MEGA.11 [47] based on the invertebrate mitochondrial genetic code. The bias of the nucleotide composition was measured via AT and GC skews as follows: AT skew = (A − T)/(A + T) and GC skew = (G − C)/(G + C), where A, T, G, and C are the occurrences of

the four nucleotides. The sequence features of the two mitochondrial circular genomes were examined using CGView [48].

2.4. Phylogenetic Analysis

A total of 26 Pteriomorphia complete mitogenomes were used in the phylogenetic analysis, with two species *Archivesica marissinica* and *Tridacna squamosa* from the Heteroconchia infraclass selected as the outgroup taxa (Table S1). The nucleotide sequences of 12 PCGs (excluding *Atp8*) and 2 rRNA genes of the Pteriomorphia species were used to reconstruct the phylogenetic relationships. The nucleotide sequences for each PCG were aligned separately based on codon position using the invertebrate mitochondrial genetic code in MEGA.11 [47]. The rRNA genes were aligned using MAFFT [49] and the ambiguously aligned sites were discarded using Gblocks [50] with default settings. Nucleotide sequences for individual PCGs and rRNA alignments were concatenated using Geneious Prime. The best partition scheme and corresponding substitution models for the dataset were calculated with Partition Finder v2.1.1 [51], using the Bayesian Information Criterion (BIC) and a user-defined search algorithm with branch lengths estimated as "linked".

Maximum Likelihood (ML) and Bayesian Inference (BI) were used to perform phylogenetic analyses. ML trees were constructed using IQTREE [52] with models, which allowed for different partitions to have different evolutionary rates (-spp option), using 10,000 ultrafast bootstrap replicates (-bb option). BI MCMC analysis was conducted using MrBayes v.3.2.7a [53], running four simultaneous Monte Carlo Markov Chains (MCMCs) for 10,000,000 generations, sampling every 1000 generations and discarding the first 25% of generations as burn-in. Two independent runs were performed to increase the chance of adequate mixing of the Markov chains and to increase the chance of detecting failure to converge, as determined using Tracer v.1.7 [54]. The effective sample size (ESS) of all of the parameters was above 200. The resulting phylogenetic trees were visualized in FigTree v.1.4.4 [55].

2.5. Gene Rearrangement Analyses

The mitochondrial gene order of the PCGs and rRNA genes was mapped onto the obtained phylogeny, and pairwise comparisons of the gene arrangement events of the superfamily Pterioidea were conducted using CREx [56]. The analyses were based on common intervals and considered reversals, transpositions, reverse transpositions, and tandem duplication random losses (TDRLs).

3. Results and Discussion

3.1. Mitogenome Composition of P. albina and P. margaritifera

As shown in Figure 1, the mitogenomes of the two pearl oysters *P. albina* and *P. margaritifera* are circular DNA molecules with lengths of 23,841 bp (GenBank Accession No.: OR529434) and 15,556 bp (GenBank Accession No.: OR529435), respectively (Figure 1). The complete mitogenome of *P. albina* encodes 38 genes, including 12 PCGs, 23 tRNA genes, and 3 rRNA genes, with two duplicates of *rrnS* that are separated by 3281 nucleotides. The *P. margaritifera* mitogenome contains the standard set of 36 mitochondrial genes, including 12 PCGs, 22 tRNA, and 2 rRNA genes. *trnT*, *trnC*, *trnW*, and *trnM* each have an additional copy in *P. albina*. The *trnM* gene has an additional copy in *P. margaritifera*. All of the mitochondrial genes were encoded on the heavy chain, consistent with the features of marine bivalve mitogenomes [57,58]. The *Atp8* gene was not detected in either of the two pearl oysters. The absence of this gene has also been reported in several bivalve mitogenomes [59–62]. Although the *Atp8* gene has been described in some species of Arcidae [63], Mytilidae [18,64], and Ostreidae [17], it has not been found in any publicly available mitogenomes of Pterioidea. The detailed annotations of the complete mitogenome are recorded in Tables 1 and 2.

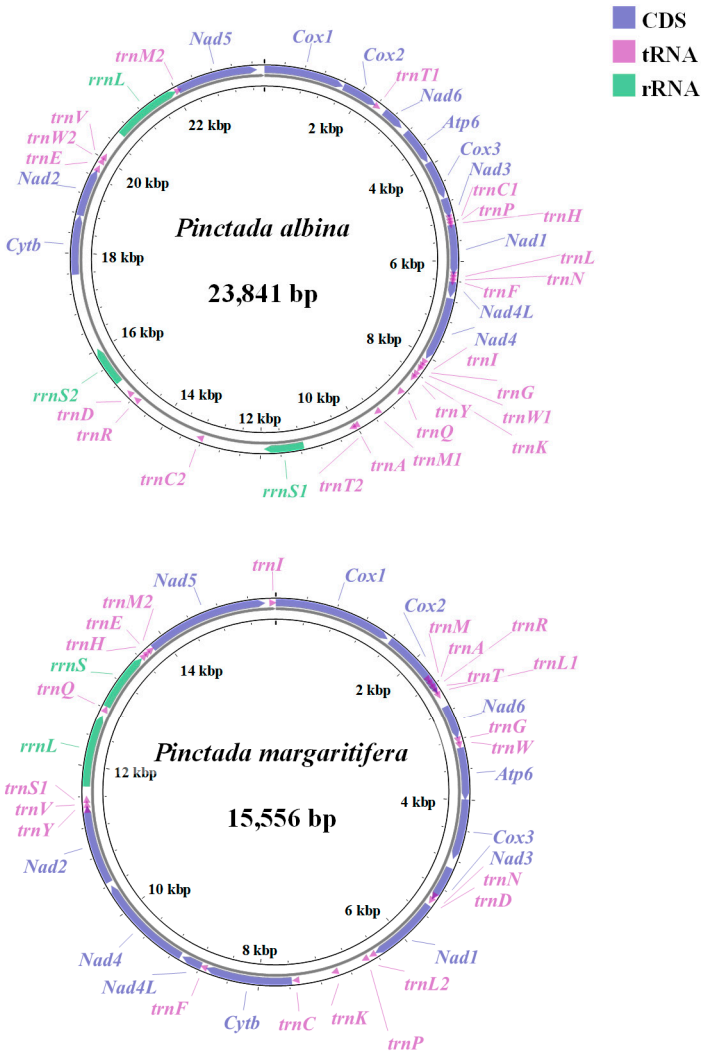

Figure 1. Mitochondrial genome map of *P. albina* and *P. margaritifera*. Gene segments are drawn to scale.

