

Article

Not peer-reviewed version

---

# Characterisation of the Mitochondrial Genome of *Capulus danieli*: Insights into Evolutionary History and Ecological Adaptations

---

Yaoyu Xie , Yida Han , Junxia Mao , [Xubo Wang](#) , [Zhenlin Hao](#) , [Ying Tian](#) \*

Posted Date: 6 August 2024

doi: 10.20944/preprints202408.0303.v1

Keywords: *Capulus danieli*; mitochondrial genome; protein-coding genes; divergence time estimation



Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Article

# Characterisation of the Mitochondrial Genome of *Capulus danieli*: Insights into Evolutionary History and Ecological Adaptations

Yaoyu Xie <sup>1</sup>, Yida Han <sup>1</sup>, Junxia Mao <sup>1</sup>, Xubo Wang <sup>1</sup>, Zhenlin Hao <sup>1</sup> and Ying Tian <sup>1,2\*</sup>

<sup>1</sup> Key Laboratory of Mariculture & Stock Enhancement in North China Sea, Ministry of Agriculture and Rural Affairs, Dalian Ocean University, Dalian, Liaoning 116023, China

<sup>2</sup> Dalian Shell Museum, Dalian, Liaoning 116023, China

\* Correspondence: Correspondence: tianying@dlou.edu.cn (T,Y)

**Abstract:** *Capulus danieli*, a distinct member of Capulidae, exhibits a unique ecological behaviour by attaching and drilling onto the pecten, distinguishing itself from other gastropods. Herein, we present the first characterisation of the mitochondrial genome of *Capulus danieli*. The results showed that the genome spans 15600 base pairs, with an A + T content of 71.12% and a G + C content of 28.88%. Within this genetic makeup, we identified 13 protein-coding genes, 22 transfer RNA genes, and 2 ribosomal RNA genes. Phylogenetic analysis revealed a close evolutionary relationship between *Capulus danieli* and members of the Ficoidea superfamily. The divergence time estimation suggested that *Capulus danieli* diverged approximately 52.29 million years ago. These findings significantly contribute to our understanding of the evolutionary history and genetic architecture of *Capulus danieli*, shedding light on its unique ecological adaptations to the marine environment.

**Keywords:** *Capulus danieli*; mitochondrial genome; protein-coding genes; divergence time estimation

## 1. Introduction

The family Capulidae belongs to the Phylum Mollusca, Subclass Caenogastropoda, Class Gastropoda, Order Littorinimorpha, and is a widely distributed marine gastropod worldwide. It comprises eighteen acknowledged genera, the preponderance of which exhibit a coiled form, although there exists a notable number of species resembling limpets [1]. Among the uncoiled groups, *Capulus* Montfort, 1810 is the most widespread genus based on current taxonomy and includes at least 20 recent species [2]. The gastropod Capulidae reached its highest level of limpetisation [3]. Capulid shell plasticity is associated with a broad range of feeding ecology. Most coiled capulids exploit water currents generated by their hosts, which is obligate suspension feeding [4]. *Capulus danieli*, a member of the family Capulidae, is a relatively large species within its genus, with diameters ranging from 20 to 30 mm [5]. Characterised by its low, limpet-shaped form, this species possesses a unique shell structure with a protoconch composed of smooth, planispiral whorls, followed by a cap-shaped teleoconch featuring a large, horseshoe-shaped muscle scar [6].

Ecologically, *Capulus danieli* shows intriguing behaviours, notably its attachment and drilling onto the shells of pecten, reflecting a xenomorphic sculpture mirroring the form of its host [5]. Despite its ecological significance, research on *Capulus danieli* remains limited.

Because most capulid species are rare and rarely collected alive, only three studies have attempted to produce a molecular phylogeny tree of the family Capulidae. [7], produced the first capulid phylogeny and investigated the larval ecology of Antarctic species. More recently, [8], proposed a new phylogenetic hypothesis using a taxonomic framework (six genera) to address the Indo-West Pacific diversity of Hyalorisia Dall, 1889. [1], also performed ancestral state reconstruction analysis on a time-calibrated phylogenetic tree within the family Capulidae and suggested that

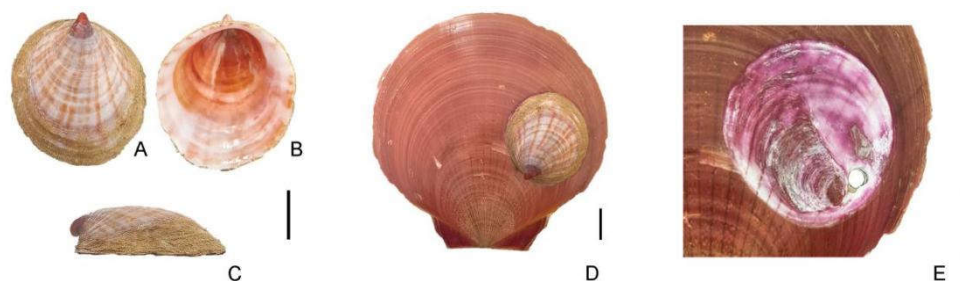
capulids evolved from a coiled suspension feeder lineage and that a shift to kleptoparasitism occurred in the family ancestor.

Here, we present the first characterisation of the mitochondrial genome of *Capulus danieli* to elucidate its gene function, phylogenetic relationships with limpet-like gastropods, and divergence time estimation. This study advances our understanding of *Capulus danieli*'s evolutionary history and ecological adaptations, contributing valuable insights into the broader field of molluscan biology.

## 2. Materials and Methods

### 2.1. Sample Collection

Four specimens of *Capulus danieli* were collected in December 2022 from Yangjiang, Guangdong Province (21°85'N, 111°95'E). Although individual capture was not feasible, the specimens were discovered on the surface of shells of the economically significant scallop *Amusium pleuronectes* (Figure 1). *Capulus* specimens are relatively rare, with approximately one found in every 30 scallops. Morphological identification involved stripping fresh tissue from the shells, excising the digestive glands, and preserving the muscle tissue in anhydrous ethanol.



