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Denser Mitogenomic Sampling for Exploring the Phylogeny of Tellinoidea (Mollusca: Bivalvia)

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Abstract: The superfamily Tellinoidea is one of the most diverse groups of marine bivalves, with significant ecological and economic value. To date, the availability of complete mitochondrial genome data within Tellinoidea remains limited, and the taxonomic coverage is still insufficient to resolve its internal controversies. The current study aims to further explore the phylogenetic relationships within Tellinoidea through denser sampling. We have newly sequenced the mitochondrial genomes of 13 species, among which seven genera are being published for the first time. Combined with the published mitogenomes and transcriptomic data, we constructed the most comprehensive Tellinoidea phylogeny to date through maximum likelihood and Bayesian Inference analyses. Our findings support the monophyly of the superfamily Tellinoidea, with Semelidae nesting as a monophyletic group within Tellinidae. We also support the paraphyly of Tellinidae based on the mitochondrial genome data for the first time, identifying that the two subfamilies (Macominae and Tellininae) are polyphyletic. Gene rearrangement analysis reveals a relatively high degree of variation in Semelidae. By expanding the mitochondrial genome dataset, this study provides new insights into the phylogeny of Tellinoidea and underscores the need for further sampling of species to reassess the phylogenetic relationships of Tellinidae and the entire Tellinoidea.

Keywords: Tellinoidea; mitogenome; phylogeny; Mollusca

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1. Introduction

The superfamily Tellinoidea (Mollusca: Bivalvia) is one of the most diverse groups within marine bivalve molluscs, consisting of five extant families, Tellinidae, Semelidae, Donacidae, Psammobiidae, and Solecurtidae, encompassing 49 genera and approximately 900 species [1]. Species within this superfamily primarily inhabit the fine-sandy or muddy substrates in the intertidal zones of temperate and tropical seas, living either as burrowers or infaunal organisms. Notably, some donacid species exhibit unique ecological adaptations, transitioning from marine to freshwater environments [2]. The varied lifestyles of this group contribute to its high biodiversity and morphological plasticity across different families. In addition to their significant ecological role in community structure, some species within this superfamily serve as economically important food resources for their value in aquaculture [3]. For instance, certain species of *Megangulus* in Tellinidae are considered valuable food resources in Japan because of their tender, flavorful meat and desirable texture [4]. Similarly, *Donax trunculus* from Donacidae is a highly valued species in Galicia (Northwestern Spain), contributing significantly to the local market [5]. Thus,

due to their important ecological and economic roles, Tellinoidea has gained attention as valuable material for the phylogenetic research, cultivation of economically important species, and management of endangered species.

In the molecular phylogenetic studies of Tellinoidea, the early research based on short genetic fragments (such as partial mitochondrial and nuclear genes) has generally supported the monophyly of the superfamily [6,7]. However, these studies mostly focused on the large-scale resolution of the bivalve tree, often using only a single or a few representatives from each family and left the monophyly of families untested. Studies utilizing the complete mitochondrial genome have also strongly supported the monophyly of Tellinoidea and suggest taxonomic revisions for certain groups [5,8–10]. For instance, the integration of mitochondrial genome data from Donacidae, alongside morphological analyses and fossil records, has resulted in the first evolutionary hypothesis for Donacidae, strongly supporting this family monophyly and forms a sister group with the other four families in Tellinoidea (Tellinidae, Semelidae, Psammobiidae, and Solecurtidae) [2,5]. Despite these studies enhancing our understanding of the evolutionary relationships within Tellinoidea, the phylogenetic positions of Tellinidae, Psammobiidae, and Solecurtidae continue to be debated. Recent studies suggest that *Sinonovacula* should be classified under the Solenoidea rather than Tellinoidea group [9]. Mapping gene order onto the phylogenetic tree revealed that Psammobiidae is paraphyletic, and species of *Gari* do not form a monophyletic sister group [10]. Notably, as the most species-rich family within Tellinoidea, Tellinidae encompasses over 500 species, accounting for approximately 60% of the total diversity of the superfamily's total diversity. However, previous studies have primarily relied on shell morphology and anatomical characteristics for classification [11–13]. For example, a phylogenetic analysis of Thailand species within the family Tellinidae, based on 43 morphological characters, concluded that while Tellinoidea and Tellinidae are monophyletic, the subfamily Tellininae is paraphyletic [11]. Although these studies provide robust support for understanding the relationships within Tellinoidea, additional molecular data are needed to further elucidate and validate these phylogenetic relationships.

Mitochondrial genomes are valued for their stable composition, rarity of recombination, maternal inheritance, and high evolutionary rate [14]. Compared with nuclear or mitochondrial gene fragments, phylogenetic analysis based on the complete mitochondrial genome can provide more informative sites, thereby improving the resolution of phylogenetic trees and increasing statistical confidence [15]. The existing studies have shown that analyzing mitochondrial genomes can accurately trace the evolution of bivalves [8]. Such analyses can enhance our understanding of bivalve evolution and diversity by overcoming the limitations of classifications based solely on morphological anatomy, including the challenges posed by convergent evolution and phenotypic plasticity. Therefore, mitochondrial genomes are now considered a cornerstone in the evolutionary research and have been widely used to reconstruct the phylogenetic relationships among different bivalve groups, including Veneroida [5], Imparidentia [16], and Mytilidae [17]. With Tellinoidea, although there have been studies utilizing the mitochondrial genomes of Tellinoidea to understand the evolutionary and phylogenetic significance of gene rearrangements, only a few mitochondrial genomes of the entire Tellinoidea have been published, with only three available for the species-rich family Tellinidae. Hence, it is still necessary to sample representative groups and construct datasets containing as many phylogenetic signals as possible to explore the phylogenetic relationships within the superfamily Tellinoidea and even within its constituent families.

In this study, we newly sequenced the mitochondrial genomes of 13 species of Tellinoidea and extracted mitochondrial genome-related data from three published transcriptomes in GenBank. We combined these data with another 18 mitochondrial genome data

obtained from NCBI to reconstruct the phylogeny of this superfamily. These data covered 20 genera across five families within Tellinoidea, among which the mitochondrial genome data of 6 genera were reported for the first time. Our aims are to: (a) reconstruct a more comprehensive phylogeny of Tellinoidea at the mitochondrial genome level to enhance the phylogenetic resolution; (b) explore the characteristics of mitochondrial genomes among different families; and (c) investigate the phylogenetic relationships within Tellinidae, with a focus on testing its monophyly and resolving the status of its subfamilies.