Table 1. Gene annotations of the complete mt genome of *P. albina*.

Gene	Strand	Location	Size (bp)	Start Codon	Stop Codon	Intergenic Nucleotides
Cox1	H	1–1626	1626	ATG	TAG	6
Cox2	H	1633–2376	744	GTG	TAG	13
trnT1	H	2390–2454	65			137
Nad6	H	2592–3071	480	ATG	TAG	116
Atp6	H	3188–3934	747	ATG	TAA	7
Cox3	H	3942–4721	780	ATT	TAA	4
Nad3	H	4726–5127	402	ATA	TAA	−20
trnC1	H	5108–5169	62			4
trnP	H	5174–5239	66			−4
trnH	H	5236–5304	69			−34
Nad1	H	5271–6281	1011	GTG	TAG	−32
trnL	H	6250–6312	63			−1
trnN	H	6312–6381	70			1
trnF	H	6383–6448	66			−36
Nad4L	H	6413–6733	321	TTG	TAG	14
Nad4	H	6748–8055	1308	ATA	TAG	54
trnI	H	8110–8176	67			18
trnG	H	8195–8261	67			5

Table 1. *Cont.*

Gene	Strand	Location	Size (bp)	Start Codon	Stop Codon	Intergenic Nucleotides
trnW1	H	8267–8331	65			69
trnK	H	8400–8466	67			13
trnY	H	8480–8545	66			320
trnQ	H	8866–8937	72			530
trnM1	H	9468–9544	77			438
trnA	H	9983–10,050	68			8
trnT2	H	10,059–10,122	64			1009
rrnS1	H	11,132–11,937	806			1275
trnC2	H	13,213–13,271	59			1420
trnR	H	14,692–14,761	70			129
trnD	H	14,891–14,953	63			265
rrnS2	H	15,219–16,024	806			
Cytb	H	17,575–18,777	1203	ATT	TAG	−11
Nad2	H	18,767–19,741	975	GTG	TAG	5
trnE	H	19,747–19,814	68			110
trnW2	H	19,925–19,990	66			28
trnV	H	20,019–20,085	67			514
rrnL	H	20,600–22,000	1401			18
trnM2	H	22,019–22,087	69			−33
Nad5	H	22,055–23,698	1644	ATA	TAG	143

Table 2. Gene annotations of the complete mt genome of *P. margaritifera*.

Gene	Strand	Location	Size (bp)	Start Codon	Stop Codon	Intergenic Nucleotides
Cox1	H	1–1563	1563	ATG	TAA	35
Cox2	H	1599–2525	927	ATG	TAG	−236
trnM1	H	2290–2343	54			−1
trnA	H	2343–2413	71			−2
trnR	H	2412–2476	65			2
trnT	H	2479–2531	53			0
trnL1	H	2532–2594	63			136
Nad6	H	2731–3189	459	ATG	TAG	−9
trnG	H	3181–3249	69			−2
trnW	H	3248–3313	66			1
Atp6	H	3315–4004	690	ATG	TAA	−11
Cox3	H	3994–4791	798	ATG	TAG	119
Nad3	H	4911–5381	471	ATG	TAA	−74
trnN	H	5308–5371	64			3
trnD	H	5375–5438	64			34
Nad1	H	5473–6414	942	ATG	TAA	11
trnL2	H	6426–6487	62			48
trnP	H	6536–6600	65			364
trnK	H	6965–7029	65			456
trnC	H	7486–7553	68			17
Cytb	H	7571–8716	1146	ATG	TAG	−1
trnF	H	8716–8780	65			0
Nad4L	H	8781–9059	279	ATG	TAA	20
Nad4	H	9080–10,387	1308	ATG	TAA	50
Nad2	H	10,438–11,481	1044	ATG	TAG	−75
trnY	H	11,407–11,471	65			0
trnV	H	11,472–11,535	64			0
trnS	H	11,536–11,601	66			131
rrnL	H	11,733–12,701	969			59
trnQ	H	12,761–12,825	65			1
rrnS	H	12,827–13,616	790			15

Table 2. *Cont.*

Gene	Strand	Location	Size (bp)	Start Codon	Stop Codon	Intergenic Nucleotides
trnH	H	13,632–13,685	54			0
trnE	H	13,686–13,747	62			−1
trnM2	H	13,747–13,810	64			−1
Nad5	H	13,810–15,420	1611	ATG	TAA	68
trnI	H	15,489–15,551	63			5

The nucleotide composition of the two pearl oyster mitogenomes show a high AT content (Table 3). The overall AT content value of the *P. albina* mtDNA was 58.0%, and the highest AT content was observed in *Cytb* (61.8%). The AT content of the total PCGs was higher than that of the total rRNA and total tRNA genes, and the AT content of PCGs' second codon (60.6%) was the highest. The AT content of the *P. margaritifera* mtDNA was 57.8%, and the AT content of the total tRNA genes (59.7%) was higher than that of the total PCGs and total rRNA genes, and the AT content of PCGs' second codon (60.5%) was higher than that of the other two codons. The two pearl oysters had a negative AT skew and a positive GC skew on the major strand, showing similar patterns to other Pteriomorphia species [62,65,66].

Table 3. List of AT content, AT skew, and GC skew of *P. albina* and *P. margaritifera* mtDNA.

