Feng *et al. BMC Genomics* (2024) 25:1055
https://doi.org/10.1186/s12864-024-10904-z

BMC Genomics

RESEARCH Open Access

Exploring the mitogenomic of Lottiidae (Patellogastropoda): phylogenetics, gene rearrangement and evolutionary divergence time estimations

Jiantong Feng[1,2,3†], Jing Miao[1†], Jiji Li[1] and Yingying Ye[1*]

Abstract

Background Lottiidae (Gray, 1840) is a derived family of Patellogastropoda and an important component of intertidal benthic communities. The mitochondrial genome (mitogenome) has been frequently used to analyze the phylogenetic relationships of Patellogastropoda. We used next-generation sequencing (NGS) to sequence the complete mitogenomes of five species. We analyzed the basic base composition characteristics of these mitogenomes, constructed a phylogenetic tree, compared the characteristics of gene rearrangement with other related species, and discussed the evolutionary patterns between gastropod species and the evolutionary relationships of each subclass. In addition, we aim to infer the differentiation time and evolutionary characteristics of various species in the order Patellogastropoda. These results will enrich the mitogenome database of Patellogastropoda and enhance our understanding of the genetic characteristics of Lottiidae and the phylogenetic relationships between gastropods.

Results We obtained the mitogenomes sequences of *Lottia peitaihoensis*, *Patelloida saccharinoides*, *Patelloida ryukyuensis*, *Nipponacmea sp.* and *Nipponacmea nigrans*, using next-generation sequencing technology. We analyzed the basic structural characteristics of their mitogenomes and found that their lengths ranged from 16.6 kbp to 19.1 kbp. While *N. nigrans* and *P. saccharinoides* contain 39 genes, *L. peitaihoensis*, *P. ryukyuensis*, and *Nipponacmea sp.* have only 38 genes, with one *trnW* less. The most abundant base among the five species is T, and most protein coding genes (PCGs) use ATT, ATG, and ATA as starting codons, and TAA and TAG as stopping codons. We selected the mitogenomes of 10 Lottiidae species for selection pressure analysis and found that all PCGs were subject to purifying selection. Phylogenetic analysis indicates that Patellogastropoda is a fundamental branch of the Gastropoda, and Lottiidae, within Patellogastropoda, is an independent branch at the outermost of the entire phylogenetic tree. Comparison of mitochondrial gene sequences of all Patellogastropoda species revealed a high degree of gene rearrangement within the family Lottiidae, eight sequences present among the 10 species examined. By estimating

---

[†] Jiantong Feng and Jing Miao contributed equally to this work.

*Correspondence:
Yingying Ye
Yeyy@zjou.edu.cn

Full list of author information is available at the end of the article

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

their divergence times, we found that the divergence of limpets occurred as early as the Permian period of the Paleozoic Era, and a large number of species diverged in the Cenozoic Era.

Conclusion The data obtained from this study will provide information on the assembly of the mitochondrial genome of the Lottiidae species, which will contribute to a better understanding of the evolutionary status and relationship among thes family of Patellogastropoda.

Keywords Lottiidae, Patellogastropoda, Mitogenome, Phylogenetic analysis, Gene rearrangement, Divergence time

Introduction

Lottiidae, which belongs to the Mollusca, Gastropoda, Patellogastropoda, and Lottioidea, represents one of the most primitive groups within the gastropod lineage, and is one of the taxa colloquially referred to as limpets [1]. Their shells range from small to medium size, usually oval-disc-shaped with radiating ribs and growth lines. The curved edges of these limpet shells allow them to move around and feed, and to adhere tightly to rock surfaces [2]. They differ from Patellidae in the position of the apex, which is not always the highest point of the shell. They are widely distributed across the global coastal intertidal zone and are essential components of the intertidal benthic community [3]. Lottiidae species can also extend beyond coastal areas to include deep-sea environments, such as *Bathyacmaea* [4–6]. Their movement is powered by undulating muscles, while their diet consists mainly of algae obtained through surface scraping and radula contraction [7]. Due to their low trophic level in the food chain and high nutritional value, limpets play a crucial ecological role in regulating energy flow and maintaining the dynamic balance of intertidal communities. Therefore, research on limpets has aroused great interest. Diverse topics of study include the histological study of gonads [8], expression patterns of *hox* genes in early development [9], population divergence and contemporary gene flow [10], 3D-printed microneedle manufacturing using their teeth [11], tooth bionics [7], and population biogeography [12].

Patellogastropoda are of particular evolutionary significance as they are the most primitive subclass of living gastropods. The classification of Patellogastropoda has undergone significant transformations over time. Initially, based on morphological identification, Patellogastropoda was categorized into three families: Acmaeidae, Patellidae, and Lepetidae [13]. By the late 1990s, the addition of Nacellidae, Lottiidae, and Neolepetidae expanded the Patellogastropoda classification [14, 15]. The 21st century saw further expansion, with the discovery of at least seven families, including Eoacmaeidae and Pectinodontidae. According to the World Register of Marine Species (WoRMS), Patellogastropoda currently has seven families from the superfamily Lottioidea: Family Acmaeidae, Eoacmaeidae, Lepetidae, Lottiidae, Neolepetopsidae, Pectinodontidae and Rhodopetalidae. The other four have undergone some taxonomic modifications, some have been converted into existing families, and some have become subtaxa of the family Lottiidae (Table S1). Besides, two families Nacellidae and Patellidae from the superfamily Patelloidea, for a total of nine extant taxa.

Traditional classification methods rely on morphological comparisons, particularly regarding shell and radular teeth characteristics. Nakayama [16] compared a group of *Lottia* species and observed differences in both the length and sharpness of their radular teeth. Zhang [5] discovered a new species, *Bathyacmaea lactea*, by observing the radular teeth of deep-sea limpets. They found that the outermost tooth tip of its lateral teeth bends outward, while the lateral tooth tip of the closely related species *B. tertia* is straight. Nakano and Ozawa [17] compared the radular teeth of four closely related species of *Patelloida* and observed obvious differences from the first lateral teeth to the third lateral teeth. Despite morphological distinctions, extensive intra-species variation often complicates species classification, leading to challenges in understanding species complexes [16]. Consequently, molecular identification techniques, combined with DNA barcoding, have emerged as effective tools for species delineation. Lin [3] extensively surveyed limpet samples in the intertidal zone of southeast coastal areas of China and identified 11 species of endemic intertidal species by sequencing the *COI* gene. Some species of *Patelloida* are often regarded as having different ecological forms. Furthermore, Nakano and Ozawa [17] employed phylogenetic tree construction based on 16 S rRNA and *COI* genes to define species differences in *Patelloida*, successfully identifying four distinct species. Additionally, molecular techniques have also facilitated the exploration of cryptic species, such as in the *Lottia kogamogai* species complex found in Japan [16], and the intertidal limpets of *Notoacmea* in New Zealand [18].

Mitochondrial DNA (mtDNA) of most organisms is a double-stranded circular structure, which is more stable and less prone to breakage than the linear structure of nuclear DNA. Their genomes are relatively small, usually including 37 genes, 13 protein-coding genes, 22 tRNAs and two rRNAs. The characteristics of maternal inheritance also facilitate evolutionary studies. The high mutation rate makes mitochondrial DNA an important marker in evolutionary biology research [19]. These characteristics provide strong support for the reconstruction of phylogenetic and evolutionary relationships [20–23]. The goal of this study is to sequence the complete mitogenomes of five species using Next-Generation-Sequencing (NGS), analyze their basic base composition characteristics, such as nucleic acid composition and codon usage, construct phylogenetic trees, compare the characteristics of gene rearrangement among species, and discuss the evolutionary patterns between species and the evolutionary relationships of each subclass in Gastropoda. Additionally, we aim to infer the divergence time and evolution characteristics of each species in Patellogastropoda. These results will enrich the mitogenome database of Patellogastropoda and improve our understanding of the genetic characteristics of Lottiidae and the phylogenetic relationships among Gastropods.