2. Materials and Methods

2.1. Sample Collection, DNA Extraction, and Next-Generation Sequencing

The specimens that were newly sequenced were obtained from shallow intertidal areas in China (Table 1). Each specimen was preserved in 95% ethanol after preliminary morphological identification and deposited in Fisheries College, Ocean University of China. Genomic DNA was extracted from the adductor muscle of 13 Tellinoidea specimens, using a modified phenol–chloroform method [18]. The concentration of total genomic DNA was measured with a Nanodrop spectrophotometer, with concentrations ranging from 50 to 200 ng/μL. Then, the qualified genomic DNA samples were submitted to Novogene Technology Co., Ltd. (Beijing, China), for library preparation and high-throughput sequencing. Sequencing libraries with an average insert size of around 300 bp were generated and sequenced using 150 bp paired-end reads on the NovaSeq 6000 platform (Illumina, San Diego, CA, USA), resulting in approximately 8 Gb of raw data generated for each library.

Table 1. List of species sequenced in this research.

Species	Order	Genbank	Length (bp)	Locality
Latona cuneata	Donacidae	PQ851673	16,921	Lingshui, Hainan, China
Semele crenulata	Semelidae	PQ238728	16,671	Lingshui, Hainan, China
Semele cordiformis	Semelidae	PQ238729	16,260	Beihai, Gangxi, China
Macoma tokyoensis	Tellinidae	PQ851675	16,606	Qingdao, Shandong, China
Megangulus venulosus	Tellinidae	PQ306545	16,143	Qingdao, Shandong, China
Jitlada culter	Tellinidae	PQ306547	16,483	Rizhao, Shandong, China
Psammacoma candida	Tellinidae	PQ221920	16,933	Qingdao, Shandong, China
Serratina perplexa	Tellinidae	PQ356192	16,673	Fangchenggang, Gangxi, China
Serratina capsoides	Tellinidae	PQ839686	16,590	Wenchang, Hainan, China
Quidnipagus palatam	Tellinidae	PQ851674	16,650	Sanya, Hainan, China
Gari maculosa	Psammobiidae	PQ276911	16,555	Fangchenggang, Gangxi, China
Nuttallia tchangsii	Psammobiidae	PQ306546	16,695	Lingao, Hainan, China
Gari virescens	Psammobiidae	PQ306548	16,116	Wenchang, Hainan, China

2.2. Mitochondrial Genome Assembly and Annotation

Trimmomatic v. 0.39 [19] was used to trim and filter the raw reads obtained from 16 samples (including 3 transcriptomic datasets and 13 newly sequenced genomic datasets), removing adapter contamination and low-quality reads with sequencing quality scores below Q20. De novo assemblies of the resulting clean reads were performed by SPAdes v. 3.15.5 [20] with *k*-mers of 21, 33, 55, and 77, and NOVOPlasty v. 4.3.1 [21]. The new mitochondrial protein-coding genes (PCGs) were annotated primarily using the MITOS web server [22] with the invertebrate mitochondrial genetic code and confirmed by Open Reading Frame Finder (https://www.ncbi.nlm.nih.gov/orffinder/, accessed on 8 June 2024). The gene boundaries were checked and adjusted in Artemis for a manual genome annotation [23]. The rRNA genes were identified through the BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/, accessed on 10 June 2024) by comparing the inferred sequences and aligning them with mitochondrial genomes from Tellinoidea, with

their boundaries presumed to lie between neighboring genes [24]. ARWEN was used to confirm the positions of tRNA genes [25]. The complete sequences of the 13 newly sequenced mitochondrial genomes are available in GenBank (Table 1).

Three raw transcriptomic datasets [26] from *Scissula similis*, *Asaphis deflorata*, and *Tagelus plebeius* were downloaded from the National Center for Biotechnology Information (NCBI) SRA database. First, the raw reads were quality-checked using FastQC v. 0.11.8 [27] and then trimmed using Trimmomatic v. 0.39. The processed data were assembled using an iterative pipeline within the mitochondrial genome reconstruction workflow (https://github.com/mozoo/mitoRNA, accessed on 1 August 2024) [28], resulting in Trinity assembly output files for 17, 13, and 14 generations, respectively. The mitochondrial PCGs and rRNA genes were identified using TBLASTX v. 2.7.1 [29], employing the nucleotide sequences of 13 PCGs and 2 rRNA genes from closely related species as query sequences.

To assess the accuracy of this approach and the resulting data, the transcriptomic data for *Scrobicularia plana* (SRR8217862) with a publicly available mitochondrial genome (MN528026) were assembled and annotated as previously outlined. The differences in positions and the similarity percentages between the mitochondrial genome sequences assembled from the transcriptomes and those in GenBank were evaluated [30,31] (Table S1).

2.3. Gene Order Analysis

Pairwise comparisons of the previously identified typical rearrangement types in the Tellinoidea and the newly sequenced ones were performed using CREx (https://usegalaxy.eu/, accessed on 30 August 2024) [32]. The complete mitochondrial gene order, including PCGs, rRNA, and tRNA genes, was considered. The comparisons were based on common intervals, taking into account rearrangement events, such as inversions, transpositions, inverse transpositions, and tandem duplication random losses (TDRLs).

2.4. Sequence Analysis and Alignment

The nucleotide composition and the 13 PCGs of newly sequenced Tellinoidea specimens were analyzed using MEGA v. 11.0.13 [33] based on the invertebrate mitochondrial genetic code. The AT and GC skews were calculated using the following formulas: AT skew = (A − T)/(A + T); GC skew = (G − C)/(G + C).

The phylogenetic analysis utilized a total of 40 mitogenomes (Table S2). Based on recent studies on the evolutionary history of Tellinoidea [10,34], six species from the Superfamily Cardioidea were selected as outgroups (Table S2). The 13 PCGs and rRNA genes were aligned using MAFFT v. 7.520 [35] with default settings. Ambiguously aligned regions were removed through Gblocks v. 0.91b [36]. Substitution saturation for the nucleotide sequences of the 13 PCGs was analyzed in DAMBE7 (http://dambe.bio.uottawa.ca, accessed on 15 September 2024) [37]. Due to high saturation, the *atp8* and the third codon positions of 12 PCGs were omitted from nucleotide-based phylogenetic studies (Table S3). The dataset consisted of nucleotide sequences from the first two codons of 12 PCGs and 2 rRNA genes. Finally, the single alignments were concatenated using FASconCAT v. 1.11 [38] for downstream phylogenetic analysis.