Speices	*P. albina*			*P. margaritifera*		
Feature	**(A + T)%**	**AT Skew**	**GC Skew**	**(A + T)%**	**AT Skew**	**GC Skew**
Whole genome	58.0	−0.13	0.30	57.8	−0.24	0.35
PCGs	58.1	−0.21	0.28	56.7	−0.31	0.36
PCGs1	53.4	−0.07	0.30	53.6	−0.13	0.37
PCGs2	60.6	−0.41	0.22	60.5	−0.42	0.28
PCGs3	60.4	−0.14	0.32	56.3	−0.35	0.42
tRNAs	55.8	−0.04	0.24	59.7	−0.03	0.30
*Cox*1	57.2	−0.21	0.20	59.1	−0.30	0.23
*Cox*2	56.0	−0.18	0.23	56.8	−0.17	0.29
*Nad*6	57.7	−0.24	0.29	59.7	−0.32	0.47
*Atp*6	57.9	−0.23	0.30	58.2	−0.33	0.48
*Cox*3	56.6	−0.27	0.25	57.2	−0.28	0.27
*Nad*3	58.7	−0.16	0.41	56.5	−0.22	0.37
*Nad*1	59.1	−0.20	0.23	55.7	−0.31	0.26
*Nad*4L	55.4	−0.10	0.38	57.3	−0.36	0.33
*Nad*4	56.6	−0.26	0.34	53.8	−0.35	0.63
Cytb	61.8	−0.19	0.19	53.1	−0.36	0.37
*Nad*2	60.1	−0.24	0.35	56.6	−0.31	0.50
*Nad*5	57.9	−0.18	0.35	56.9	−0.31	0.42
rrnS	56.2	0.10	0.20	59.7	−0.01	0.22
rrnL	59.4	0.05	0.30	58.5	−0.04	0.33
rRNAs	57.7	0.07	0.25	59.1	−0.03	0.28

3.2. Protein-Coding Genes

Most of the PCGs in the two pearl oyster mitogenomes used typical start codons (ATG and ATA), while a few genes in *P. albina* used alternative start codons, such as *Cox1*, *Nad1*, and *Nad2* using GTG, *Cytb* and *Cox*3 using ATT, and *Nad4L* using TTG. For the termination codons, TAA and TAG were used in all PCGs of the two mitogenomes. The most frequently detected amino acid in the PCGs of the two species' mitogenomes was Leu and the least was Gln (Figure 2), which is in accordance with the features of the invertebrate mitochondrial genome [67,68]. Both species showed significant synonymous codon usage

bias (Tables 4 and 5, and Figure S1), preferring codons containing bases A, T, and G, which reflects the high AT content of marine bivalves.

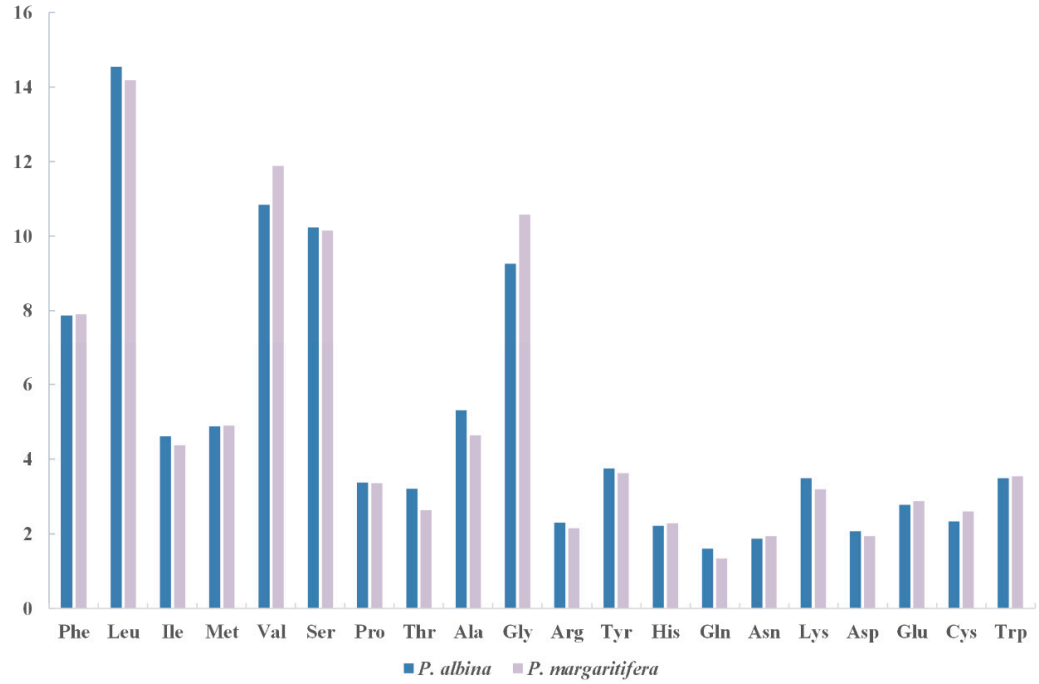

Figure 2. Amino acid compositions of *P. albina* and *P. margaritifera* mitochondrial genomes.

Table 4. Codon and relative synonymous codon usage (RSCU) of 12 protein-coding genes (PCGs) in the mtDNA of *P. albina*.