**Figure 1.** *Capulus danieli* and its host *Amusium pleuronectes*. A, The back view of *Capulus danieli*. B, The ventral view of *Capulus danieli*. C, The lateral view of *Capulus danieli*. D, *Capulus danieli* attached on *Amusium pleuronectes*. E, The shell of *A. pleuronectes* with drillhole from *Capulus danieli*.

### 2.2. DNA Extraction, Library Preparation and Next Generation Sequence

The genomic DNA (gDNA) was extracted using the MagPure Bacterial DNA Kit from Magen (Guangzhou, China) and pre-grinding in liquid nitrogen. The concentration of the extracted DNA was tested using a Qubit dsDNA HS assay kit from Sangon (Shanghai, China), and its integrity was confirmed using 1% agarose gel electrophoresis. Subsequently, library preparation and next-generation sequencing were performed by Sangon Biotech (Shanghai) Co., Ltd. For the library preparation, 500 ng of the quantified DNA was randomly fragmented using Covaris (Woburn, USA). The Hieff NGS MaxUp II DNA Library Prep Kit for Illumina from YEASEN (Shanghai, China) was utilised for the following steps. The process included repairing the ends and adding a 3' end A tail, followed by the ligation of adaptors using an enhancer and Fast T4 DNA ligase. Index primers were added through PCR, and the resulting amplified product (approximately 400 bp) was selected using DNA selection beads. The concentration and size of the library were confirmed using the Qubit 4.0 (Thermo, Waltham, USA) and 2% agarose gel electrophoresis, respectively, and the libraries were pooled and loaded onto a Novaseq 6000 from Illumina (San Diego, USA) or DNBseq-T7 from BGI (Shenzhen, China) sequencer using a 2 × 150 bp paired-end sequence kit, following the manufacturer's instructions [9].

### 2.3. Sequence Assembly and Annotation of Mitochondrial Genome

Raw sequencing data of at least 6 GB were used for subsequent analyses. All raw reads were trimmed using Fastqc (version 0.11.2) [10]. SPAdes software (version 3.15) [11], was used to assemble the raw sequence reads into contigs. The complete mitochondrial genome was obtained by aligning the contigs against a reference mitochondrial genome, serving as the seed sequence for the software

MITObim (version 1.9.1) with 30 iterations. An in-house genome annotation pipeline, utilizing NCBI-BLAST, was applied to annotate the protein-coding genes, tRNA, and rRNA genes. Subsequently, circular gene maps of *Capulus danieli* were generated using Circos software.

#### 2.4. Sequence Analyses of Mitogenomes

The nucleotide composition of the entire mitogenome, protein-coding genes (PCGs), rRNA and tRNA genes, and A-T content were analysed using MEGA 7.0 [12]. Additionally, codon usage and relative synonymous codon usage (RSCU) of the PCGs were investigated. Base skew values were calculated using the formulas A-T skew =  $(A - T)/(A + T)$  and G-C skew =  $(G - C)/(G + C)$  [13,14]. Furthermore, DnaSP 6.0 [15,16], was selected to analyse the non-synonymous (Ka) and synonymous (Ks) substitution rates of mitogenomes in *Capulus danieli* to explore their evolutionary adaptation.

#### 2.5. Systematic Analysis

We selected 25 species to construct a phylogenetic tree based on their complete mitochondrial genomes. The sequences of 23 species belonging to Haliotidae, Neritidae, Patellidae, Nacellidae, Calyptraeidae, Muricidae, Ficidae, Naticidae, Strombidae, Struthiolariids, and Xenophoridae in the Gastropoda class were obtained from the NCBI database (Table 1). Some species were selected based on their similarity in shell morphology to *C. danieli* [17]. Others were selected based on their close phylogenetic relationship with *C. danieli* as determined by the alignment of their mitochondrial genomes available at NCBI.

Additionally, two bivalve species, *Chlamys farreri* and *Mizuhopecten yessoensis*, were included as outgroups, with GenBank accession numbers EF473269.1 and FJ595959.1, respectively. Prior to tree construction, PhyloSuite v1.2.3 [18,19], was utilised to extract the protein-coding genes (PCGs) from each sequence, followed by multiple sequence alignment using MAFFT [20,21]. The alignment results of the protein-coding gene sequences were optimised using MACSE [22], and Gblocks [23], were employed for sequence pruning. The extracted PCGs were concatenated into a comprehensive dataset, and ModelFinder [24,25], was used to partition the data and select the optimal evolutionary model. A phylogenetic tree was constructed using the MrBayes method [26].

**Table 1.** List of species analyzed in this study and their GenBank accession numbers.

Classification(family)	Species name	Size(bp)	GenBank accession
Haliotidae	<i>Haliotis iris</i>	17131	KU310895.1
	<i>Haliotis rubra</i>	16907	AY588938.1
Neritidae	<i>Clithon sowerbianum</i>	15919	MT230542.1
	<i>Theodoxus fluviatilis</i>	15667	MT628587.1
Patellidae	<i>Patella ferruginea</i>	14400	MH916654.1
	<i>Patella vulgata</i>	14808	MH916653.1
Nacellidae	<i>Cellana radiata</i>	16194	MH916651.1
	<i>Cellana toreuma</i>	16268	ON018805.1
	<i>Nacella clypeater</i>	16742	KT990124.1
	<i>Nacella magellanica</i>	16663	KT990125.1
Calyptraeidae	<i>Desmaulus extintorium</i>	16608	OQ511529.1
Muricidae	<i>Concholepas concholepas</i>	15495	JQ446041.1
	<i>Indothais sacellum</i>	15237	NC 063938.1
Capulidae	<i>Capulus danieli</i>	15640	OR687630.1
	<i>Capulus ungaricus</i>	525	MT525803.1
	<i>Hyalorisia tosaensis</i>	405	MT525849.1
	<i>Hyalorisia galea</i>	526	MT525835.1
	<i>Cryocapulus subcompressus</i>	760	KR364850.1
	<i>Torellia exilis</i>	529	MT525847.1
	<i>Torellia smithi</i>	714	KR364868.1
	<i>Torellia insignis</i>	769	KR364865.1