Materials and methods

Sample collection and DNA extraction

We collected five samples of Lottiidae from the intertidal zone in the coastal area of southeast China for phylogenetic analysis (Table 1). The preliminary morphological classification of the samples was conducted using the "Atlas of the Famous Shell in the World for Identification and Appreciation" [24], "China Marine Shellfish Atlas" [25], and related limpet literature [3]. We stripped the fresh tissue from the shell, removed the viscera and digestive glands, stored muscle tissue in absolute ethanol at -20 °C, and extracted total DNA using the salting-out method [26].

DNA sequencing and assembly

We sequenced the mitogenomes on the Illumina Novaseq ^{TM} platform of Shanghai Origingene Bio-pharm Technology Co., Ltd, China. The specific sequencing process involved detecting and constructing a DNA library with an initial amount of 1 μg DNA. We then broke the DNA into 300–500 bp fragments using ultrasound, constructed a sequencing library, amplified the sequences by bridge PCR, and finally sequenced them using Illumina NovaSeq6000. After post-sequencing data filtering, we filtered the original data, including trimming adapter sequences, removing low-quality reads, high-N-rate sequences, and short-length sequences, to obtain high-quality sequencing data. We used GetOrganelle (https://github.com/Kinggerm/GetOrganelle) [27] to obtain the initial assembly results, corrected bases using Pilon v1.23, and determined the starting position and direction of the mitochondrial genome according to the reference genomes of related species. Finally, we obtained the final mitogenome sequence, and obtained the best assembly result through multiple calibration iterations.

Table 1 Information of five species of the family Lottiidae

Species	Sampling date	Location	Coordinate
Lottia peitaihoensis	October 2019	Qingdao, Shandong	N36°05′37″, E120°37′09″
Patelloida saccharinoides	December 2020	Yangjiang, Guandong	E111°98′62″, N21°64′84″
Patelloida ryukyuensis	August 2019	Fuding, Fujian	N27°28′35″, E120°21′18″
Nipponacmea nigrans	December 2020	Shantou, Guandong	E116°78′71″, N23°32′83″
Nipponacmea sp.	November 2020	Gouqi Island, Zhejiang	E122°82′82″, N30°73′09″

Genome annotation and bioinformatics analysis

We used the MITOS web server (http://mitos2.bioinf.uni-leipzig.de/index.py) [28] for genome annotation, selecting the invertebrate codon table from the codon table. We referred to published species to determine the start and stop codons of the five newly sequenced species [20], and uploaded the correct sequences to the National Center for Biotechnology Information (NCBI) to obtain the Genbank accession numbers. We generated circular mitogenome visualizations of the five species using the online website Prokess (https://proksee.ca/). We calculated the content of ATCG bases in the mitogenome using DAMBE 7 [29]. We used MEGA X [30] to calculate the frequency of amino acid usage and the relative synonymous codon usage (RSCU) in protein coding genes (PCGs), and DnaSP6.0 [31] to calculate the Ka/Ks (non-synonymous to synonymous substitution) ratio based on 13 PCGs of all Lottiidae species. We calculated AT-skew and GC-skew according to the formulas AT-skew = (A-T)/(A + T) and GC-skew = (G-C)/(G + C) [32].

Phylogenetic analysis

We used the mitogenome sequences of 121 species, including the newly sequenced five Lottiidae and sequences of 116 species in Gastropoda downloaded from the NCBI database, to construct a phylogenetic tree based on Gastropoda. These species included Vetigastropoda, Neritimorpha, Caenogastropoda, Neomorpholeones, Heterobranchia, and Patellogastropoda (Table 2). Additionally, we used two bivalve species, *Gari togata* and *Donax dysoni* (Genbank accession numbers: MN164429 and MZ3622601), as the outgroup. We used the software DAMBE 7 [29] to identify the PCGs of each sequence and concatenated the PCGs of all species into a large data set. We aligned and trimmed the sequences using MEGA X [30], and evaluated the suitability of these sequences for constructing phylogenetic trees by calculating nucleotide substitution saturation using the software DAMBE 7 [29].

In this study, phylogenetic analysis was performed using both Bayesian inference (BI) in MrBayes 3.2.7a [33] and maximum likelihood (ML) in IQ-tree 2.1.3 [34]. The analysis was partitioned by codon position, with the third

Table 2 List of gastropod species used in phylogenetic analysis with their GenBank accession numbers

Subclass	Family	Species	Size (bp)	Accession no.
Patellogastropoda	Lottiidae	✻*Patelloida saccharinoides*	19,048	OP537520
		✻*Patelloida ryukyuensis*	16,666	MZ329339
		Patelloida conulus	17,030	MZ048283
		✻*Lottia peitaihoensis*	19,096	MZ362261
		Lottia luchuana	17,199	MZ329341
		Lottia digitalis	26,835	DQ238599
		Scurria scurra	18,482	MZ196208
		✻*Nipponacmea* sp.	17,564	OL944392
		Nipponacmea fuscoviridis	18,720	MK395167
		✻*Nipponacmea nigrans*	19,636	OL944393
	Acmaeidae	*Niveotectura pallida*	18,670	OQ133458
		Acmaea mitra	17,903	OQ133456
	Neolepetopsidae	*Eulepetopsis* sp.	19,080	MZ888771
		Paralepetopsis sp.	19,844	ON526992
	Lepetidae	*Sagamilepeta sagamiensis*	18,608	OM976644
	Pectinodontidae	*Bathyacmaea nipponica*	16,792	MF095859
		Bathyacmaea lactea	18,446	MW309841
	Nacellidae	*Nacella concinna*	16,761	KT990126
		Nacella magellanica	16,663	KT990125
		Nacella clypeater	16,742	KT990124
		Cellana grata	16,181	MW722939
		Cellana nigrolineata	16,153	LC600801
		Cellana radiata	16,194	MH916651
		Cellana toreuma	16,260	MZ329338
	Patellidae	*Patella ferruginea*	14,400	MH916654
		Patella vulgata	14,808	MH916653
		Patella pellucida	14,949	OU795045
Heterobranchia	Aegiridae	*Notodoris gardineri*	14,424	DQ991934
	Polyceridae	*Roboastra europaea*	14,472	NC_004321
		Nembrotha kubaryana	14,395	NC_034920
	Aplysiidae	*Aplysia vaccaria*	14,130	DQ991928
		Aplysia dactylomela	14,128	DQ991927
		Aplysia californica	14,117	NC_005827
	Siphonariidae	*Siphonaria pectinata*	14,065	AY345049
		Siphonaria gigas	14,518	JN627205
	Volvatellidae	*Ascobulla fragilis*	14,745	AY345022
	Plakobranchidae	*Thuridilla gracilis*	14,259	DQ991939
		Elysia chlorotica	14,132	EU599581
		Elysia timida	14,088	NC_035490
		Elysia cornigera	14,118	NC_035489
	Onchidiidae	*Onchidella celtica*	14,150	AY345048
		Onchidella borealis	14,510	DQ991936
	Ellobiidae	*Ovatella vulcani*	14,274	JN615139
		Auriculastra duplicata	13,920	NC_036959
		Auriculinella bidentata	14,135	NC_016168
		Myosotella myosotis	14,246	NC_012434
	Pyramidellidae	*Pyramidella dolabrata*	13,856	NC_012435
Caenogastropoda	Turritellidae	*Turritella bacillum*	15,868	NC_029717
	Pomatiopsidae	*Oncomelania quadrasi*	15,184	LC276227
	Naticidae	*Mammilla mammata*	15,319	NC_046597
		Mammilla kurodai	15,309	NC_046596
		Euspira pila	15,244	NC_046703
		Euspira gilva	15,315	NC_046593