Amino Acid	Codon	Count (RSCU)	Amino Acid	Codon	Count (RSCU)
Phe	UUU	246.0 (1.67)	Ala	GCU	83.0 (1.68)
	UUC	48.0 (0.33)		GCC	40.0 (0.81)
Leu	UUA	176.0 (1.94)		GCA	47.0 (0.95)
	UUG	106.0 (1.17)		GCG	28.0 (0.57)
	CUU	80.0 (0.88)	Gly	GGU	67.0 (0.77)
	CUC	24.0 (0.27)		GGC	55.0 (0.64)
	CUA	93.0 (1.03)		GGA	72.0 (0.83)
	CUG	64.0 (0.71)		GGG	152.0 (1.76)
Ile	AUU	135.0 (1.57)	Arg	CGU	23.0 (1.07)
	AUC	37.0 (0.43)		CGC	12.0 (0.56)
Met	AUA	98.0 (1.08)		CGA	24.0 (1.12)
	AUG	84.0 (0.92)		CGG	27.0 (1.26)
Val	GUU	134.0 (1.32)	Tyr	UAU	97.0 (1.39)
	GUC	44.0 (0.43)		UAC	43.0 (0.61)
	GUA	114.0 (1.13)	His	CAU	52.0 (1.25)
	GUG	113.0 (1.12)		CAC	31.0 (0.75)
Ser	UCU	73.0 (1.53)	Gln	CAA	29.0 (0.97)
	UCC	21.0 (0.44)		CAG	31.0 (1.03)
	UCA	30.0 (0.63)	Asn	AAU	40.0 (1.14)
	UCG	7.0 (0.15)		AAC	30.0 (0.86)
	AGU	48.0 (1.01)	Lys	AAA	75.0 (1.15)
	AGC	26.0 (0.54)		AAG	55.0 (0.85)
	AGA	76.0 (1.59)	Asp	GAU	52.0 (1.35)
	AGG	101.0 (2.12)		GAC	25.0 (0.65)

Table 4. *Cont.*

Amino Acid	Codon	Count (RSCU)	Amino Acid	Codon	Count (RSCU)
Pro	CCU	47.0 (1.49)	Glu	GAA	32.0 (0.62)
	CCC	23.0 (0.73)		GAG	72.0 (1.38)
	CCA	35.0 (1.11)	Cys	UGU	57.0 (1.31)
	CCG	21.0 (0.67)		UGC	30.0 (0.69)
Thr	ACU	50.0 (1.67)	Trp	UGA	37.0 (0.57)
	ACC	15.0 (0.50)		UGG	93.0 (1.43)
	ACA	36.0 (1.20)	*	UAA	3.0 (0.50)
	ACG	19.0 (0.63)		UAG	9.0 (1.50)

"*" in this table means stop codon.

Table 5. Codon and relative synonymous codon usage (RSCU) of 12 protein-coding genes (PCGs) in the mtDNA of *P. margaritifera*.

Amino Acid	Codon	Count (RSCU)	Amino Acid	Codon	Count (RSCU)
Phe	UUU	239.0 (1.62)	Ala	GCU	89.0 (2.06)
	UUC	56.0 (0.38)		GCC	24.0 (0.55)
Leu	UUA	136.0 (1.54)		GCA	23.0 (0.53)
	UUG	187.0 (2.12)		GCG	37.0 (0.86)
	CUU	76.0 (0.86)	Gly	GGU	108.0 (1.09)
	CUC	19.0 (0.22)		GGC	51.0 (0.52)
	CUA	46.0 (0.52)		GGA	68.0 (0.69)
	CUG	66.0 (0.75)		GGG	168.0 (1.70)
Ile	AUU	132.0 (1.62)	Arg	CGU	29.0 (1.45)
	AUC	31.0 (0.38)		CGC	11.0 (0.55)
Met	AUA	58.0 (0.63)		CGA	12.0 (0.60)
	AUG	125.0 (1.37)		CGG	28.0 (1.40)
Val	GUU	190.0 (1.71)	Tyr	UAU	93.0 (1.38)
	GUC	44.0 (0.40)		UAC	42.0 (0.62)
	GUA	87.0 (0.78)	His	CAU	43.0 (1.01)
	GUG	123.0 (1.11)		CAC	42.0 (0.99)
Ser	UCU	70.0 (1.48)	Gln	CAA	14.0 (0.56)
	UCC	21.0 (0.44)		CAG	36.0 (1.44)
	UCA	26.0 (0.55)	Asn	AAU	50.0 (1.39)
	UCG	19.0 (0.40)		AAC	22.0 (0.61)
	AGU	67.0 (1.41)	Lys	AAA	52.0 (0.87)
	AGC	34.0 (0.72)		AAG	67.0 (1.13)
	AGA	45.0 (0.95)	Asp	GAU	48.0 (1.33)
	AGG	97.0 (2.05)		GAC	24.0 (0.67)
Pro	CCU	59.0 (1.89)	Glu	GAA	31.0 (0.58)
	CCC	20.0 (0.64)		GAG	76.0 (1.42)
	CCA	26.0 (0.83)	Cys	UGU	80.0 (1.65)
	CCG	20.0 (0.64)		UGC	17.0 (0.35)
Thr	ACU	45.0 (1.84)	Trp	UGA	41.0 (0.62)
	ACC	16.0 (0.65)		UGG	91.0 (1.38)
	ACA	16.0 (0.65)	*	UAA	7.0 (1.17)
	ACG	21.0 (0.86)		UAG	5.0 (0.83)

"*" in this table means stop codon.

3.3. tRNA and rRNA Genes

The mitogenome of most metazoans contains 22 tRNA genes, including two copies of *trnL* and two of *trnS*. However, the number of tRNA genes is highly variable in bivalves [69,70]. Duplication of the *trnM* genes has been found in many bivalve mitogenomes [71], which is consistent with our findings.

In the literature, the duplication of the *trnW* gene has also been observed in Ostreoidea [17], while additional copies of *trnT* and *trnC* have not been reported. In addition,

one of the *trnL* and both of the *trnS* genes have not yet been found in the mitogenome of *P. albina*. The duplication of *trnS* has not been detected in *P. margaritifera*. In this study, the secondary structure of tRNAs was investigated and the majority of them were found to have a typical cloverleaf structure, except for *trnC2* in *P. albina* and *trnS* and *trnM1* in *P. margaritifera* (Figure 3). The D-arm of *trnC2* in *P. albina* and *trnS* in *P. margaritifera* was absent, and *trnM1* in *P. margaritifera* lacked the T-arm. These tRNA genes ranged from 53 to 73. The mitogenome of *P. albina* was 8285 bp larger than that of *P. margaritifera*, which may be related to the duplication of *rrnS* and the additional mitogenome ORFs [12]. *Pinctada albina* have an almost identical extra copy of the *rrnS*, which was not detected in *P. margaritifera*. Multiple studies have shown that duplication of *rrnS* is a common feature of Ostreoidea [65], which was previously observed in *Pinctada imbricata* [40] and was also observed in this study, and which may be related to gene rearrangement.