	<i>Torellia mirabilis</i>	745	KR364856.1
	<i>Trichamathina violaceus</i>	529	MT525806.1
	<i>Trichamathina bicarinata</i>	529	MT525846.1
Ficidae	<i>Ficus subintermedia</i>	16255	OR522697.1
	<i>Ficus variegata</i>	15736	NC 056153.1
Naticidae	<i>Neverita didyma</i>	15629	NC 046594.1
	<i>Notocochlis qualtieriana</i>	15176	NC 046705.1
Strombidae	<i>Canarium labiatum</i>	15843	NC 084213.1
	<i>Laevistrombus canarium</i>	15626	NC 053786.1
Struthiolariidae	<i>Struthiolaria papulosa</i>	15475	NC 059921.1
Xenophoridae	<i>Onustus exutus</i>	16043	MK327366.1
	<i>Xenophora japonica</i>	15684	MW244823.1

### 2.6. Estimation of Differentiation Time of Capulidae

We used the 16S genes of 11 Capulidae species (Table 1) with the topological structure of the Bayesian tree as the framework to estimate the divergence time within the Capulidae family. We used BEAST v2.7.6 software [27], and a relaxed clock model for this analysis. The Yule process was used to determine the tree's prior branch evolution rate.

We used the oldest confidently attributable species to the genus *Capulus*, i.e., *Capulus onyxoides* Cossmann, 1879, from the Ypresian period to incorporate fossil evidence into the analysis (Lower Eocene, 56–47.8 Ma) as a standard point. Similarly, we referenced *Capulus (Hyalorisia) nettlesii* Robinson, 1983† from the Upper Eocene period (41.2–33.9 Ma) for the genus *Hyalorisia* [1]. Markov chain Monte Carlo (MCMC) analysis was performed with 100 million generations sampled every 1000 generations. The TreeAnnotator v1.8.4 component of the BEAST software package was used to discard the first 25% of aging samples as burn-in. Convergence of the chain was confirmed using Tracer v1.7, [28], ensuring effective sample size (ESS) values greater than 200. The resulting divergence time estimates were validated against Timetree fossil records, and the results were reported to verify the accuracy. We used TVBOT as a graphic beautification tool (<https://www.chiplot.online/tvbot.html>) [29].

## 3. Results

### 3.1. Distribution Characteristics of *Capulus danieli* Mitochondria

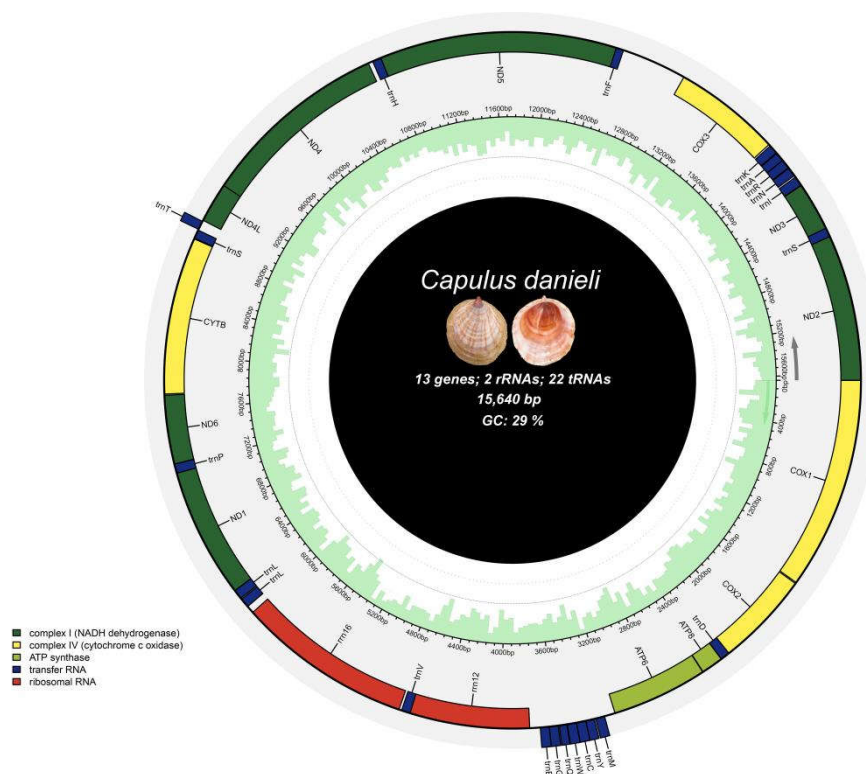
The complete mitochondrial genome of *Capulus danieli* spanned 15,640 bp (GenBank accession number: NC 084349.1) and comprised a typical circular, closed, double-stranded molecule with a control region and 37 coding genes (Figure 2). Among these, there were 13 protein-coding genes (COX1, COX2, ATP8, ATP6, ND1, ND6, CYTB, ND4L, ND4, ND5, COX3, ND3, and ND2), including 3 cytochrome oxidase subunit genes, 7 NADH dehydrogenase subunit genes, 2 ATP synthase subunit genes, and 1 cytochrome b gene. Additionally, there were 22 tRNA genes (including, trnM, trnY, and trnC) and 2 rRNA genes (s-rRNA and l-rRNA).

The arrangement and location of genes in the mitochondrial genome of *C. danieli* were similar to those observed in most known neogastropods. The gene structure was densely packed with intervals and overlaps. The longest gene in the mitochondrial DNA was *nad5* (1734 bp), whereas the shortest was *trnQ* (55 bp). Notably, the length of tRNA genes was generally less than 100 bp, with the anticodon positioned within the tRNA gene.

The mitochondrial genome of *C. danieli* contained 23 intergenic spacers with a combined length of 829 bp. The longest intergenic spacer was situated between *trnF* and *COX3*, spanning 473 bp, followed by the spacer between *ATP6* and *trnM*, which was 77 bp in length. Three gene overlaps totalling 27 bp were observed, with the longest overlap occurring between *ND5* and *trnF* (18 bp), followed by *nad4l* and *nad4* (7 bp). Additionally, there were 11 regions with neither an overlap nor an interval.