Table 2 (continued)

Subclass	Family	Species	Size (bp)	Accession no.
	Conidae	*Conus capitaneus*	15,829	NC_030354
		Conus borgesi	15,536	EU827198
		Conus betulinus	16,240	NC_039922
	Muricidae	*Menathais tuberosa*	15,294	NC_031405
		Concholepas concholepas	15,495	NC_017886
		Rapana venosa	15,272	NC_011193
		Indothais lacera	15,272	NC_037221
		Chicoreus torrefactus	15,359	NC_039164
		Chicoreus asianus	15,361	MN793976
		Boreotrophon candelabrum	15,265	NC_046505
		Ocinebrellus inornatus	15,324	NC_046577
		Ocinebrellus falcatus	15,326	NC_046052
		Ceratostoma rorifluum	15,338	NC_046526
		Ceratostoma burnetti	15,334	NC_046569
Neritimorpha	Neritidae	*Neritona juttingae*	15,950	PP958837
		Neritina iris	15,618	MZ189742
		Neripteron violaceum	15,710	KY021066
		Neritina violacea	15,710	KY021066
		Clithon oualaniense	15,706	MT568501
		Clithon sowerbianum	15,919	MT230542
		Clithon squarrosum	15,905	MZ297477
		Clithon lentiginosum	15,885	MZ152905
		Clithon corona	15,975	MZ189741
		Nerita albicilla	15,314	MK516738
		Nerita yoldii	15,719	MK395169
		Nerita japonica	15,875	MN747116
		Nerita tessellata	15,741	KF728889
		Nerita fulgurans	15,343	KF728888
		Nerita costata	15,604	NC_068078
		Nerita undata	15,583	MN477254
		Nerita insculpta	15,721	NC_068079
		Nerita balteata	15,571	MN477253
		Nerita histrio	15,538	NC_068083
		Nerita chamaeleon	15,716	MT161611
Neomphaliones	Neomphalidae	*Cyathermia naticoides*	16,424	BK064858
		Symmetromphalus regularis	16,249	BK064859
		Lamellomphalus manusensis	15,990	OK552681
	Melanodrymiidae	*Melanodrymia galeronae*	18,013	OR852748
		Melanodrymia aurantiaca	17,629	BK064863
		Melanodrymia brightae	17,506	BK064862
	Peltospiridae	*Gigantopelta aegis*	16,097	MW442948
		Chrysomallon squamiferum	15,388	AP013032
		Depressigyra globulus	16,222	BK064865
		Pachydermia laevis	16,033	BK064860
		Dracogyra subfuscus	15,744	NC_067061
		Nodopelta heminoda	15,583	NC_067937
		Peltospira operculate	15,269	BK064864
		Peltospira delicata	15,405	BK064861
Vetigastropoda	Phasianellidae	*Phasianella solida*	16,698	NC_028709
		Phasianella australis	18,397	KX298888
	Angariidae	*Angaria neglecta*	19,470	NC_028707
		Angaria delphinus	19,554	NC_031860
	Haliotidae	*Haliotis iris*	17,131	NC_031361

Table 2 (continued)

Subclass	Family	Species	Size (bp)	Accession no.
		Haliotis rubra	16,907	AY588938
		Haliotis ovina	16,531	NC_056350
	Trochidae	*Umbonium thomasi*	15,998	MH729882
		Monodonta labio	16,440	NC_035682
		Stomatella planulata	17,151	MK240320
		Gibbula umbilicalis	16,277	NC_035682
	Tegulidae	*Tectus pyramis*	18,439	NC_036068
		Tegula lividomaculata	17,375	NC_029367
		Tegula brunnea	17,690	NC_016954
		Omphalius rusticus	18,067	NC_056356
		Omphalius nigerrimus	17,755	KX298895
	Turbinidae	*Astralium haematragum*	16,310	KX298891
		Bolma rugosa	17,432	NC_029366
		Lunella granulate	17,190	KX298890
		Lunella correensis	17,308	MN604179

The newly sequenced species were marked with✻

codon position excluded to reduce noise and saturation. The ML analysis included 1000 ultrafast likelihood bootstrap repeats, and the best fitting model of each partition (first and second codon positions) was determined as GTR+I+R4 based on the Bayesian Information Criterion (BIC). For the BI method, the format conversion was performed using PAUP 4.0b [35], and MrMTgui was used to select the best fit model GTR+I+G under the AIC by combining the results of PAUP 4.0, Modeltest 3.7 [36] and MrModeltest 2.3 [37]. Markov chain Monte Carlo (MCMC) sampling was used with three hot chains and one cold chain run simultaneously for 2 million generations, with samples taken every 1000 generations. The first 25% of trees were discarded as burn-in, and the convergence of independent operations was evaluated by the mean standard deviation of the split frequency (<0.01). Finally, the evolutionary tree was edited and visualized using Figtree v1.4.3 [38].

Divergence time estimation

We estimated the divergence time for 27 species in Patellogastropoda by using 13 PCGs. We used BEAST v1.8.4 [39] software to analyze the topological structure of the ML tree. We chose an uncorrelated relaxed clock as the molecular clock model and calculated prior branch evolution rate of the tree using the Yule process. The distribution of fossil standard points was determined using a normal distribution, with a standard deviation set to 2.0, as the prior. To calibrate the phylogenetic tree, we meticulously selected three calibration points anchored in fossil records obtained from TimeTree (http://www.timetree.org/). The separation of genus *Nipponacmea* from genus *Lottia* is traced back to the Cretaceous-Paleogene boundary, around 49.5 million years ago (MYA), with an interval of 39.0 to 60.1 MYA. Additionally, the divergence between genus *Cellana* and *Nacella* within the Nacellidae, a commonly used calibration point for estimating limpet divergence, is estimated at 25.71 MYA, with a broader interval of 13.99 to 93.00 MYA. The divergence of the Patellidae from other families in the same branch is estimated to have occurred during the Permian-Triassic boundary, approximately 257.67 MYA, with a confidence interval ranging from 185.20 to 325.34 MYA [40]. We performed Markov chain (MCMC) analysis for 100 million generations, sampling every 1000 generations. We used the TreeAnnotator v1.8.4 component of BEAST v1.8.4 [39] software package to remove 10% of the sampled trees as "burn-in". We confirmed the convergence of the chain using Tracer v.1.6 [41] and determined that its effective sample sizes (ESSs) were greater than 200. Finally, we verified the accuracy of the divergence time using Timetree fossil records and reported results. We edited the results with FigTree v1.4.3 [38], displaying 95% of the highest posterior density (95% HPD) and the scale axis representing the time point.