2.5. Phylogenetic Analysis

In this study, phylogenetic relationships within Tellinoidea were inferred using both maximum likelihood (ML) and Bayesian Inference (BI) methods. The ML tree was constructed using IQ-TREE v. 1.6.1 [39] with 1000 ultrafast bootstrap replicates (-bb 1000) and an automatic algorithm. The BI tree was inferred using MrBayes v. 3.1.2 [40] with four parallel Markov Chain Monte Carlo (MCMC) chains run for 10,000,000 generations, sampling every 1000 generations, and discarding the first 25% of generations as burn-in on the CIPRES Science Gateway v. 3.3. Tracer v. 1.7 [41] was used to examine all the parame-

ters, ensuring an effective sample size (ESS) greater than 200. The resulting phylogenetic tree was visualized in FigTree v. 1.4.4 [42]. The optimal partitioning scheme and best-fit substitution models for the dataset were determined using PartitionFinder 2.1.1 [43] with the Bayesian Information Criterion (BIC). The selected optimal partitions and models are provided in Table S4.

3. Results and Discussion

3.1. Phylogenetic Analysis

The phylogenetic relationships within the Tellinoidea and the monophyly of some families have long been a subject of uncertainty. Our study provides the most comprehensive phylogenetic framework for Tellinoidea to date, based on mitochondrial genome data. By using nucleotide sequences from the first two codons of 12 protein-coding genes (excluding the *atp8* gene) and 2 rRNA genes (*rrnS* and *rrnL*), we constructed a concatenated dataset comprising 8795 sites to reconstruct the phylogenetic tree of Tellinoidea. Both ML and BI analyses produced congruent tree topologies, with high support for most nodes (Figure 1). The phylogenetic tree confirmed that Tellinoidea is monophyletic as the sister group to Cardioidea, a finding that is consistent with previous molecular and morphological studies [6,7,27,34]. Within Tellinoidea, Donacidae forms a single clade, positioned as a sister group to all other four families, except for the basal-position species *Nuttallia obscurata* and *Gari togata* (Psammobiida), which aligns with the most recent bivalve phylogenetic studies [5,9,10]. The mitogenome of *G. togata* may represent a male-type (M-type) mitogenome, as suggested by Burzyński et al. [44]. This could explain its unusual phylogenetic behavior, as M-type mitogenomes often exhibit distinct evolutionary patterns due to doubly uniparental inheritance (DUI). The remaining families' divergence occurs in the following order: (Semelidae + Tellininae) and (Psammobiidae + Solecurtidae).

Unexpectedly, our results show that Semelidae forms a monophyletic group, nested within Tellinidae with moderate support (BS: 82%, PP: 0.99). This finding contrasts sharply with the previous findings, as previous studies based on mitochondrial genomes have consistently emphasized that Semelidae forms a sister group to (Tellinidae + (Psammobiidae + Solecurtidae)) [5,8–10], though these studies included only a single species (*Semele scabra*). Studies that included two or more species have not recovered Semelidae as a monophyletic group, whether based on short fragments or mitochondrial genetic data [6,7,16]. Therefore, our study highlights the potential challenges in the internal systematics of Tellinidae, underscoring the need for further investigation into these intriguing phylogenetic relationships. Notably, the Tellinidae data in this study include two subfamilies (Macominae and Tellininae), both of which exhibit polyphyly in the phylogenetic tree. *Psammacoma candida* (Macominae) was found to be the sister group to some species within Tellininae, with strong support in both the ML and BI trees. However, it is important to note that our study lacks additional representative species of Macominae, which may somewhat limit the broader applicability of our conclusions regarding the phylogenetic positioning of *Psammacoma*. Within Tellininae, *Serratina* and *Quidnipagus* form a highly supported clade (BS: 100%, PP: 1.00), which is sister to (Semelidae + (*Macoma* + other Tellininae species)), resulting in a paraphyletic structure for Tellinidae. This finding contradicts previous studies, primarily due to the limited availability of mitochondrial genome data for Tellinidae, with most research involving only two species—*Macoma balthica* (Macominae) and *Iridona iridescens* (Tellininae)—which form well-supported sister branches [5,8,10]. However, previous studies based on morphology, anatomy, and short DNA fragment sequencing have consistently found that multiple Tellininae sampling groups do not form a monophyletic clade, which is in agreement with our findings [6,7,34]. Thus, we look forward to incorporating more

representative groups of Tellinidae in future studies to better understand the phylogenetic placement of the subfamilies.