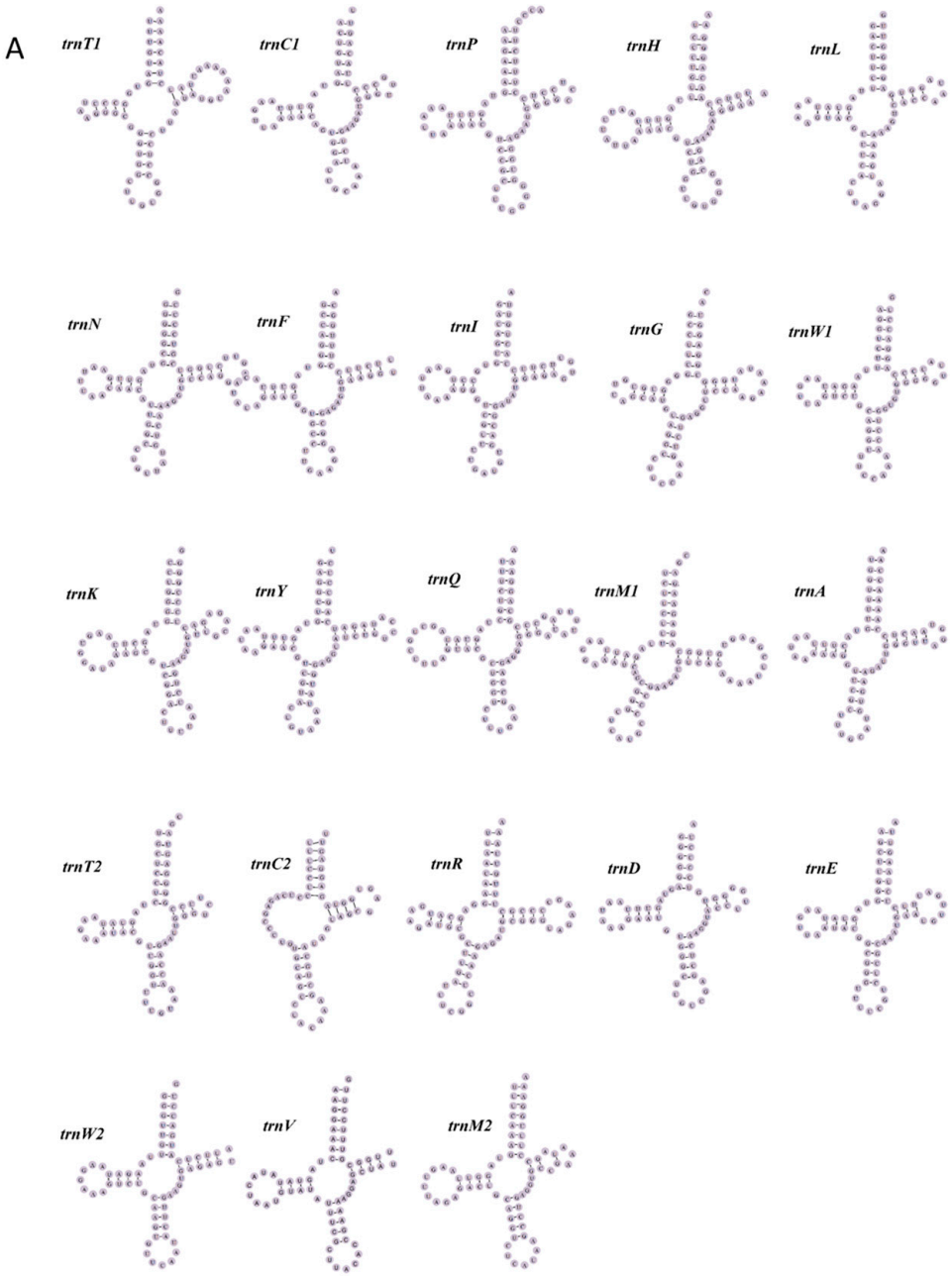

Figure 3. *Cont.*

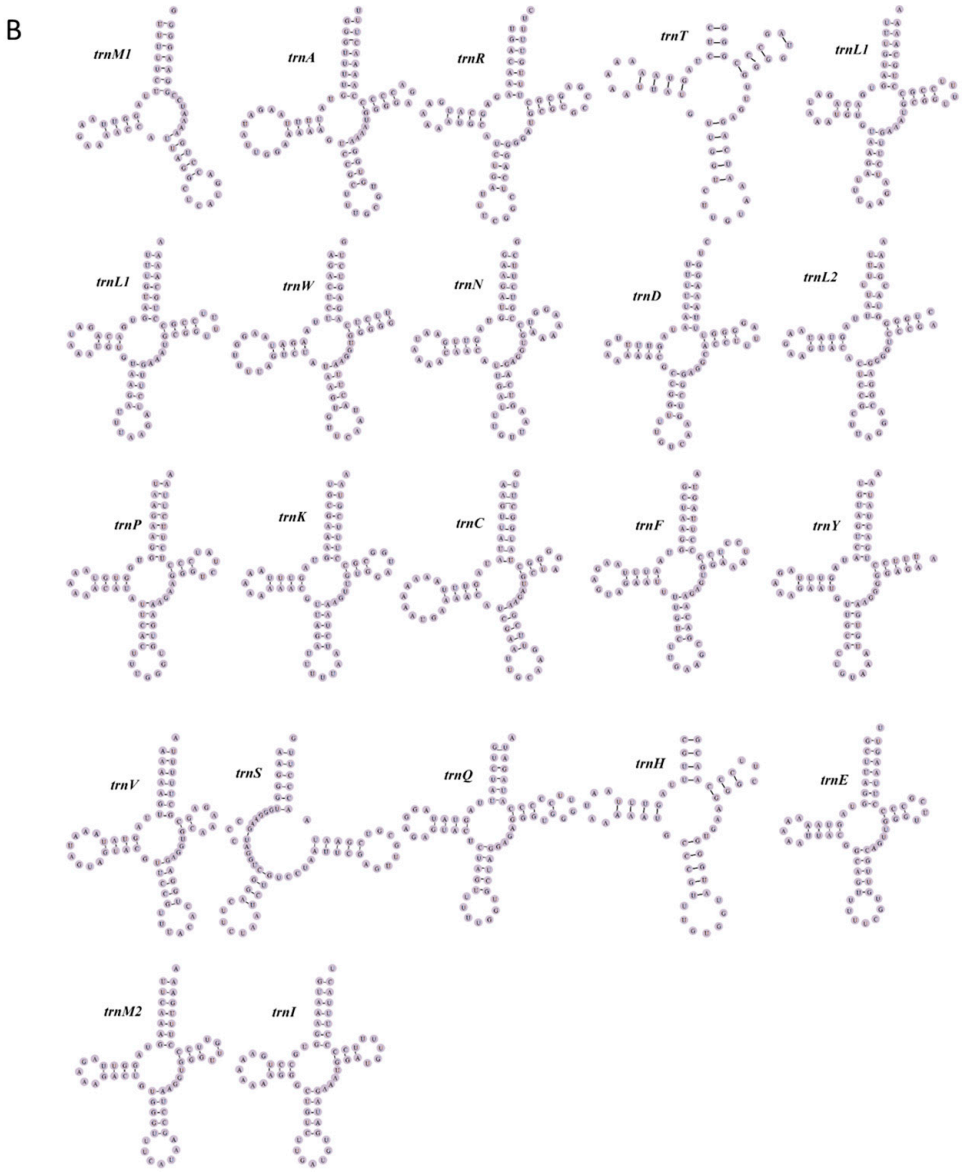

Figure 3. Putative secondary structures of the tRNA genes in the mitogenome of *P. albina* (**A**) and *P. margaritifera* (**B**).

3.4. Phylogenetic Analysis

According to BIC, the best partition scheme for PCGs was the one combining subunits within genes into a single partition, but analyzing each codon position separately, while the best partition scheme for rRNAs was the one combining the two genes (Table S2). ML (−lnL = 279,788.758) and BI (−lnL = 279,693.89 for run 1; −lnL = 279,695.61 for run 2) analyses arrived at almost identical topologies (Figure 4).

The phylogenetic tree showed that the eight species of Pterioidea formed a strongly supported and monophyletic clade. The infraclass Pteriomorphia was comprised of two clades. The first clade only included the superfamily Mytiloidea, while the second one consisted of superfamilies Pectinoidea, Pinnoidea, Ostreoidea, Pterioidea, and Arcoidea, which is consistent with the results of Wu et al. [72] based on mitochondrial PCGs. However, the study of Wu et al. [72] showed that Pterioidea and Pinnoidea formed a clade, which was a sister to Pectinoidea. This finding differed from ours. The phylogenetic relationship reconstructed in our study indicated that Pterioidea formed its own clade, which was the sister group of Pinnoidea + Ostreoidea. The branch formed by these three superfamilies was most closely related to Pectinoidea and followed by Arcoidea.