Results

Structural characteristics of five mitogenomes

The mitogenome sequences of the five species have been uploaded to NCBI and GenBank accession numbers have been obtained (Table 2). The basic structure of the circular mitochondrial genome is shown in Fig. 1. The genomes vary in length from 16.6 kbp to 20 kbp, with the difference mainly attributable to variations in non-coding regions. Notably, *N. nigrans* exhibits three large intergenic regions, which are a 983 bp interval between *trnY* and *trnL1*, an 800 bp interval between *trnC* and *trnW1*, and a 775 bp interval between *trnK* and *trnS1*, leading to a significantly longer mitogenome than the other species. Three species, *L. peitaihoensis*, *P. ryukyuensis*, and

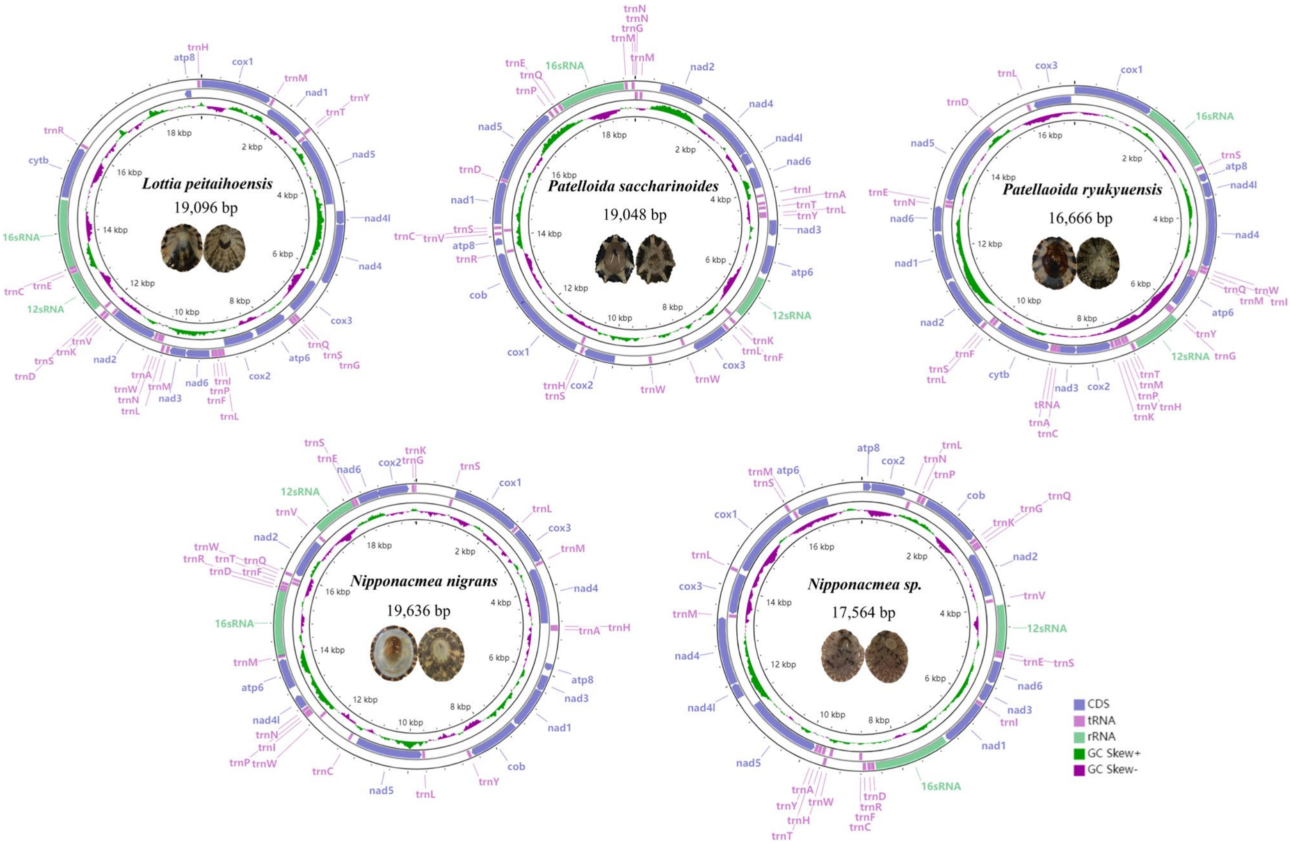

Fig. 1 Complete circular mitogenome map of five species, the ring indicates gene arrangement and distribution

Nipponacmea sp. encode a total of 38 genes, including 13 PCGs, two rRNA, and 23 tRNA, while *N. nigrans* and *P. saccharinoides* encode an additional *trnW*, bringing the total number of genes to 39. Among the five species, *ND5* is the longest PCG, encoding approximately 567 amino acids over approximately 1700 bp, whereas *ATP8* is the shortest PCG, encoding approximately 53 amino acids over approximately 160 bp. Furthermore, tRNA length ranges from 64 to 74 bp, while the length of *12 S rRNA* exceeds 900 bp, and that of *16 S rRNA* ranges between 1400 and 1500 bp.

Base content and skewness

After analyzing the nucleotide composition of the mitogenome in five species, we found an uneven proportion of the four bases (Table 3). The T base content is significantly higher, while the C base content is the lowest. In *L. peitaihoensis*, three genes have T base content exceeding 40%, namely *Cytb* (40.89%), *ND6* (43.93%), and *ND3* (40.11%), and *P. saccharinoides* has the lowest content of base C (9.15%). The total number of AT bases exceeds 50%, and in some genes, it even exceeds 60%. However, the *P. ryukyuensis ATP8* gene has a total AT base content of only 44.65%, and its A base content is relatively low at 14.47%. The calculation of skewness values of five species showed that the AT-skew values of PCGs are negative (Table 4), indicating an obvious T-base bias. *P. saccharinoides* and *P. ryukyuensis* have an AT-skew of -0.37, demonstrating a significant shift in T bases. The *COII* of *P. ryukyuensis* and *L. peitaihoensis* is relatively unique, having positive AT-skew values of 0.02 and 0.07, respectively, indicating a slight shift in the A base.

Amino acid content and codon usage

We analyzed the amino acid content and codon usage frequency of the mitogenomes of five species. Gly, Val, Ser2, and Leu1 are the four most frequently used amino acids, accounting for 9.67%, 6.72%, 8.55%, and 6.85% of the total amino acid content, respectively (Fig. 2). Most other amino acids are present at levels below 5.00%, and the content of His and Gln is lowest among the five species, with an average of only 1.75%. The RSCU values of the 13 PCGs show that GGG (Gly), UUU (Phe), AGG (Ser2), and AGA (Ser2) are the most frequently used codons, with usage frequencies exceeding 1.40%. Conversely, UCG (Ser1), CUC (Leu1), and CCG (Pro) are the least frequently used codons, each with frequencies below 0.50%. All five species use TAA or TAG as the stop codon (Table 5), and most genes use ATA or ATG as the start codon, with a few genes using ATT. Notably, we