All 13 protein-coding genes in the mitochondrial DNA of *C. danieli* contained initiation and termination codons. The initiation codon was ATG, whereas TAG was the termination codon for

ND4L, ND4, and ND3. The remaining genes contained the TAA termination codon, except for ND2 and ND5, which featured an incomplete codon T (Table 2).



**Figure 2.** Gene map of the complete mitogenomes for *Capulus danieli*.

**Table 2.** Gene organization and annotation of *Capulus danieli* mitochondrial genome.

Gene	Location	Length	amino acid	start codon	terminal codon	Intergenic nucleotide*(bp )	strand
COX1	1-1542	1542	514	ATG	TAA	8	+
COX2	1551-2243	693	231	ATG	TAA	-1	+
trnD	2242-2309	68				1	+
ATP8	2311-2469	159	53	ATG	TAA	2	+
ATP6	2472-3167	696	232	ATG	TAA	77	+
trnM	3245-3310	66				9	-
trnY	3320-3382	63				0	-
trnC	3383-3450	68				0	-
trnW	3451-3517	67				3	-
trnQ	3521-3575	55				7	-
trnG	3583-3647	65				0	-
trnE	3648-3712	65				74	-
s-rRNA	3787-4653	867				0	+
trnV	4654-4717	64				18	+
l-rRNA	4736-6005	1270				54	+
trnL	6060-6128	69				8	+
trnL	6137-6205	69				0	+
ND1	6206-7147	942	314	ATG	TAA	0	+
trnP	7148-7214	67				1	+
ND6	7216-7713	498	166	ATG	TAA	8	+
CYTB	7722-8861	1140	380	ATG	TAA	5	+

trnS	8867-8930	64				5	+
trnT	8936-8999	64				8	-
ND4L	9008-9304	297	99	ATG	TAG	-7	+
ND4	9298-10668	1371	457	ATG	TAG	34	+
trnH	10701-10764	64				0	+
ND5	10765-12498	1734	578	ATG	T	-18	+
trnF	12482-12537	56				473	+
COX3	13010-13789	780	260	ATG	TAA	12	+
trnK	13802-13868	67				4	+
trnA	13873-13938	66				0	+
trnR	13939-14005	67				4	+
trnN	14010-14076	67				12	+
trnI	14089-14159	71				2	+
ND3	14162-14515	354	118	ATG	TAG	-2	+
trnS	14514-14580	67				0	+
ND2	14581-15640	1060	353	ATG	T	0	+

Intergenic nucleotide\*(bp): positive values indicated the interval sequence of adjacent genes, and negative values indicated the overlapping of adjacent genes.

### 3.2. Base Content and Base Shift Degree

There were 4938, 6186, 2333, and 2183 A, T, G, and C bases of mtDNA in *Capulus danieli*, accounting for 31.57%, 39.55%, 14.92%, and 13.96% of the total bases, respectively. The A + T content was 71.12%, the G + C content was 28.88%, the AT-skew was -0.112, and the GC-skew was 0.033. The protein-coding gene length of *Capulus danieli* was 11247 bp, the tRNA length was 1439 bp, and the rRNA length was 2137 bp (Table 3). mtDNA showed AT base bias, and A + T was 70.03%, 71.72%, and 73.04% in the PCGs, tRNA, and rRNA genes, respectively.

In the mitochondrial DNA (mtDNA) of *Capulus danieli*, the number of A, T, G, and C bases was 4938, 6186, 2333, and 2183, respectively, constituting 31.57%, 39.55%, 14.92%, and 13.96% of the total bases. The A + T content was higher (71.12%) than the G + C content is lower (28.88%). The AT skew was -0.112, indicating a bias towards A over T, whereas the GC skew was 0.033. The length of the protein-coding gene (PCG) in *Capulus danieli* was 11,247 bp, with tRNA and rRNA lengths of 1439 and 2137 bp, respectively. mtDNA exhibited an AT base bias, with A + T content of 70.03%, 71.72%, and 73.04% in PCGs, tRNA, and rRNA genes, respectively. rRNA genes display the most significant bias towards A + T content (73.04%), exceeding G + C content (26.96%), with AT-skew at 0.083 and GC-skew at 0.139. Within protein-coding genes, the proportion of A bases was lower than that of T bases, with the largest difference observed in the AT base content percentages. In contrast, the difference in CG base content percentages is minimal, with AT-skew at -0.180 and GC-skew at 0.017 (Table 3). The control region segments typically exhibit a higher A + T content in mitochondrial gene sequences, making them prone to base mutations, thereby qualifying them as highly variable regions.

**Table 3.** Nucleotide composition of the mitochondrial genome of *Capulus danieli*.

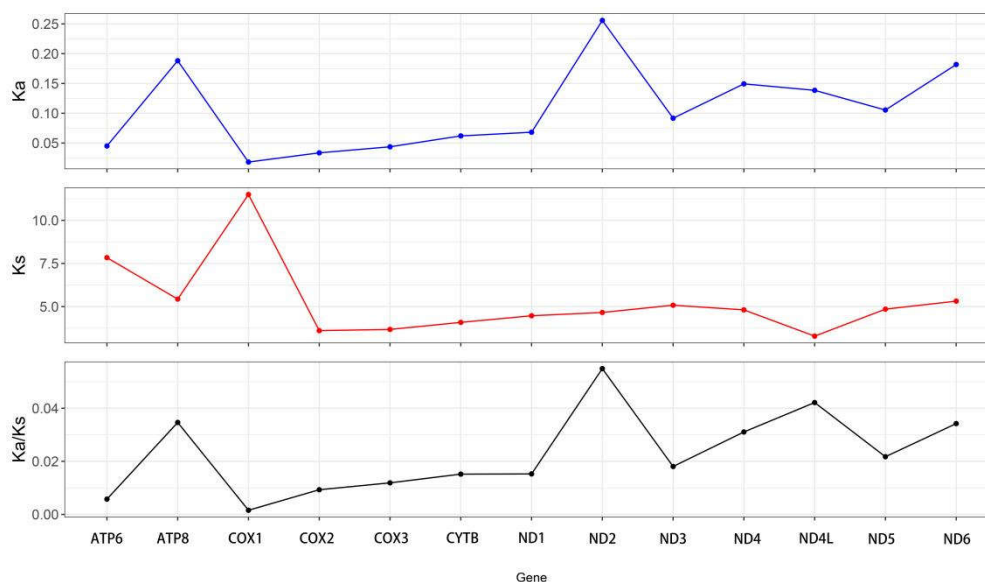
Item	length/bp Size	A%	T%	G%	C%	(A+T)%	(C+G)%	AT-skew	GC-skew
mtDNA	15640	31.57	39.55	14.92	13.96	71.12	28.88	-0.112	0.033
PCGsgenes	11247	28.71	41.32	15.23	14.73	70.03	29.96	-0.180	0.017
tRNA	1439	36.28	35.44	15.77	12.51	71.72	28.28	0.012	0.115
rRNA	2137	39.54	33.50	15.35	11.61	73.04	26.96	0.083	0.139