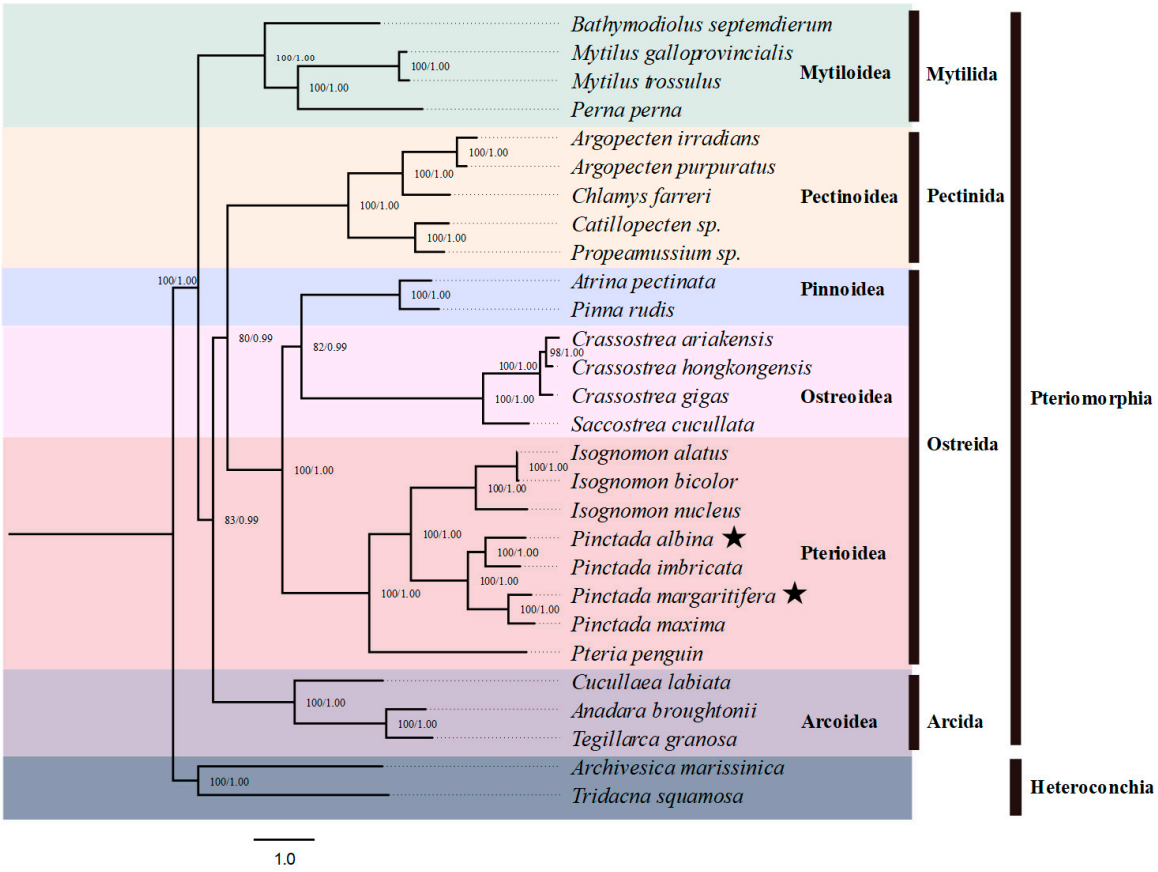

Figure 4. Phylogenetic relationships of 8 Pterioidea species relative to other Pteriomorphia species, based on the concatenated nucleotide sequences of 12 mitochondrial protein-coding genes and two ribosomal RNA genes. Numbers at the nodes correspond to ML bootstrap proportions and the Bayesian posterior probabilities. Order and Superfamily affiliations of Pteriomorphia species are indicated on the the tree. Species marked with stars were sequenced in this study.

The relationship between the four superfamilies Pterioidea, Pinnoidea, Ostreoidea, and Pectinoidea has long been controversial. Gaitán-Espitia et al. [39] analyzed Pteriomorphia based on 12 PCGs, which showed that the Pterioidea was more closely related to Ostreoidea, and their MRCA was a sister group to Pinnoidea. This result was supported by phylogenies derived from transcriptomes [73], 18S rDNA [74], and a combined dataset from Tëmkin [36]. However, research by Adamkewicz et al. [75] at the class bivalve level based on 18S rDNA showed that Pterioidea was more closely related to Pinnoidea, and they formed a clade as a sister group to Ostreoidea. Meanwhile, our study revealed a closer relationship between Pinnoidea and Ostreoidea, which was also supported by Zhan et al. [40] based on 12 PCGs, by Ozawa et al. [76] using 12 PCGs and two rRNAs, and by Matsumoto [38] based on COI. The monophyly of the genera *Pinctada*, *Isognomon*, and *Pteria* was well supported in Pterioidea by our research, with *Pinctada* being most closely related to *Isognomon*. This result is consistent with the study by Tëmkin [36] on molecular data sets composed of DNA sequences for nuclear and mitochondrial loci, and anatomical and shell morphological characteristics. The monophyly of the genera *Pinctada* and *Pteria* is also supported by Zhan et al. [40]. Our phylogenetic tree revealed that the genus *Pinctada* can be divided into two groups: *P. albina* + *P. imbricata* and *P. maxima* + *P. margaritifera*, which is consistent with previous morphological classification based on shell morphology and anatomical characteristics [77–79]. The morphological identification showed that *P. albina* and *P. imbricata* have small shells and hinge teeth, while *P. maxima* and *P. margaritifera* have larger shells without hinge teeth.

3.5. Mitochondrial Gene Rearrangements within Pterioidea

The mitochondrial gene order in metazoans is relatively conserved. However, a large number of gene rearrangements have been found in mitochondrial studies on bivalves [30,32,64]. Based on the types of genes, genome rearrangements can be characterized as minor (tRNAs only) or major (PCGs and rRNA genes) rearrangements [80]. In general, rearrangements of tRNAs are common, while PCGs are relatively conserved. There were still substantial gene rearrangement events in the PCGs and rRNA genes of Pterioidea as we deleted all tRNAs (Table 6). The CREx analysis of the PCGs and rRNA genes' order in Pterioidea suggested that when assuming the gene orders of *P. margaritifera* and *P. albina* to be the ancestral ones, those of other species could be obtained with a minimum number of changes. However, the *rrnS* gene in the mitogenome of *P. albina* contained an extra copy, which required an additional deletion event leading to other species or duplication in *P. albina*. Moreover, there were high numbers of common intervals between *P. margaritifera* and other species (Figure 5A). Therefore, the PCGs and rRNA gene order of *P. margaritifera* were assumed to be most similar to the ancestral order of Pterioidea (Figure 5B,C).

Table 6. CREx analysis of the most ancestral gene order in Pterioidea. The arrangements of PCGs and rRNAs are considered. The mitogenomes of the three species in Isognomon have the same gene order, so *Isognomon bicolor* is used to represent them. The gene rearrangement events are abbreviated as follows: Transp., transposition; Rev., reversal; Rev. transp., reverse transposition; TDRL, tandem duplication-random loss.

From	To	Transp.	Rev.	Rev.transp.	TDRL	Total Events
P. albina	*P. imbricata*	1	0	0	0	1
	P. margaritifera	2	0	0	0	2
	P. maxima	0	0	0	2	2
	P. penguin	0	0	0	2	2
	I. bicolor	3	4	0	0	7
P. imbricata	*P. albina*	1	0	0	0	1
	P. margaritifera	2	0	0	0	2
	P. maxima	3	0	0	0	3
	P. penguin	0	0	0	3	3
	I. bicolor	3	4	0	0	7
P. margaritifera	*P. albina*	0	0	0	1	1
	P. imbricata	2	0	0	0	2
	P. maxima	1	0	0	0	1
	P. penguin	0	0	0	3	3
	I. bicolor	3	4	0	0	7
P. maxima	*P. albina*	1	0	0	1	2
	P. imbricata	3	0	0	0	3
	P. margaritifera	1	0	0	0	1
	P. penguin	0	0	0	3	3
	I. bicolor	2	8	0	0	10
P. penguin	*P. albina*	1	0	0	2	3
	P. imbricata	1	0	0	2	3
	P. margaritifera	1	0	0	2	3
	P. maxima	3	0	0	1	4
	I. bicolor	1	1	1	2	5
I. bicolor	*P. albina*	3	4	0	0	7
	P. imbricata	3	4	0	0	7
	P. margaritifera	3	4	0	0	7
	P. maxima	2	8	0	3	13
	P. penguin	2	1	1	2	6