Table 3 Base content of protein coding genes in five species

Gene	***N.* nigrans**					**Nipponacmea sp.**				
	A %	**T %**	**C %**	**G %**	**A+T %**	**A %**	**T %**	**C %**	**G %**	**A+T %**
Mito	28.91	29.61	16.52	24.96	58.52	28.92	31.21	16.26	23.62	60.13
COI	24.71	35.80	16.50	23.52	59.97	24.48	34.61	22.60	18.31	59.09
COII	25.73	34.45	16.57	23.26	60.18	27.88	32.26	18.83	21.02	60.14
ATP8	27.67	35.85	16.35	20.13	63.52	29.59	33.33	18.24	18.7	62.92
ATP6	25.73	33.92	13.16	27.19	59.65	24.31	34.60	21.69	19.36	58.90
ND5	26.83	33.09	25.34	14.75	59.92	25.11	35.03	24.17	15.69	60.14
ND4	24.30	32.82	25.04	17.84	57.12	26.34	34.98	14.55	24.36	61.32
ND4L	26.60	33.67	12.79	26.94	60.27	26.6	34.01	10.77	28.62	60.61
Cytb	24.41	35.61	16.36	23.62	60.02	25.21	37.09	15.98	21.71	62.30
ND6	25.05	36.16	13.94	24.85	61.21	26.00	35.56	14.67	23.78	61.56
ND1	24.92	36.46	14.89	23.73	61.38	27.29	34.74	14.56	23.41	62.03
COIII	23.50	35.97	16.19	24.34	59.47	25.12	34.38	21.64	18.87	59.50
ND3	25.52	34.75	14.41	25.42	60.27	24.01	35.03	14.41	26.55	59.04
ND2	24.89	35.04	24.57	15.49	59.93	22.88	38.61	22.26	16.25	61.49
Gene	***P. saccharinoides***					***P. ryukyuensis***				
	A %	**T %**	**C %**	**G %**	**A+T %**	**A %**	**T %**	**C %**	**G %**	**A+T %**
Mito	22.41	30.75	16.05	30.78	53.16	23.64	30.69	17.86	27.81	54.33
COI	19.31	35.95	15.87	28.87	55.26	19.39	36.90	17.70	26.01	56.29
COII	21.17	35.62	14.89	28.32	56.79	28.01	27.03	26.05	18.91	55.04
ATP8	20.26	30.07	9.15	40.52	50.33	14.47	30.19	18.87	36.48	44.65
ATP6	19.00	34.27	16.51	30.22	53.27	24.74	29.33	25.33	20.59	54.07
ND5	19.07	35.50	14.50	30.93	54.57	26.83	27.59	28.82	16.75	54.42
ND4	23.33	28.07	30.85	17.76	51.40	17.00	36.14	15.52	31.34	53.14
ND4L	21.53	31.25	28.47	18.75	52.78	16.84	34.02	15.81	33.33	50.86
Cytb	20.78	35.74	17.29	26.18	56.52	25.00	29.21	28.68	17.11	54.21
ND6	23.78	29.43	32.55	14.23	53.21	18.56	37.92	15.17	28.34	56.49
ND1	16.61	36.23	14.15	33.01	52.84	17.26	38.91	13.08	30.76	56.16
COIII	16.96	34.31	15.94	32.78	51.27	23.22	28.68	24.87	23.22	51.90
ND3	20.76	35.96	14.04	29.24	56.72	26.96	30.72	24.93	17.39	57.68
ND2	17.78	33.84	12.09	36.28	51.62	17.46	37.90	11.01	33.63	55.36
Gene	***L. peitaihoensis***									
	A %	**T %**	**C %**	**G %**	**A+T %**					
Mito	28.45	31.64	15.74	24.16	60.09					
COI	24.36	36.22	15.32	24.10	60.58					
COII	31.53	27.48	24.17	16.82	59.01					
ATP8	27.67	33.33	21.38	17.61	61.01					
ATP6	28.22	32.28	23.88	15.62	60.50					
ND5	29.39	31.54	24.67	14.40	60.93					
ND4	20.59	39.52	13.80	26.09	60.11					
ND4L	20.46	38.28	12.21	29.04	58.75					
Cytb	21.82	40.89	16.32	20.96	62.71					
ND6	18.88	43.93	13.49	23.70	62.81					
ND1	26.24	34.52	22.37	16.88	60.75					
COIII	26.69	31.70	22.73	18.88	58.39					
ND3	21.47	40.11	11.58	26.84	61.58					
ND2	28.56	30.53	24.82	16.10	59.09					

found a rare TTG starting codon in the *COI* gene of *N. nigrans* and the *ND4* gene of *P. saccharinoides*, while *L. peitaihoensis* uses GTG as the starting codon for its *COI* gene.

Selection pressure analysis

We analyzed the selection pressure of Lottiidae by using the Ka/Ks ratio (non-synonymous to synonymous substitution). The 13 PCGs were assessed for 10 species in the family. The results indicated that *ATP8* had the highest

Table 4 AT-skew values of protein coding genes in five species

Gene	*N.* nigrans	Nipponacmea sp.	*P.* saccharinoides	*P.* ryukyuensis	L. peitaihoensis
Mito	-0.01	-0.04	-0.16	-0.13	-0.05
COI	-0.19	-0.17	-0.30	-0.31	-0.20
COII	-0.14	-0.07	-0.25	0.02	0.07
ATP8	-0.13	-0.06	-0.19	-0.35	-0.09
ATP6	-0.14	-0.18	-0.29	-0.08	-0.07
ND5	-0.10	-0.16	-0.30	-0.01	-0.04
ND4	-0.15	-0.14	-0.09	-0.36	-0.31
ND4L	-0.12	-0.12	-0.18	-0.34	-0.30
Cytb	-0.19	-0.19	-0.26	-0.08	-0.30
ND6	-0.18	-0.16	-0.11	-0.34	-0.40
ND1	-0.19	-0.12	-0.37	-0.39	-0.14
COIII	-0.21	-0.16	-0.34	-0.11	-0.09
ND3	-0.15	-0.19	-0.27	-0.07	-0.30
ND2	-0.17	-0.26	-0.31	-0.37	-0.03

Ka/Ks ratio of 0.733, while *COX3* had the lowest value of 0.179 (Fig. 3). All the Ka/Ks values of the 13 PCGs were less than 1, suggesting that the species in the family were influenced by purifying selection during the evolutionary process.

Phylogenetic relationship

We used maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analysis methods to concatenate 13 PCGs from 115 species in six subclasses of Gastropoda, including Patellogastropoda, Heterobranchia, Caenogastropoda, Neritimorpha, Neomphaliones, and Vetigastropoda. The bivalves *Gari togata* and *Donax dysoni* were used as outgroups (Fig. 4). Both methods produced almost identical topologies, with high confidence for most branches. Overall, each subclass of the entire gastropod family is a branch of its own, among which Vetigastropoda and Neritimorpha are sister groups to each other, followed by Caenogastroopoda, Neritimorpha and Heterobranchia, while Patellogastropoda is located at the outermost of the entire phylogenetic tree. However, the six families except the family Lottiidae are clustered into one branch, and the Lottiidae is independent of the outermost branch of all gastropods. The analysis results show that the Gastropoda subclass has a stable evolutionary tree topology (Fig. 4). Except for Patellogastropoda, which is a polyphyletic group, the remaining subclasses form monophyletic groups, and the posterior probability values of most branches are 1 and the bootstrap values are 100. Combining the analysis of multiple evolutionary branches, the phylogenetic relationship of this subclass is (Patellogastropoda + (Patellogastropoda + (Heterobranchia + (Neomphaliones + (Caenogastroopoda + (Neritimorpha + Vetigastropoda))))). In Patellogastropoda, *Paralepetopsis* sp. of Neolepetopsidae and *Sagamilepeta sagamiensis* of Lepetidae are clustered together, and then, they are clustered together with Pectinodontidae. *Eulepetopsis* sp. of Neolepetopsidae is a sister group to Acmaeidae. The four families Neolepetopsidae, Lepetidae, Pectinodontidae and Acmaeidae form a monophyletic group. And they are sister groups to the family Nacellidae, outside them are the three Patellidae species. The largest and oldest branch in Patellogastropoda is the Lottiidae, among which *Lottia digitalis* and *Scurria scurra* are clustered into one branch, and then form a monophyletic group with the three species of the genus *Nipponacmea*. *Lottia Peitaihoensis* and *Lottia luchuana* of the same genus are clustered into one branch, located outside them. The three species of the genus *Lottia* are not completely clustered into one branch but are instead clustered with *L. digitalis* formed a paraphyletic group, and more data are still needed to verify the reasons for their evolution into a paraphyletic group. The genus *Patelloida* is located at the outermost side of the entire Lottiidae. The phylogenetic relationship is abbreviated as (Lottiidae + ((((Acmaeidae + Neolepetopsidae) + (Neolepetopsidae + Lepetidae) + Pectinodontidae) + Nacellidae) + Patellidae)). Nacellidae and Patellidae belong to the superfamily Patelloidea, and the other five families are from the superfamily Lottioidea. In particular, the Lottiidae is independent of the superfamily to which it belongs and is a sister group to other families in the Patellogastropoda subclass. In the evolutionary analysis of limpets by previous researchers, the long branch attraction phenomenon is easy to occur in the Lottiidae, which is separated from other species of Patellogastropoda on both sides of the Heterobranchia subclass. This situation is a rare phenomenon in the relatively stable evolution of gastropods, which deserves our further exploration.