### 3.3. Mitochondrial Gene Codon Usage

In total, 3755 codons were present in the protein-coding genes (PCGs) of *C. danieli*. Among these, the UUA (L) codon exhibited the highest frequency of usage, occurring 351 times, followed by the relatively high-frequency UUU (F), AUU (I), and AUA (M) codons. The UAG codon was the least



lowest (0.002), whereas that of ND2 was the highest (0.256). Conversely, the Ks value of cox1 was the highest (11.49), whereas that of ND4L was the lowest (3.288). Although there were variations in the Ka and Ks values among the 13 PCGs, all Ka/Ks values were less than 1, with ND2 exhibiting the highest Ka/Ks value (0.055) and cox1 showing the lowest Ka/Ks value (Ka/Ks = 0.002). This indicated that atp8 had the highest evolutionary rate under purifying selection pressure, followed by ND4L (Ka/Ks = 0.042). Conversely, cox1 had the slowest evolutionary rate, suggesting that it was the most conserved during evolution.



**Figure 4.** Selection pressure analysis of *Capulus danieli* ( Ka is non-synonymous substitution value, Ks is synonymous substitution value ).

### 3.5. Mitochondrial Dna Microsatellites

Microsatellites, also known as simple sequence repeats (SSRs), arise from DNA slippage and misalignment during DNA replication or repair or from unequal exchange between sister chromatids during mitosis or meiosis. These short, repetitive sequences are valuable for species-specific identification.

In this study, three SSRs were identified in the mitochondrial genome of *Capulus danieli* (Table 5). All these SSRs consist of single nucleotide repeats and belong to the A/T motif type.

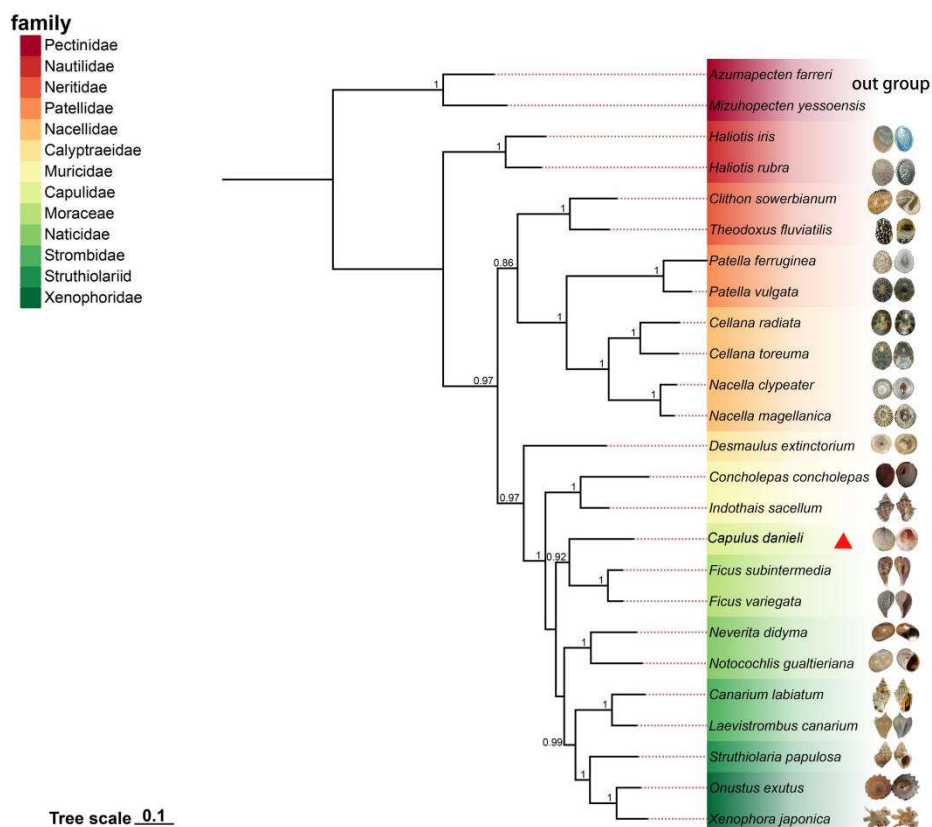
**Table 5.** SSR in the mitochondrial sequence of *Capulus danieli*.