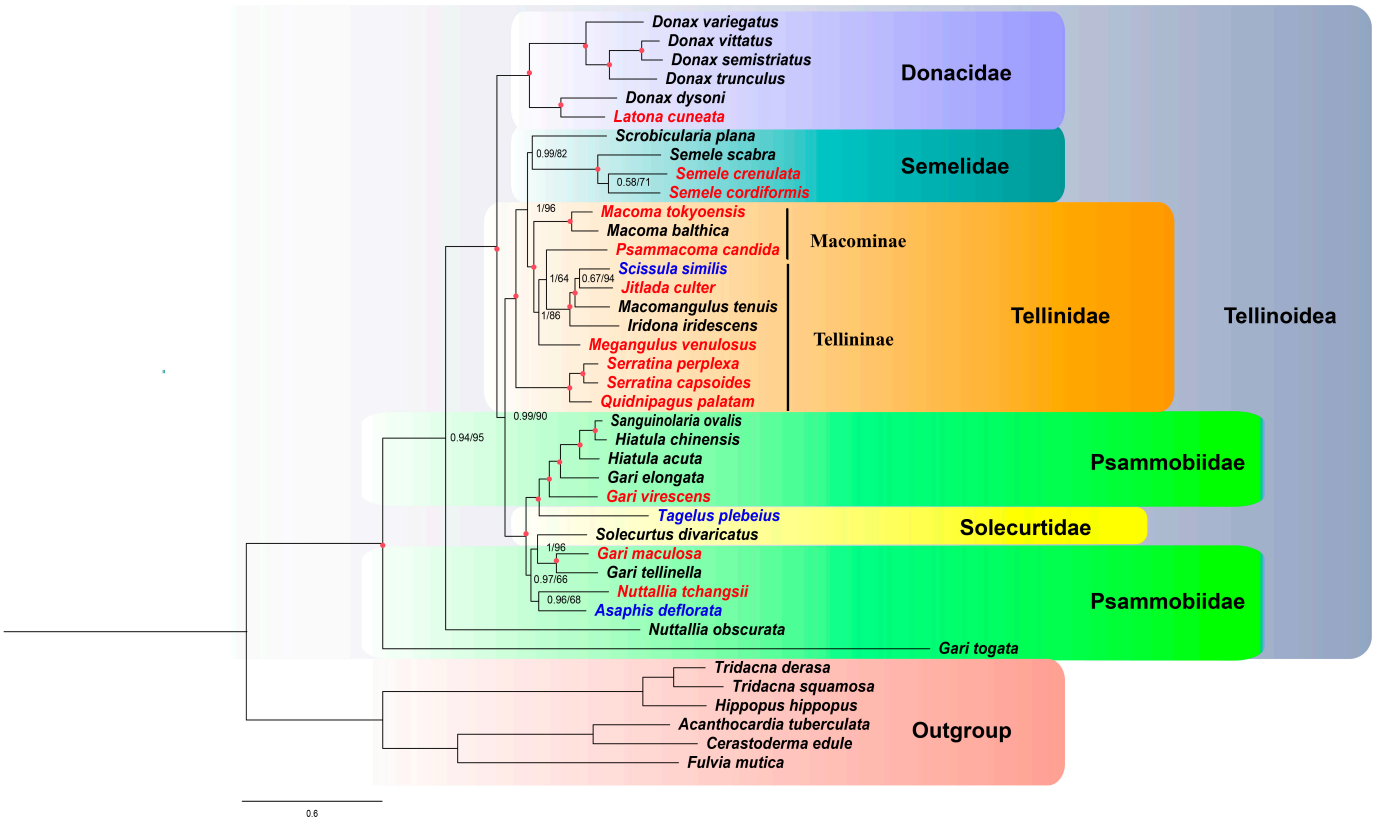

Figure 1. Phylogenetic relationships of Tellinoidea inferred from both maximum likelihood (ML) and Bayesian Inference (BI) analyses, based on concatenated datasets of nucleotide sequences from 12 protein-coding genes and two rRNA genes in the mitochondrial genomes. Numbers at nodes are statistical support values for BI (posterior probabilities)/ML (bootstrap proportions in percentage). Solid red circles represent nodes with posterior probabilities = 1 and bootstrap proportions = 100. The newly sequenced mitochondrial genomes are depicted in red, while the utilized transcriptomes are presented in blue.

In our phylogenetic tree, two species of Solecurtidae did not cluster together, but instead were nested within two sister branches of Psammobiidae. Additionally, Psammobiidae also displayed a paraphyletic relationship, consistent with previous molecular studies [9,10]. The five species of the genus *Gari* did not form a monophyletic group, a result that aligns with the findings of Sun et al. [10], although the study included only two species. Moreover, *N. obscurata*, located at the base of the phylogenetic tree, did not form a sister group with the newly sequenced *Nuttallia tchangsii* from this study. Therefore, our research underscores the importance of incorporating more comprehensive and validated molecular data in future studies to reassess the phylogenetic positions of the *Gari* and *Nuttallia* within the Psammobiidae.

3.2. Gene Arrangements of the Mitochondrial Genome

Mitochondrial genome arrangements are generally conserved across metazoans, yet bivalves—particularly marine species—exhibit frequent and extensive gene rearrangements [45,46]. This phenomenon has been attributed to unidirectional replication and transcription mechanisms inherent to their single-stranded mitochondrial genomes, which may accelerate rearrangement events during evolution [45]. Building on the foundational work of Sun et al. [10], who classified four rearrangement types (Types I–IV) in Tellinoidea

and identified Type IV as the typical gene order (TTGO), our study expands this framework by analyzing newly sequenced mitochondrial genomes. Comparative analysis of the 13 newly sequenced mitochondrial genomes revealed that most conform to TTGO. However, they also exhibit four types of rearrangements (Types V–VIII) characterized by translocations and inversions, which represent a higher degree of gene rearrangement than previously recognized, as illustrated in Figure 2.

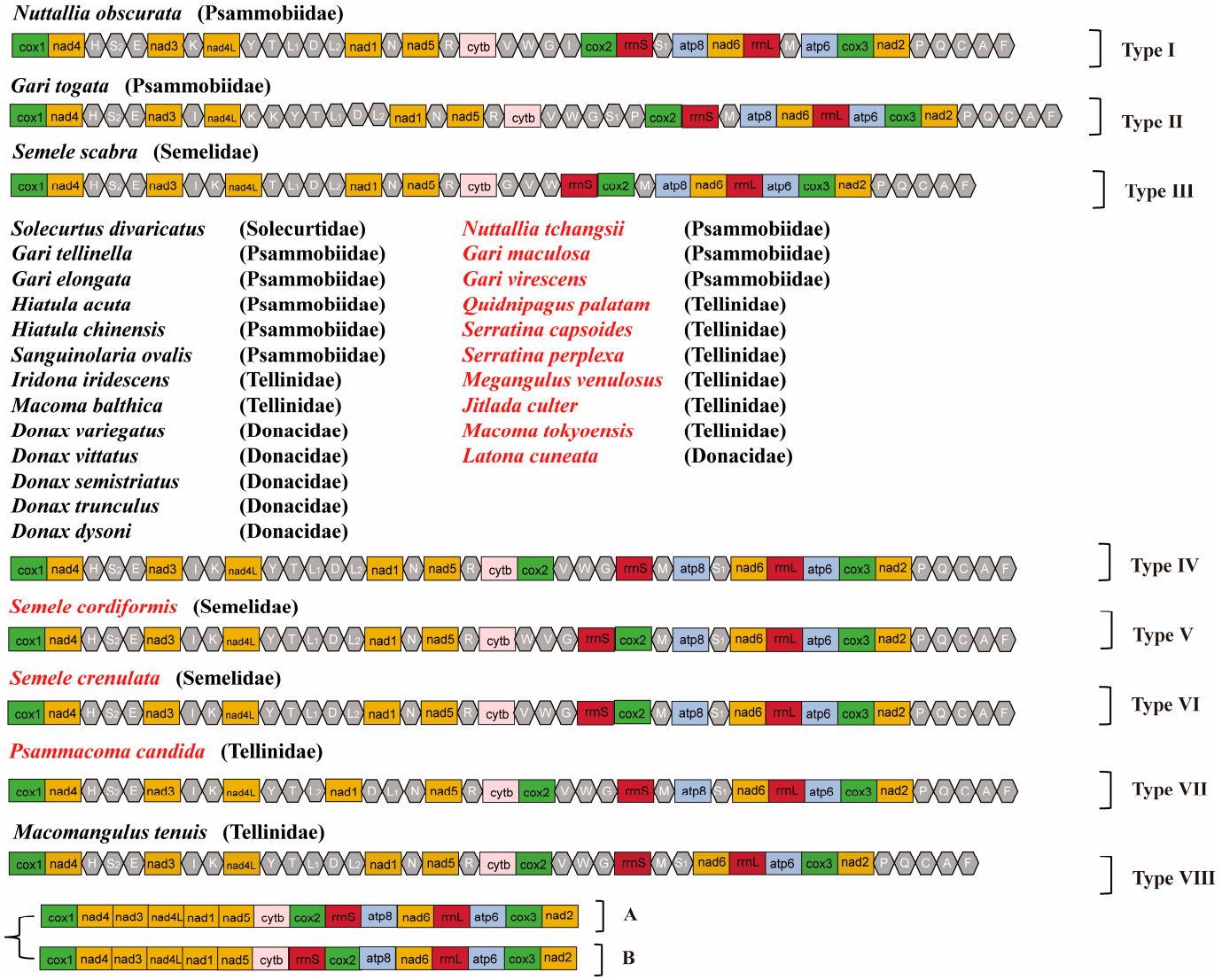

Figure 2. Gene rearrangement types in the mitochondrial genomes of Tellinoidea. The figure illustrates the different gene rearrangement types (Types V–VIII) observed in the newly sequenced mitochondrial genomes, compared to the typical gene order (TTGO) proposed by Sun et al. (2020) [10]. The arrangement of the 13 protein-coding genes is indicated by A and B. The newly sequenced mitochondrial genomes are depicted in red.