Gene rearrangement

We compared the gene sequence of all known Patellogastropoda species with that of the ancestors of Gastropods,

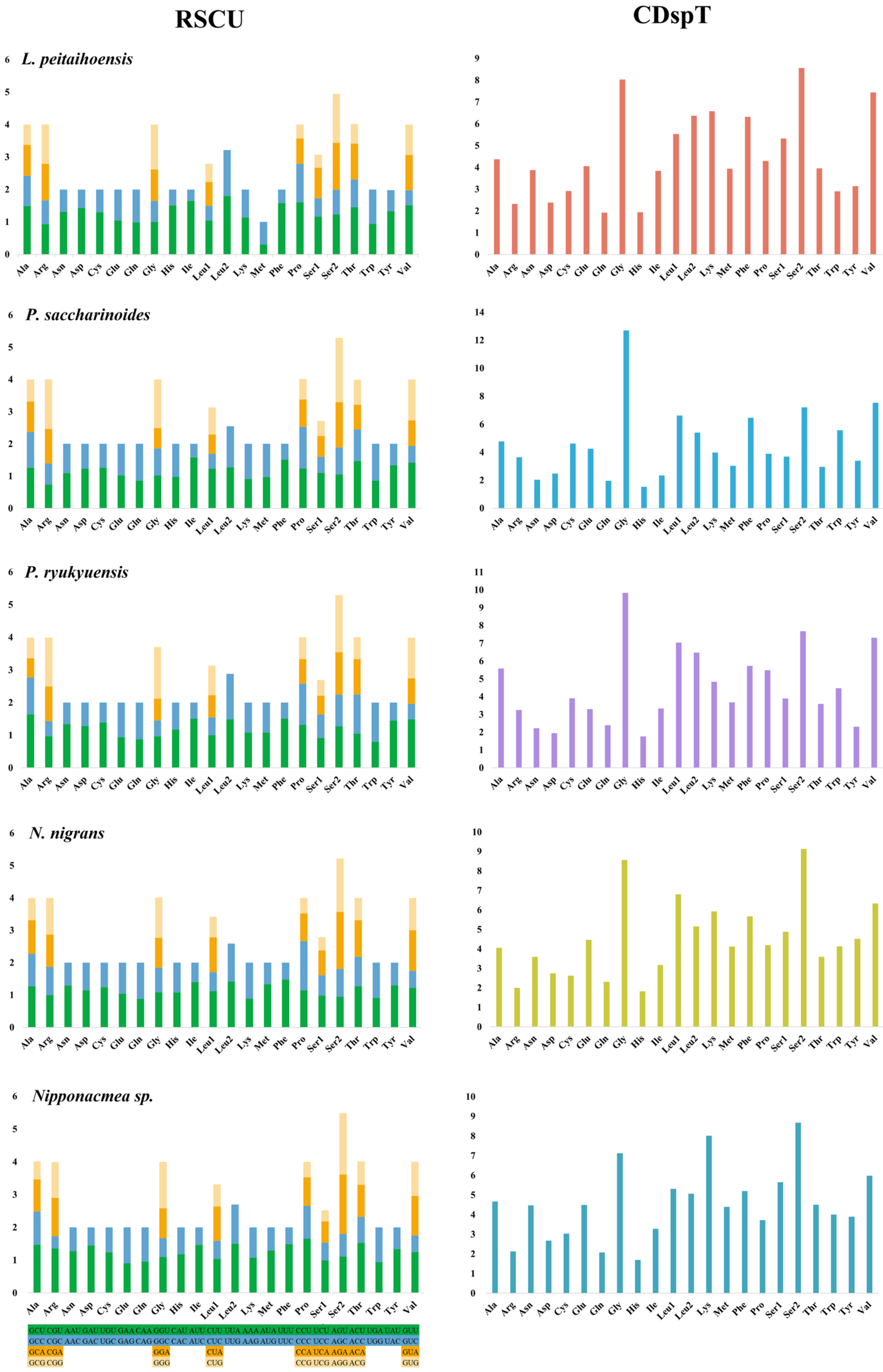

Fig. 2 Amino acid content and relative synonymous codon usage (RSCU) of PCGs of five new sequenced species

Table 5 Usage of start/stop codons of 13 PCGs from five species

	N. **nigrans**		**Nipponacmea sp.**		***P.*** **saccharinoides**		***P.*** **ryukyuensis**		**L. peitaihoensis**	
codon	**start**	**stop**	**start**	**stop**	**start**	**stop**	**start**	**stop**	**start**	**stop**
COI	TTG	TAG	ATG	TAG	ATG	TAA	ATT	TAG	GTG	TAG
COII	ATG	T(AA)	ATG	T(AA)	ATG	T(AA)	ATG	TAA	ATG	TAA
ATP8	ATG	TAG	ATG	TAA	ATA	TAG	ATT	TAA	ATG	TAA
ATP6	ATA	TAG	ATG	TAA	ATG	TAA	ATA	TAG	ATG	TAA
ND5	ATA	TAA	ATT	TAG	ATG	TAG	ATG	TAA	ATT	TAA
ND4	ATG	TAA	ATG	TAG	TTG	TAG	ATG	TAG	ATG	TAG
ND4L	ATG	TAA	ATG	TAA	ATG	TAA	ATA	TAA	ATG	TAG
Cytb	ATG	TAG	ATG	TAA	ATG	TAA	ATG	TAA	ATG	TAA
ND6	ATT	TAA	ATA	TAG	ATA	TAG	ATG	TAG	ATG	TAA
ND1	ATG	TAG	ATG	TAA	ATG	TAG	ATG	TAG	ATG	TAG
COIII	ATG	TAA	ATG	TAA	ATG	T(AA)	ATG	TA(A)	ATG	TAA
ND3	ATG	TAG	ATG	TAA	ATG	TAG	ATG	TAG	ATG	TAA
ND2	ATT	TAA	ATT	TAA	ATT	TAA	ATG	TAG	ATT	TAA