Numbering	Gene	Initial position/bp	Termination position/bp	length/bp	SSR
1	COX	188	197	10	(T)10
2	ND1	6955	6964	10	(T)10
3	COX3	12653	12667	15	(A)15

### 3.6. Phylogenetic Relationship

The Bayesian inference (BI) method was used to build a tree of 13 PCGs sequences from 25 species, of which two species, *Chlamys farreri* and *Mizuhopecten yessoensis*, were used as outgroups (Figure 5). The phylogenetic tree contained 13 families, each of which formed a monophyletic branch. Previous studies have demonstrated that this is reasonable [30–32]. The posterior probability value of most branches was one, and the overall topology was stable. From the overall perspective of the phylogenetic tree, the following evolutionary relationships were observed: (Neritimorpha + Patellogastropoda) + Vetigastropoda + Caenogastropoda). From the phylogenetic relationship of gastropoda, it could be seen that Haliotidae in Vetigastropoda was located in the basal position of

the phylogenetic tree and was the earliest branch of the Limpet-like shell. The gastropods that appeared after this were mainly divided into two major branches: (Neritimorpha + Patellogastropoda) and Caenogastropoda. *Capulus danieli* has the closest genetic relationship with *Ficus*, which also belongs to the Littorinimorph. *Capulus danieli* had a distant phylogenetic relationship with the limpet-like shell species Neritimorpha, Patellogastropoda, and Caenogastropoda, and was not within the same major branch. A Limpet-like shell appeared in multiple branches of the phylogenetic tree, indicating that it has evolved independently several times in Gastropoda. Statistically, limpet-like shells occur in at least 54 families of gastropods [17].



**Figure 5.** Phylogenetic tree inferred using Bayesian inference methods based on concatenated sequences of 13 PCGs from 23 gastropod mitogenomes, The sequences of two bivalves were chosen as the outgroup.

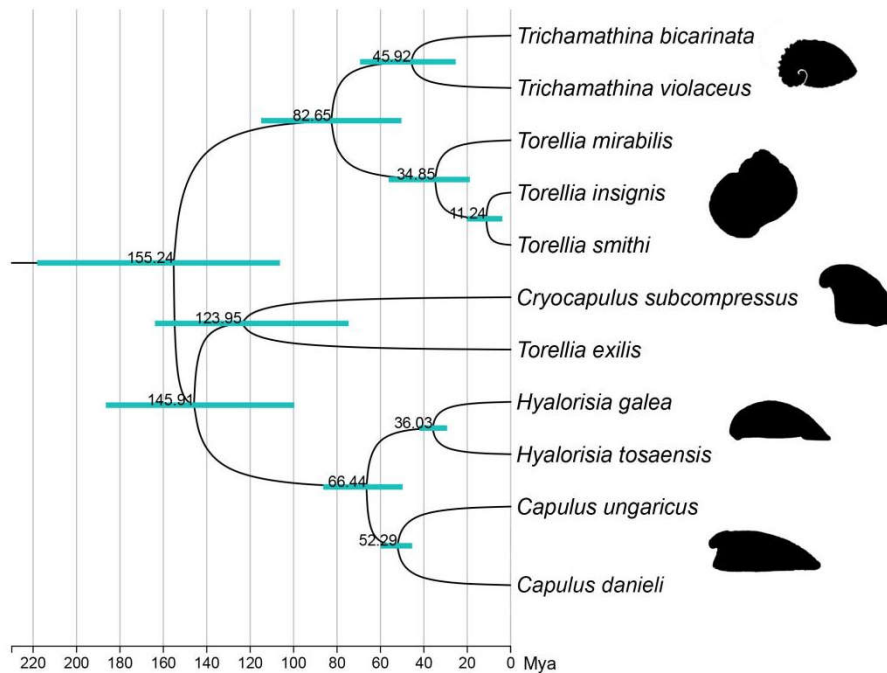
### 3.7. Divergence Time Estimation

We reconstructed a divergence time tree of Capulidae based on the analysis of the 16S gene sequence to explore its evolutionary process (Figure 6). According to the results, the origin of the Capulidae can be traced back to the end of the Callovian stage (Middle Jurassic) period about 155.25 million years ago. This finding provided us with an important time node for the origin of this family.

It began to evolve to the first limpet-like shell, which is reflected in the branch of *Cryocapulus subcompressus*, at 123.95 Mya (Early Cretaceous). Subsequently, the second evolution to the limpet-like shell, which dates back to about 82.65 million years ago (Late Cretaceous), resulted in a divergence between the cap-shaped genus *Trichamathina* and the spiral genus *Torellia*. About 66.44 million years ago (Late Cretaceous), there was a divergence between *Hyalorisia* and *Capulus*. The genus *Trichamathina* has a limpet-like shell type. It is mainly manifested in symmetrical and enlarged body snails but still retains some of the curling tower characteristics, which reveals its unique evolutionary path. About 66.44 million years ago (Late Cretaceous), there was a divergence between *Hyalorisia* and *Capulus*. Although there are differences in the taxonomic status of the two species, they

show similar and highly capped shell-like characteristics, which further highlights the diversity and complexity of the evolution of the family. Finally, during the Eocene (Paleogene), about 52.29 to 36.03 million years ago, the differentiation of Capulidae reached its peak, and Capulidae gradually evolved a shell type similar to that of limpets. This period marked an important stage in the evolution of the family and provided valuable information about its evolution.

Tree scale 20



**Figure 6.** Estimating the divergence time of Capulidae based on 11 16s sequences. bars indicate 95% highest posterior density intervals for node ages (Ma), and number at node the median (Ma).

#### 4. Discussion

The present study, leveraging mitochondrial protein-coding genes (PCGs), sheds light on the phylogenetic relationships within the Gastropoda class, particularly focusing on the Capulidae family. Our analysis, constrained by the absence of other Capulidae mitochondrial genomes in the NCBI public database, used the mitochondrial genome of *Capulus danieli* to establish a phylogenetic framework. The findings have significant implications for our understanding of the evolutionary history and morphological adaptations within this diverse group of marine gastropods. The system phylogenetic tree constructed in this study places *Capulus danieli* close to *Ficus* within the Order Littorinimorpha.

The recurrent emergence of the limpet-like shell within Capulidae, as observed in the *Cryocapulus*, *Capulus* + *Hyalorisia*, and *Trichamathina* lineages, underscores the complex evolutionary pressures that have shaped the diversity of shell forms in this family. Our results suggested that *Capulus*, which includes at least 20 extant limpet-like species [2], may be polyphyletic and have evolved through multiple independent lineages. This polyphyly indicated convergence in shell morphology, which is likely a response to similar ecological challenges or opportunities.