In general, tRNA rearrangements are common, while PCGs and rRNA genes are relatively conserved. This is likely due to the flexibility provided by their secondary structure, which facilitates more frequent translocations [47]. However, in Tellinoidea, rearrangements are not limited to tRNA genes but also involve PCGs and rRNA genes. The newly sequenced *Semele* (Semelidae) species, *Semele crenulata* and *Semele cordiformis*, exhibit distinct gene arrangements compared to TTGO. In *S. cordiformis*, exhibits a unique cluster (*trnW-trnV-trnG-rrnS-cox2*) classified as Type V, resulting from translocations of *trnV-trnW-trnG*, *rrnS* and *cox2*. In contrast, *S. crenulata* displays a distinct Type VI rearrangement

involving only *rrnS* and *cox2*. Notably, these patterns differ markedly from the Type III arrangement previously reported for *Semele scabra* [10]. The primary differences lie in the positions of the three tRNA genes, *trnV*, *trnW*, and *trnG*. Generally, gene arrangement differences within the same genus are rarely reported, but recent bivalve studies have observed significant differences between species within certain genera, such as *Atrina*, *Pinctada*, and *Barbatia* [45]. Further complexity was observed in *P. candida*, which exhibits inversions in multiple genes, including *trnL1*, *trnD*, *trnL2*, and *nad1* (Type VII). Intriguingly, *Macomangulus tenuis* retains TTGO for all annotated genes except *atp8* (Type VIII). After excluding tRNA genes, two distinct PCG/rRNA arrangements (A and B) were identified across Tellinoidea (Figure 2). Arrangement A, exclusive to Semelidae, features *rrnS* and *cox2* translocations relative to arrangement B. However, limited sampling within Semelidae (only three *Semele* species) precludes definitive conclusions about whether this arrangement is genus-specific or family-wide.

These findings highlight a higher degree of mitochondrial genome plasticity in Tellinoidea than previously recognized, particularly within Semelidae. Future studies incorporating additional genera—especially underrepresented groups, like Macominae—will be critical to resolving the evolutionary drivers and taxonomic implications of these rearrangements.

3.3. Mitochondrial Genome Structure and Organization

The main features of the 13 newly sequenced complete mitochondrial genomes of Tellinoidea are summarized in Table S5. Each genome contains 13 PCGs, 22 tRNAs, and 2 rRNAs, with all coding genes located on the forward strand—a prominent characteristic of marine bivalve mitochondrial genomes [10]. The complete mitochondrial genomes of these 13 bivalves varied in size from 16,116 bp (*Gari virescens*) to 16,933 bp (*P. candida*). Both the AT content and AT-skew are consistent with those observed in the bivalves of the Tellinoidea [9,10]. The nucleotide composition of the newly sequenced tellinoidean mitogenomes is highly AT-rich (>55%) and exhibits a similar pattern across species. *Jitlada culter* has the highest AT content (65.4%), while *Megangulus venulosus* has the lowest (58%) (Table 2). Nucleotide skews in these mitogenomes follow patterns similar to those observed in pteriomorphians and other heterodonts, characterized by a negative AT-skew and a positive GC-skew on the forward strand [10]. The analysis revealed that nucleotide compositions were all strongly skewed away from C in favor of G (the GC-skews range from 0.303 to 0.465) and from A in favor of T (the AT skews range from −0.283 to −0.128).

Table 2. List of AT content, AT skew, GC skew and rRNA length.

Species	(A+T) %		AT Skew		GC Skew		rRNA Length	
	Mitogenome	PCGs	Mitogenome	PCGs	Mitogenome	PCGs	rrnS	rrnL
Latona cuneata	62.2	61.4	−0.212	−0.274	0.323	0.328	858	1255
Semele crenulata	60.6	60.1	−0.261	−0.341	0.442	0.455	892	1373
Semele cordiformis	62	62	−0.252	−0.313	0.465	0.484	915	1301
Macoma tokyoensis	63.1	62.5	−0.217	−0.299	0.441	0.355	878	1330
Megangulus venulosus	58	57.3	−0.262	−0.33	0.357	0.362	892	1331
Jitlada culter	65.4	65.5	−0.223	−0.282	0.372	0.374	894	1362
Psammacoma candida	60.3	60	−0.23	−0.29	0.38	0.41	883	1349
Serratina perplexa	63.1	62.8	−0.261	−0.322	0.377	0.382	891	1466
Serratina capsoides	63	63.1	−0.283	−0.341	0.395	0.408	893	1440
Quidnipagus palatam	61.3	61.5	−0.272	−0.34	0.39	0.387	894	1458
Gari maculosa	63.3	62.5	−0.261	−0.338	0.341	0.376	880	1438
Nuttallia tchangsii	60.8	60.5	−0.128	−0.289	0.303	0.301	880	1367
Gari virescens	62.4	61.9	−0.24	−0.318	0.307	0.312	865	1323

The *atp8* gene is highly sensitive to natural selection, characterized by a high mutation rate and length variability [48]. Consequently, it is not highly conserved in bivalves, and many marine bivalve species, including those from the Arcidae family and certain mussel species, lack the *atp8* gene [15,49]. However, recent studies suggest that the absence of *atp8* in bivalves is often attributed to challenges in genome annotation [50]. In the newly sequenced and previously published mitochondrial genomes of Tellinoidea, *atp8* was detected in all species except *M. tenuis* (Tellinidae), where the gene was not annotated. Although the positions of *atp8* varied across the genomes, it is present in the mitochondrial genomes of all other species. Most PCGs initiate with standard start codons ATN (ATG, ATA, and ATT), while a few employ GTG or TTG as start codons (Table S6). Termination is generally achieved with the stop codons TAA and TAG, but truncated stop codons are also observed. These occur in *nad5* (in *Quidnipagus palatam*, *Macoma tokyoensis*, *Serratina capsoides*, *Serratina perplexa*, *J. culter*, *M. venulosus*, *S. cordiformis*, and *S. crenulata*), *nad1* (in *Q. palatam*, *S. capsoides*, and *S. perplexa*), and *nad4l* (in *G. virescens*). In most marine bivalves, many protein-coding genes lack clearly conserved start or stop positions. In the absence of other stop codons, truncated T stop codons are used, or several amino acid residues are avoided to prevent overlap with another protein-coding gene [51]. This is a common phenomenon in metazoan mitochondrial genomes, where functional stop codons can be generated post-transcriptionally through polycistronic transcript cleavage and polyadenylation processes [52].