A

	Pa	Pi	Pmar	Pmax	Pp	Lb	Ln	La
Pinctada albina	204	116	108	74	10	52	52	52
Pinctada imbricata	116	204	108	74	10	52	52	52
Pinctada margaritifera	108	108	176	98	6	36	36	36
Pinctada maxima	74	74	98	176	8	32	32	32
Pteria penguin	10	10	6	8	176	10	10	10
Isognomon bicolor	52	52	36	32	10	176	176	176
Isognomon nucleus	52	52	36	32	10	176	176	176
Isognomon alatus	52	52	36	32	10	176	176	176

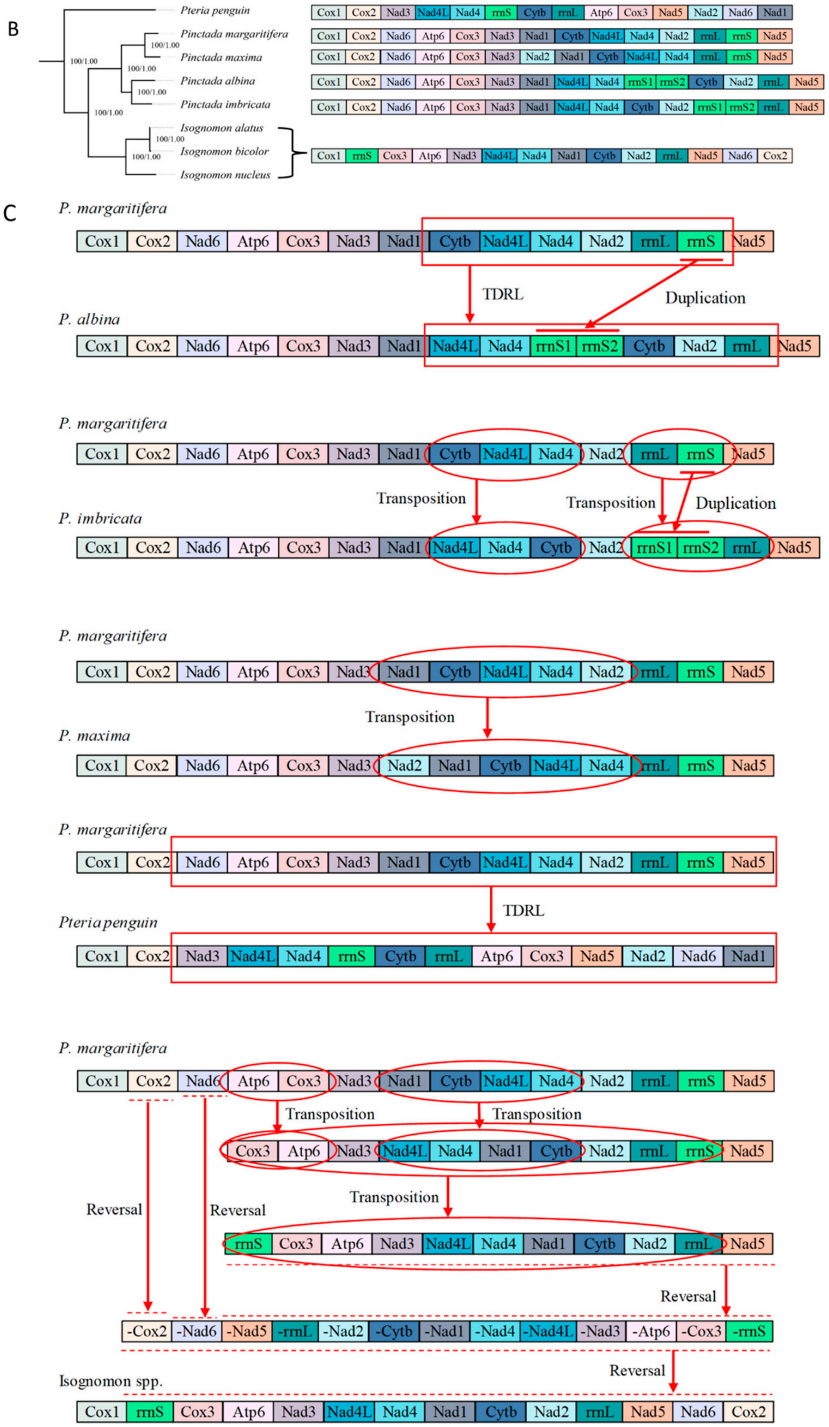

Figure 5. (**A**) Pairwise comparisons of mitochondrial gene arrangements with all tRNAs removed in Pterioidea using CREx. The numbers indicate the similarity of the compared gene orders. The larger

the number, the more similar the gene order between the two compared sequences. (**B**) Linearised PCGs and rRNA gene orders of Pterioidea, based on the phylogenetic tree. (**C**) The putative evolutionary patterns of Pterioidea mitochondrial PCGS and rRNA gene rearrangements.

4. Conclusions

The newly sequenced complete mitogenomes of *P. albina* and *P. margaritifera* showed similar patterns for genome size and composition compared with those of other pterioid species. However, the presence of an extra copy of *rrnS* in *P. albina* is an informative characteristic that has otherwise only been detected in the *P. imbricata* mitogenome. The results of our phylogenetic analysis support the monophyly of Pterioidea placed in the Ostrea order and provide a robust phylogenetic framework for Pteriomorphia. An analysis of the rearrangement events of PCGs and rRNA within Pterioidea species was conducted and the ancestral gene order was inferred. The present study indicates that the complete mitochondrial genome is a useful tool with which to understand the evolution of marine bivalve Pteriomorphia.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8100528/s1, Figure S1: Relative synonymous codon usage (RSCU) of mitochondrial genome of *P. albina* (Left column for each amino acid) and *P. margaritifera* (Right column for each amino acid); Table S1: List of species used in this study; Table S2: Best fit partitions and substitution models.

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