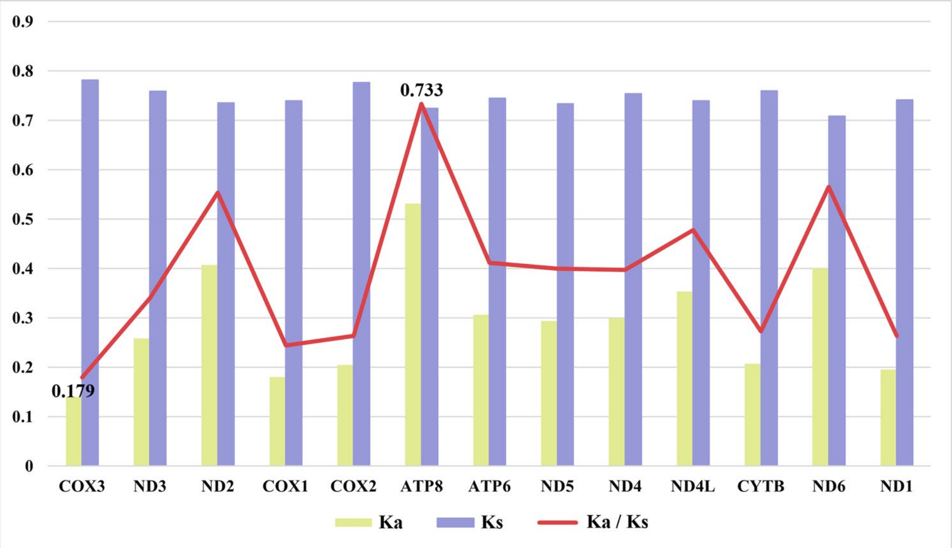

Fig. 3 Ka/Ks ratios for all 13 PCGs of all Lottiidae species, yellow corresponds to Ka value, purple corresponds to Ks value, and red line graph refers to Ka/Ks value

starting from the *COI* gene (Fig. 5). The ancestors had 37 genes in their mitogenome, including 13 PCGs, two rRNAs, and 22 tRNA genes. Among the four studied families of Patellogastropoda, Nacellidae showed relatively conservative gene sequences, with all species having consistent mitogenome sequences and all PCGs consistent with their ancestors, with only a few tRNA positions changed. Acmaeidae and Pectinodontidae had a similar rearrangement to Nacellidae, completely retaining the PCGs sequence of their ancestors, with only a small range of tRNA rearrangement. The gene order of Paralepetopsis sp. of Neolepetopsidae is basically the same as that of Sagamilepeta sagamiensis of Lepetidae, except that the tRNA sequences on both sides of the *COIII* gene have changed a little. Compared with the ancestral PCG sequence, they only have one change after the *ND5* gene is adjusted to the *ND1* gene. The gene order of Eulepetopsis sp. of Neolepetopsidae has changed greatly. The ATP8 gene located between *COII* and *ATP6* has moved to between *ND1* and *rrnS*, two rRNAs have been transposed, and *ND2* has been placed between *rrnL* and *ND3*. That is, the gene interval segment has changed from *rrnL-rrn*S*-COIII-ND3-ND2* to *rrnS-rrnL-ND2-COIII-ND3*. All tRNAs had also undergone a huge rearrangement. In Patellidae, there were 35 genes in the mitogenome, which was missing *trnC* and *trnQ* compared to the ancestral Gastropoda, and the gene sequence was significantly different from the ancestral sequence, especially in tRNA. The PCGs fragments from *Cytb* to *ATP8* were preserved, and *rrnL-rrnS* and *ND*3-*ND2* fragments were reversed. Lottiidae had the highest gene rearrangement rate in Patellogastropoda. The mitogenome of each species in the family has been rearranged, and the number of genes had also increased

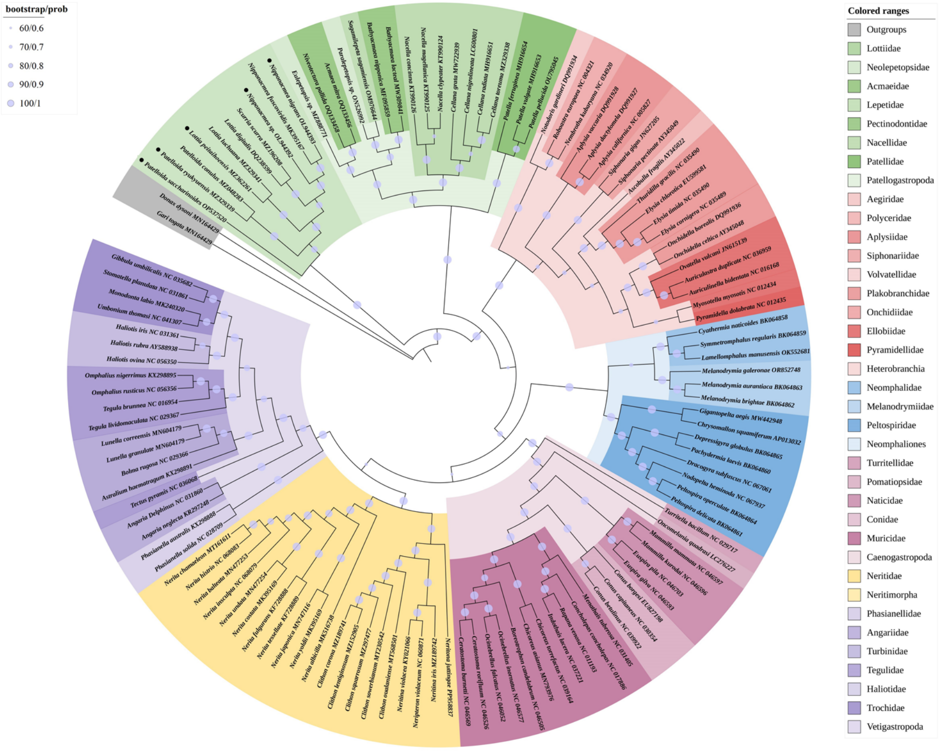

Fig. 4 Bayesian inference (BI) and maximum likelihood (ML) methods were used to infer phylogenetic trees based on 13 PCGs. The five species newly sequenced are marked with black dots, the light purple dot on each branch is the posterior probability of BI /bootstrap support in ML, and the two species of the gray branch are outgroups

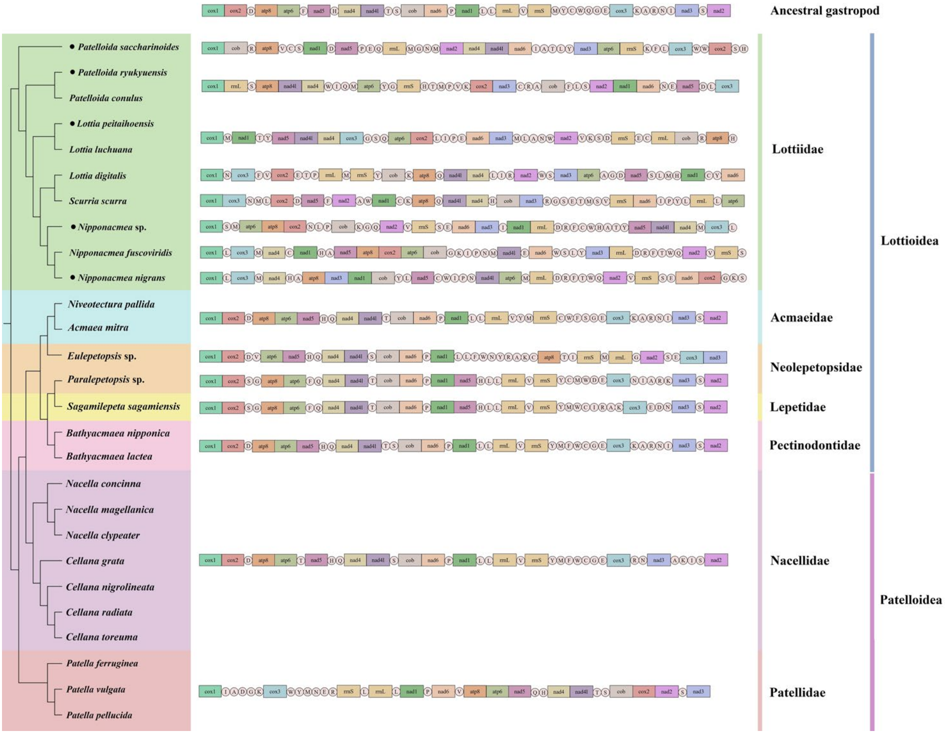

Fig. 5 Complete mitogenome alignment map of all species in the Patellogastropoda, the rectangular pattern represents PCGs, the circle represents tRNA, and tRNA is labeled with amino acid abbreviations