The 13 mitochondrial genomes analyzed contain a complete set of 22 tRNA genes. While the mitochondrial genome of *S. scabra* (previously the only sequenced species in Semelidae) lacks *trnY* and *trnS1* genes, our newly sequenced genomes of *S. crenulata* and *S. cordiformis* contain both tRNAs. Specifically, *trnY* was located between *nad4l* and *trnT*, while *trnS1* was found between *atp8* and *nad6*, consistent with the typical positions reported for mitochondrial genomes in the Tellinoidea [10]. The rRNA genes (*rrnL* and *rrnS*) were identified using BLAST searches, and their lengths fall within the range observed in sequenced molluscan mitochondrial genomes, although their exact boundaries could not be determined [53]. The length of *rrnL* ranges from 1255 bp (*Latona cuneata*) to 1466 bp (*S. perplexa*), flanked by *nad6* and *atp6*, while the length of *rrnS* varies between 858 bp (*L. cuneata*) and 915 bp (*S. cordiformis*) (Table 2). In this study, the *rrnS* gene is located between *trnG* and *cox2* in *S. cordiformis* and *S. crenulata*, whereas in other tellinoidean mitochondrial genomes, it is positioned between *trnG* and *trnM*. A previous study by Sun et al. [10] also found that in the mitochondrial genome of *G. togata*, the *rrnS* gene is located between *cox2* and *trnM*, indicating that the gene's position is relatively unconserved.

4. Conclusions

In this study, we reconstructed the most comprehensive phylogenetic tree of Tellinoidea to date. The phylogenetic analysis strongly supports the monophyly of Tellinoidea. For the first time, the paraphyly of Tellinidae is revealed, and the two subfamilies involved in Tellinidae (Macominae and Tellininae) are not monophyletic. Furthermore, Semelidae is recovered as a monophyletic group, forming a sister group with certain Tellinidae species. In Psammobiidae, *Gari* and *Nuttallia* do not form a sister group either. Compared to the typical gene order in Tellinoidea, we discovered four new types (Types V–VIII) of rearrangement patterns. In addition to tRNA rearrangements, species in the family Semelidae also exhibit translocations in *rrnS* and *cox2*. Therefore, further studies utilizing additional mitogenomic data are required to enhance our understanding of the phylogeny within Tellinidae and its subfamilies, as well as to clarify the taxonomic status of relevant genera in Semelidae and Psammobiidae.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17050303/s1. Table S1 presents a comparative analysis of mitochondrial genes obtained from the *Scrobicularia plana* transcriptome data (SRR8217862) against mitochondrial genes published in GenBank. Table S2 list of species dowloaded from Genbank [34,54–60]. Table S3 provides substitution saturation values for the three codon positions of the 13 PCGs. Table S4 lists the optimal partitioning schemes and models selected using PartitionFinder 2.1.1 with the Bayesian Information Criterion (BIC). Table S5 lists the mitochondrial genome features for the 13 newly sequenced species. Table S6 summarizes the annotation details of the mitochondrial PCGs for the 13 newly sequenced species.

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Data Availability Statement: All 13 newly sequenced mitochondrial genomes have been submitted to NCBI, and the sequences are available for download via the GenBank accession numbers listed in Table 1. Mitochondrial genes from transcriptomic data of Tellinoidea species, obtained from the NCBI SRA database, are available on Figshare at the following link: https://figshare.com/s/74935a4bd0d161d2b368, accessed on 24 February 2025. The raw next-generation sequencing (NGS) data for Tellinoidea species have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject ID PRJNA1227165.

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References

1. MolluscaBase. Taxon Tree. Available online: https://www.molluscabase.org (accessed on 1 March 2024).
2. Moncada, E.; Lord, A.; Simone, L.R.L.; Adjei-Boateng, D.; Bouchet, P.; Strong, E.E.; Bieler, R.; Giribet, G. Marine surf to freshwater: A molecular phylogeny of Donacidae (Bivalvia: Heterodonta). *Invertebr. Syst.* **2022**, *36*, 984–1001. [CrossRef]
3. Vitonis, J.E.V.V.; Zaniratto, C.P.; Machado, F.M.; Passos, F.D. Comparative studies on the histology and ultrastructure of the siphons of two species of Tellinidae (Mollusca: Bivalvia) from Brazil. *Zoologia* **2012**, *29*, 219–226. [CrossRef]
4. Kawashima, H.; Ohnishi, M. Fatty acid compositions of various tissue lipids in the marine bivalves, *Megangulus venulosus* and *Megangulus zyonoensis*, from coastal waters of Hokkaido, Northern Japan. *J. Oleo Sci.* **2003**, *52*, 309–315. [CrossRef]
5. Fernández-Pérez, J.; Nantón, A.; Ruiz-Ruano, F.J.; Camacho, J.P.M.; Méndez, J. First complete female mitochondrial genome in four bivalve species genus *Donax* and their phylogenetic relationships within the Veneroida order. *PLoS ONE* **2017**, *12*, e0184464. [CrossRef]
6. Taylor, J.D.; Williams, S.T.; Glover, E.A.; Dyal, P. A molecular phylogeny of heterodont bivalves (Mollusca: Bivalvia: Heterodonta): New analyses of 18S and 28S rRNA genes. *Zool. Scr.* **2007**, *36*, 587–606. [CrossRef]
7. Combosch, D.J.; Collins, T.M.; Glover, E.A.; Graf, D.L.; Harper, E.M.; Healy, J.M.; Kawauchi, G.Y.; Lemer, S.; McIntyre, E.; Strong, E.E.; et al. A family-level tree of life for bivalves based on a Sanger-sequencing approach. *Mol. Phylogenet. Evol.* **2017**, *107*, 191–208. [CrossRef]
8. Ozawa, G.; Shimamura, S.; Takaki, Y.; Yokobori, S.; Ohara, Y.; Takishita, K.; Maruyama, T.; Fujikura, K.; Yoshida, T. Updated mitochondrial phylogeny of Pteriomorph and Heterodont Bivalvia, including deep-sea chemosymbiotic *Bathymodiolus* mussels, vesicomyid clams and the thyasirid clam *Conchocele cf. bisecta*. *Mar. Genom.* **2017**, *31*, 43–52. [CrossRef]