The molecular clock analysis estimated the origin of the Capulidae to be in the Middle Jurassic period, specifically during the Callovian stage, approximately 155.25 million years ago. [1], estimated that Capulidae originated 112.87 million years ago. This timeframe precedes the oldest known Capulidae fossil record from the Late Cretaceous-Campanian stage [33]. The discrepancy between molecular and fossil evidence underscores the need for further research to reconcile these divergent timelines and better understand the early evolutionary history of Capulidae.

The divergence time for *Capulus danieli*, dated to around 52.29 million years ago during the Palaeocene-Eocene transition, coinciding with a period of global warming and elevated sea surface temperatures [34]. The paleoenvironmental conditions of this era, characterised by transient eutrophication and ocean acidification [35–37], likely exerted selective pressures that favoured the adoption of a parasitic lifestyle in *Capulus danieli*. This ecological shift may have facilitated the transition to a limpet-like shell form, providing the species with enhanced access to nutrients via kleptoparasitising bivalves.

The current study was limited by the scarcity of mitochondrial genomic data for other Capulidae species, which restricted a comprehensive analysis of intrafamilial differences. Future studies should aim to fill this gap by sequencing additional mitochondrial genomes in this family. Additionally, ecological observations and molecular data are essential to validate the hypothesised adaptive transitions and provide a more nuanced understanding of the evolutionary trajectory of Capulidae.

## 5. Conclusions

In this study, we reported the characterisation of the mitochondrial genome of *Capulus danieli*, which is the first complete mitochondrial genome recorded in the family Capulidae. We analyzed the characteristics of the mitochondrial sequence of *Capulus danieli*, mainly including: AT skew, codon usage, selection pressure, microsatellites and elucidated the evolutionary relationship between *Capulus danieli* and limpet-like gastropod through phylogenetic analysis. Through the estimation of divergence time, the evolution period of Capulidae is estimated, and the process of its transformation into limpet-like shell is described. These findings significantly contribute to our understanding of the evolutionary history and genetic architecture of *Capulus danieli*, shedding light on its unique ecological adaptations to the marine environment.

**Author Contributions:** Yao-Yu Xie: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Software (equal); Validation (equal); Visualization (equal); Writing - original draft (equal). Yi-Da Han: Resources (equal). Jun-Xia Mao: Resources (equal). Xu-Bo Wang: Resources (equal). Zhen-Lin Hao: Resources (equal). Ying Tian: Project administration (equal); Supervision (equal); Writing - review & editing (equal).

**Funding:** This research was supported by the National Key Research and Development Program of China (2021YFB2600200); the Central Government Subsidy Project for Liaoning Fisheries (2023).

**Institutional review board statement:** The experimental protocol was designed in accordance with the recommendations of the Regulations of the Laboratory Animal—Guideline for Ethical Review of Animal Welfare (National Standards of P. R. China, GB/T 35823—2018) and reviewed and approved by the animal care and use committee of Dalian Ocean University (DLOU-2023007).

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** The authors are also grateful to the anonymous reviewers for the great elaboration of the manuscript through their critical reviewing and comments.

**Data availability statement:** The sequenced mitochondrial genome has been uploaded to NCBI (accession number of OR687630.1).

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Fassio, G.; Bouchet, P.; Lozouet, P.; Modica, M.V.; Russini, V.; Schiaparelli, S.; Oliverio, M. Becoming a limpet: An 'intermittent limpetization' process driven by host features in the kleptoparasitic gastropod family Capulidae. *Mol. Phylogenet. Evol.* **2020a**, *155*, 107014. <https://doi.org/10.1016/j.ympev.2020.107014>
2. Worms. Available online: <https://www.marinespecies.org/aphia.php?p=taxdetails&id=137729> (accessed on 24 July 2014).
3. Simone, L.R.L. Main processes of body modification in gastropods: the limpetization. *Malacopedia* **2018**, *1*, 23–35.
4. Iyengar, E.V. Suspension feeding and kleptoparasitism within the genus *Trichotropis* (Gastropoda: Capulidae). *J. Mollus. Stud.* **2007**, *74*, 55–62. <https://doi.org/10.1093/mollus/eym043>