9. Yuan, Y.; Li, Q.; Yu, H.; Kong, L. The complete mitochondrial genomes of six heterodont bivalves (Tellinoidea and Solenoidea): Variable gene arrangements and phylogenetic implications. *PLoS ONE* **2012**, *7*, e32353. [CrossRef]
10. Sun, S.; Jiang, L.; Kong, L.; Li, Q. Comparative mitogenomic analysis of the superfamily Tellinoidea (Mollusca: Bivalvia): Insights into the evolution of the gene rearrangements. *Comp. Biochem. Physiol. Part D* **2020**, *36*, 100739. [CrossRef]
11. Simone, L.R.L.; Wilkinson, S. Comparative morphological study of some Tellinidae from Thailand (Bivalvia: Tellinoidea). *Zool. J. Linn. Soc.* **2008**, *154*, 167–191.
12. Marques, R.C.; Da Silva, A.M.; Simone, L.R.L. Cladistic analysis of the transisthmian genus *Eurytellina* (Bivalvia: Tellinoidea) based on morphological and morphometric data. Organ. *Divers. Evol.* **2022**, *22*, 857–891. [CrossRef]
13. Batistão, A.R.; Audino, J.A.; Passos, F.D. Comparative anatomy of siphons in tellinoidean clams (Bivalvia, Tellinoidea). *J. Morphol.* **2024**, *285*, e21762. [CrossRef]
14. Boore, J.L. Animal mitochondrial genomes. *Nucleic Acids Res.* **1999**, *27*, 1767–1780. [CrossRef] [PubMed]
15. Kong, L.; Li, Y.; Kocot, K.M.; Yang, Y.; Qi, L.; Li, Q.; Halanych, K.M. Mitogenomics reveals phylogenetic relationships of Arcoida (Mollusca, Bivalvia) and multiple independent expansions and contractions in mitochondrial genome size. *Mol. Phylogenet. Evol.* **2020**, *150*, 106857. [CrossRef]
16. Wang, Y.; Yang, Y.; Kong, L.; Sasaki, T.; Li, Q. Phylogenomic resolution of Imparidentia (Mollusca: Bivalvia) diversification through mitochondrial genomes. *Mar. Life Sci. Technol.* **2023**, *5*, 326–336. [CrossRef] [PubMed]
17. Lee, Y.; Kwak, H.; Shin, J.; Kim, S.C.; Kim, T.; Park, J.K. A mitochondrial genome phylogeny of Mytilidae (Bivalvia: Mytilida). *Mol. Phylogenet. Evol.* **2019**, *139*, 106533. [CrossRef]
18. Li, Q.; Park, C.; Kijima, A. Isolation and characterization of microsatellite loci in the Pacific abalone, Haliotis discus hannai. *J. Shellfish Res.* **2002**, *21*, 811–816.
19. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, 2114–2120. [CrossRef]
20. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Prjibelski, A.D.; et al. SPAdes: A new genome assembly algorithm and its applications to single—Cell sequencing. *J. Comput. Biol.* **2012**, *19*, 455–477. [CrossRef]
21. Dierckxsens, N.; Mardulyn, P.; Smits, G. NOVOPlasty: De novo assembly of organelle genomes from whole genome data. *Nucleic Acids Res.* **2016**, *45*, e18.
22. Bernt, M.; Donath, A.; Jühling, F.; Externbrink, F.; Florentz, C.; Fritzsch, G.; Pütz, J.; Middendorf, M.; Stadler, P.F. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mol. Phylogenet. Evol.* **2013**, *69*, 313–319. [CrossRef] [PubMed]
23. Rutherford, K.; Parkhill, J.; Crook, J.; Horsnell, T.; Rice, P.; Rajandream, M.-A.; Barrell, B. Artemis: Sequence visualization and annotation. *Bioinformatics* **2000**, *16*, 944–945. [CrossRef]
24. Boore, J.L.; Macey, J.R.; Medina, M. Sequencing and comparing whole mitochondrial genomes of animals. *Methods Enzymol.* **2005**, *395*, 311–348. [PubMed]
25. Laslett, D.; Canbäck, B. ARWEN: A program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. *Bioinformatics* **2008**, *24*, 172–175. [CrossRef]
26. Lemer, S.; Bieler, R.; Giribet, G. Resolving the relationships of clams and cockles: Dense transcriptome sampling drastically improves the bivalve tree of life. *Proc. R. Soc. B* **2019**, *286*, 20182684. [CrossRef]
27. Thrash, A.; Arick, M.; Peterson, D.G. Quack: A quality assurance tool for high throughput sequence data. *Anal. Biochem.* **2018**, *548*, 38–43. [CrossRef] [PubMed]
28. Forni, G.; Puccio, G.; Bourguignon, T.; Evans, T.; Mantovani, B.; Rota-Stabelli, O.; Luchetti, A. Complete mitochondrial genomes from transcriptomes: Assessing pros and cons of data mining for assembling new mitogenomes. *Sci. Rep.* **2019**, *9*, 14806. [CrossRef]
29. Camacho, C.; Coulouris, G.; Avagyan, V.; Ma, N.; Papadopoulos, J.; Bealer, K.; Madden, T.L. BLAST+: Architecture and applications. *BMC Bioinform.* **2009**, *10*, 421. [CrossRef]
30. Abalde, S.; Tenorio, M.J.; Uribe, J.E.; Zardoya, R. Conidae phylogenomics and evolution. *Zool. Scr.* **2019**, *48*, 194–214. [CrossRef]
31. Xu, T.; Qi, L.; Kong, L.; Li, Q. Mitogenomics reveals phylogenetic relationships of Patellogastropoda (Mollusca, Gastropoda) and dynamic gene rearrangements. *Zool. Scr.* **2022**, *51*, 147–160. [CrossRef]
32. Bernt, M.; Merkle, D.; Ramsch, K.; Fritzsch, G.; Perseke, M.; Bernhard, D.; Schlegel, M.; Stadler, P.F.; Middendorf, M. CREx: Inferring genomic rearrangements based on common intervals. *Bioinformatics* **2007**, *23*, 2957–2958. [CrossRef]
33. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef] [PubMed]

34. Bieler, R.; Mikkelsen, P.M.; Collins, T.M.; Glover, E.A.; González, V.L.; Graf, D.L.; Harper, E.M.; Healy, J.; Kawauchi, G.Y.; Sharma, P.P.; et al. Investigating the bivalve tree of life—An exemplar—Based approach combining molecular and novel morphological characters. *Invertebr. Syst.* **2014**, *28*, 32. [CrossRef]
35. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef]
36. Talavera, G.; Castresana, J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* **2007**, *56*, 564–577. [CrossRef]
37. Xia, X. DAMBE7: New and improved tools for data analysis in molecular biology and evolution. *Mol. Biol. Evol.* **2018**, *35*, 1550–1552. [CrossRef] [PubMed]
38. Kück, P.; Meusemann, K. FASconCAT: Convenient handling of data matrices. *Mol. Phylogenet. Evol.* **2010**, *56*, 1115–1118. [CrossRef]
39. Nguyen, L.-T.; Schmidt, H.A.; Von Haeseler, A.; Minh, B.Q. IQ—TREE: A fast and effective stochastic algorithm for estimating maximum—likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef]
40. Ronquist, F.; Teslenko, M.; Van Der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef]
41. Rambaut, A.; Drummond, A.J.; Xie, D.; Baele, G.; Suchard, M.A. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* **2018**, *67*, 901–904. [CrossRef]
42. Rambaut, A. FigTree v1.4.4. 2014. Available online: http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 12 October 2024).
43. Lanfear, R.; Frandsen, P.B.; Wright, A.M.; Senfeld, T.; Calcott, B. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* **2016**, *34*, 772–773. [CrossRef] [PubMed]
44. Burzyński, A.; Śmietanka, B.; Fernández-Pérez, J.; Lubośny, M. The Absence of Canonical Respiratory Complex I Subunits in Male-Type Mitogenomes of Three *Donax* Species. *Sci. Rep.* **2024**, *14*, 14465. [CrossRef]
45. Ren, J.; Shen, X.; Jiang, F.; Liu, B. The mitochondrial genomes of two scallops, Argopecten irradians and *Chlamys farreri* (Mollusca: Bivalvia): The most highly rearranged gene order in the family Pectinidae. *J. Mol. Evol.* **2010**, *70*, 57–68. [CrossRef]
46. Zhang, Y.; Heng, X.; Li, F.; Liu, C.; Li, Q.; Wang, A.; Yang, Y. Comparative mitogenomic analyses of the infraclass Pteriomorphia (Mollusca: Bivalvia) provides novel insights into gene rearrangement and phylogeny. *Comp. Biochem. Physiol. Part D* **2024**, *53*, 101361. [CrossRef] [PubMed]
47. Cantatore, P.; Gadaleta, M.N.; Roberti, M.; Saccone, C.; Wilson, A.C. Duplication and remoulding of tRNA genes during the evolutionary rearrangement of mitochondrial genomes. *Nature* **1987**, *329*, 853–855. [CrossRef]
48. Gissi, C.; Iannelli, F.; Pesole, G. Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. *Heredity* **2008**, *101*, 301–320. [CrossRef] [PubMed]
49. Śmietanka, B.; Burzyński, A.; Wenne, R. Comparative genomics of marine mussels (*Mytilus* spp.) gender associated mtDNA: Rapidly evolving *atp8*. *J. Mol. Evol.* **2010**, *71*, 385–400. [CrossRef]
50. Zhao, B.; Gao, S.; Zhao, M.; Lv, H.; Song, J.; Wang, H.; Zeng, Q.; Liu, J. Mitochondrial genomic analyses provide new insights into the "missing" *atp8* and adaptive evolution of Mytilidae. *BMC Genom.* **2022**, *23*, 738. [CrossRef]
51. Williams, S.T.; Foster, P.G.; Hughes, C.; Harper, E.M.; Taylor, J.D.; Littlewood, D.T.J.; Dyal, P.; Hopkins, K.P.; Briscoe, A.G. Curious bivalves: Systematic utility and unusual properties of anomalodesmatan mitochondrial genomes. *Mol. Phylogenet. Evol.* **2017**, *110*, 60–72. [CrossRef]
52. Ojala, D.; Montoya, J.; Attardi, G. tRNA punctuation model of RNA processing in human mitochondria. *Nature* **1981**, *290*, 470–474. [CrossRef]
53. Maynard, B.T.; Kerr, L.J.; McKiernan, J.M.; Jansen, E.S.; Hanna, P.J. Mitochondrial DNA sequence and gene organization in the Australian blacklip abalone *Haliotis rubra* (Leach). *Mar. Biotechnol.* **2005**, *7*, 645–658. [CrossRef]
54. Capt, C.; Bouvet, K.; Guerra, D.; Robicheau, B.M.; Stewart, D.T.; Pante, E.; Breton, S. Unorthodox features in two venerid bivalves with doubly uniparental inheritance of mitochondria. *Sci. Rep.* **2020**, *10*, 1087. [CrossRef]
55. Dreyer, H.; Steiner, G. The complete sequences and gene organisation of the mitochondrial genomes of the heterodont bivalves *Acanthocardia tuberculata* and *Hiatella arctica*—And the first record for a putative ATPase subunit 8 gene in marine bivalves. *Front. Zool.* **2006**, *3*, 13. [CrossRef]
56. Imanishi, Y.; Tanaka, M.; Fujiwara, M. Complete mitochondrial genome sequence of Japanese cockle *Fulvia mutica* (Cardiidae). *Fish. Sci.* **2013**, *79*, 949–957. [CrossRef]
57. Ma, H.; Lin, L.; Zhang, Y.; Chen, S.; Shi, W.; Yu, Z. The complete mitochondrial genome sequence of the giant clam *Tridacna derasa* (Tridacnidae: *Tridacna*). *Mitochondrial DNA Part B* **2018**, *3*, 911–912. [CrossRef] [PubMed]
58. Ma, H.; Zhang, Y.; Xiao, S.; Chen, S.; Zhang, Y.; Xiang, Z.; Li, J.; Yu, Z. The complete mitochondrial genome of giant clam, *Hippopus hippopus* (Cardiidae: Tridacninae). *Conserv. Genet. Resour.* **2019**, *11*, 263–266. [CrossRef]

59. Ma, Y.; Li, J.; Ye, Y. Complete sequence and gene organization of the mitochondrial genome of clam *Donax dysoni*. *Oceanol. Limnol. Sin.* **2023**, *54*, 246–258.
60. Saunier, A.; Garcia, P.; Becquet, V.; Marsaud, N.; Escudié, F.; Pante, E. Mitochondrial genomes of the Baltic clam *Macoma balthica* (Bivalvia: Tellinidae): Setting the stage for studying mito-nuclear incompatibilities. *BMC Ecol. Evol.* **2014**, *14*, 259. [CrossRef]