5. Orr, V. The drilling habit of *Capulus danieli* (Crosse) (Mollusca:Gastropoda). *Veliger* **1962**, *5*, 63–67.
6. Ponder, W.F.; Lindberg, D.R. Towards a phylogeny of gastropod molluscs: an analysis using morphological characters. *Zool. J. Linn. Soc.* **1997**, *119*, 83–265. <https://doi.org/10.1111/j.1096-3642.1997.tb00137.x>
7. Fassio, G.; Modica, M.V.; Alvaro, M.C.; Schiaparelli, S.; Oliverio, M.J.H. Developmental trade-offs in Southern Ocean mollusc kleptoparasitic species. *Hydrobiologia* **2015**, *761*, 121–141.
8. Fassio, G.; Russini, V.; Buge, B.; Schiaparelli, S.; Modica, M.V.; Bouchet, P.; Oliverio, M. High cryptic diversity in the kleptoparasitic genus *Hyalorisia* Dall, 1889 (Littorinimorpha: Capulidae) with the description of nine new species from the Indo-West Pacific. *J. Mollus. Stud.* **2020b**, *86*, 401–421. <https://doi.org/10.1093/mollus/eyaa028>
9. Dierckxsens, N.; Mardulyn, P.; Smits, G. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic Acids Res.* **2017**, *45*, e18. <https://doi.org/10.1093/nar/gkw955>
10. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, *34*, i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>
11. 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. <https://doi.org/10.1089/cmb.2012.0021>
12. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. <https://doi.org/10.1093/molbev/msw054>
13. Hassanin, A.; Léger, N.; Deutsch, J. Evidence for multiple reversals of asymmetric mutational constraints during the evolution of the mitochondrial genome of Metazoa, and consequences for phylogenetic inferences. *Syst. Biol.* **2005**, *54*, 277–298. <https://doi.org/10.1080/10635150590947843>
14. Perna, N.T.; Kocher, T.D. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *J. Mol. Evol.* **1995**, *41*, 353–358. <https://doi.org/10.1007/BF00186547>
15. Rozas, J.; Rozas, R. DnaSP, DNA sequence polymorphism: An interactive program for estimating population genetics parameters from DNA sequence data. *Comput. Appl. Biosci.* **1995**, *11*, 621–625. <https://doi.org/10.1093/bioinformatics/11.6.621>
16. Librado, P.; Rozas, J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **2009**, *25*, 1451–1452. <https://doi.org/10.1093/bioinformatics/btp187>
17. Vermeij, G.J. The limpet form in gastropods: evolution, distribution, and implications for the comparative study of history. *Biol. J. Linn. Soc.* **2017**, *120*, 22–37. <https://doi.org/10.1111/bij.12883>
18. Zhang, D.; Gao, F.; Jakovlic, I.; Zou, H.; Zhang, J.; Li, W.X.; Wang, G.T. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Mol. Ecol. Resour.* **2020**, *20*, 348–355. <https://doi.org/10.1111/1755-0998.13096>
19. Xiang, C.; Gao, F.; Jakovlić, I.; Lei, H.; Hu, Y.; Zhang, H.; Zou, H.; Wang, G.; Zhang, D. Using PhyloSuite for Molecular Phylogeny and Tree-based Analyses. *iMeta.* **2023**, *2*, e87. <https://doi.org/10.1002/imt2.87>
20. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Phylogenetics Evol.* **2013**, *30*, 772–780. <https://doi.org/10.1093/molbev/mst010>
21. Abascal, F.; Zardoya, R.; Telford, M.J. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic Acids Res.* **2010**, *38*, W7–13. <https://doi.org/10.1093/nar/gkq291>
22. Ranwez, V.; Douzery, E.J.P.; Cambon, C.; Chantret, N.; Delsuc, F. MACSE v2: toolkit for the alignment of coding sequences accounting for frameshifts and stop codons. *Mol. Biol. Evol.* **2018**, *35*, 2582–2584. <https://doi.org/10.1093/molbev/msy159>
23. Talavera, G.; Castresana, J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* **2007**, *56*, 564–577. <https://doi.org/10.1080/10635150701472164>
24. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.F.; von Haeseler, A.; Jermini, L.S. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods.* **2017**, *14*, 587–589. <https://doi.org/10.1038/nmeth.4285>
25. Shapiro, B.; Rambaut, A.; Drummond, A.J. Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. *Mol. Biol. Evol.* **2006**, *23*, 7–9. <https://doi.org/10.1093/molbev/msj021>
26. 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. <https://doi.org/10.1093/sysbio/sys029>
27. Drummond, A.J.; Suchard, M.A.; Xie, D.; Rambaut, A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **2012**, *29*, 1969–1973. <https://doi.org/10.1093/molbev/mss075>
28. 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. <https://doi.org/10.1093/sysbio/syy032>
29. Peng, Y.Y.; Yan, H.H.; Guo, L.C.; Deng, C.; Wang, C.L.; Wang, Y.B.; Kang, L.P.; Zhou, P.P.; Yu, K.Q.; Dong, X.L.; et al. Referencegenome assemblies reveal the origin and evolution of allohexaploid oat. *Nat. Genet.* **2022**, *54*, 1248–1258. <https://doi.org/10.1038/s41588-022-01127-7>

30. Zhong, S.; Huang, L.; Huang, G.; Liu, Y.; Wang, W. The first complete mitochondrial genome of MAMMILLA from *Mammilla mammata* (Littorinimorpha: Naticidae). *Mitochondrial DNA Part B* **2020**, *5*, 96–7. <https://doi.org/10.1080/23802359.2019.1698350>
31. Qi, Y.; Zhong, Z.; Liu, X.; He, X.; Zhou, Y.; Zhang, L.; Chen, C.; Linse, K.; Qiu, J.W.; Sun, J. Phylogenomic analyses reveal a single deep-water colonisation in Patellogastropoda. *Mol. Biol. Evol.* **2024**, *190*, 107968. <https://doi.org/10.1016/j.ympev.2023.107968>
32. Ma, Y.; Zheng, B.; Li, J.; Meng, W.; Xu, K.; Ye, Y. Characterization of the complete mitochondrial genome of *Desmaulus extincorium* (Littorinimorpha, Calyptraeidea, Calyptraeidae) and molecular phylogeny of Littorinimorpha. *PLoS One* **2024**, *19*, e0301389. <https://doi.org/10.1371/journal.pone.0301389>
33. Saul, L.R.; Squires, R.L. Cretaceous trichotropid gastropods from the Pacific slope of North America: possible pathways to calyptraeid morphology. *Nautilus* **2008**, *122*, 115–142.
34. Tripathi, A.; Zachos, J.C.; Bice, K.; Marinovich, L. Late Paleocene arcticcoastal climate inferred from molluscan stable and radiogenic isotope ratios. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **2001**, *17*, 101–113. [https://doi.org/10.1016/S0031-0182\(01\)00230-9](https://doi.org/10.1016/S0031-0182(01)00230-9)
35. Scheibner, C.; Speijer, R.P.; Marzouk, A.M. Turnover of largerforaminifera during the Paleocene-Eocene thermal aximumand paleoclimatic control on the evolution of platform ecosystems. *Geology* **2005**, *33*, 493–496. <https://doi.org/10.1130/G21237.1>
36. Scheibner, C.; Speijer, R.P. Late Paleocene-Early Eocene Tethyan carbonate platform evolution: A response to long and shortterm paleoclimatic change. *Earth-Sci. Rev.* **2008**, *90*, 71–102. <https://doi.org/10.1016/j.earscirev.2008.07.002>
37. Alegret, L.; Ortiz, S. Global extinction event in benthic foraminifera across the Paleocene/Eocene boundary at the dababiyastratotype section. *Micropaleontology* **2007**, *52*, 433–447. <https://doi.org/10.2113/gsmicropal.52.5.433>

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.