by one or two tRNAs. In this analysis of the 10 species of the Lottiidae, eight gene sequencing compositions were found. In the complex rearrangement of the family Lottiidae species, it can be found that *P. saccharinoides* only retains the ancestral gene segment *ND4-ND4L*. The other two species of the genus *Patelloida* have the same gene order, and *ND4* and *ND4L* are transposed compared to the ancestral order. Similarly, *L. peitaihoensis* and *L. luchuana* have the same gene order and have the gene segment *ND4L-ND4*. *L. digitalis* and *S. scurra* have clustered into one in evolution, and we can find that they have the same PCG segment *COI-COIII-COII*, and the *ATP8-trnQ-ND4L-ND4* segment. Among the three *Nipponacmea* genera, *Nipponacmea* sp. has an identical gene segment *ND5-ND4L-ND4-COIII* with *Lottia peitaihoensis* and *L. luchuana*. The other two *Nipponacmea* have identical segments *COI-trnL-COIII-trnM-ND4*, and a long segment *rrnL-trnD-trnR-trnF-trnT-trnW-trnQ-ND2-trnV-rrnS-trnS*. In general, similar gene segments can be found in the same genus of Lottiidae, and transposition of *ND4* and *ND4L* often occurs in this family. Their high mutation from the ancestral gene sequence may be the reason for being the outermost monophyletic branch in the phylogenetic tree.

Nacellidae and Pectinodontidae showed conservative evolution with minor tRNA changes and retention of ancestral PCGs. Patellidae showed significant divergence, including gene loss and sequence reversal. Lottiidae had extensive gene rearrangements and increased number of tRNA genes, indicating high genomic variability. This analysis highlights the importance of examining tRNA rearrangements and overall gene composition to better understand evolutionary dynamics. Further research could explore the functional implications of these genomic changes and their implications for the biology and adaptation of these species.The conserved gene order observed in Nacellidae and Pectinodontidae indicates evolutionary stability and a possible lack of selective pressure to change, suggesting that their existing gene arrangements are well adapted to their ecological niches. Extensive gene rearrangements in families such as Lottiidae may reflect adaptive responses to different environmental pressures or lifestyles, indicating strong evolutionary plasticity. Unique gene orders can serve as phylogenetic markers, to resolve evolutionary relationships and refine classification within and between families. Gene rearrangements may indicate speciation events or adaptive radiations, providing insight into the evolutionary history and diversification of the group.

Estimation of divergence time

Based on divergence time estimates of the Patellogastropoda, the studied 27 species of this group diverged during the Permian period of the Paleozoic, about 282.92 MYA (Fig. 6). The genera within the Lottiidae began to differentiate about 148.08 MYA during the Mesozoic

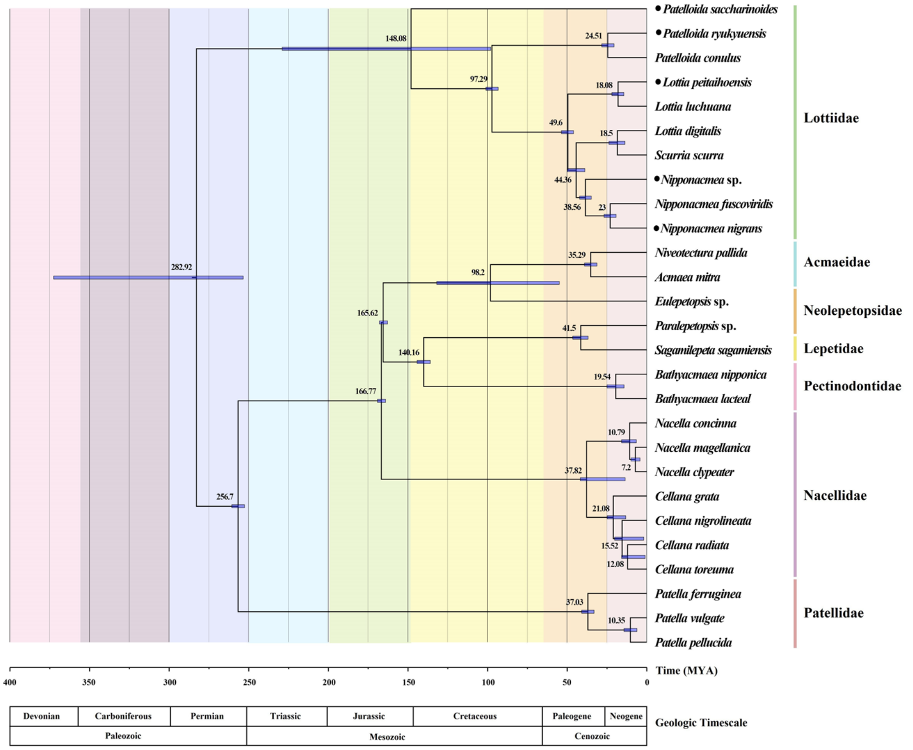

Fig. 6 Estimation of divergence time based on 13 PCGs of all species in the Patellogastropoda

Cretaceous. The Patellidae, which is the second evolved branch, emerged during the late Permian, around 256.7 MYA. In the Mesozoic Jurassic, approximately 166.77 MYA, Nacellidae began to diverge. In addition, the divergence accelerated during the Cenozoic period, and a large number of new species appeared. The species from Neolepetopsidae and Lepetidae, as well as the species within Acmaeidae, Nacellidae and Patellidae, first diverged in the Paleogene, while the two species of the Pectinodontidae first diverged in the Neogene. The most recent divergence was between *Nacella magellanica* and *Nacella clypeater*, only around 7.2 MYA. Among the five species in this study, *P. saccharinoides* was the first species to diverge, which occurred at about 148.08 MYA, followed by *Nipponacmea* sp. about 38.56 MYA; the divergence time of *P. ryukyuensis* and *N. nigrans* both occurred at the boundary between the Paleogene and Neogene in the Cenozoic Era, while the divergence time of *L. peitaihoensis* was the latest, occurring around 18.08 MYA.

Discussion

Basic characteristics of mitogenomes of Lottiidae species

In this study, we sequenced and analyzed the complete mitogenome of five Lottiidae species (*P. ryukyuensis*, *P. saccharinoides*, *L. peitaihoensis*, *N. nigrans*, and *Nipponacmea* sp.). We examined the basic characteristics of the genome, such as base content, codon preference, amino acid content, and selection pressure value of each gene. The mitogenomes of the five species were all less than 20 kbp in length, and the number of genes in Lottiidae species increased from the 37 genes of the ancestral Gastropoda to 38 or 39 genes, with individual tRNA genes such as *trnW*, *trnM*, and *trnL* appearing in multiple copies. This phenomenon was also observed in previously published species such as *Nipponacmea fuscoviridis* and *S. scurra* [20, 42]. Our analysis of Ka/Ks values of the Lottiidae mitogenomes showed an overall level of less than 1, indicating the pressure of purifying selection. Feng et al. [20] reported that the Ka/Ks values of the mitogenomes of *L. goshimai* and *N. fuscoviridis* were mostly close to or greater than 1. However, our study, which included a larger number of species, showed most PCGs had Ka/Ks values less than 0.5. Despite the effects of purifying selection and selection pressure, the degree of mitogenome rearrangement in Lottiidae is quite significant and unusual for Gastropoda. Further investigation is necessary to determine the mechanism causing this high degree of rearrangement.

Reconstruction of Patellogastropoda phylogenetic relationship

In morphological analysis, Patellogastropoda was placed at the bottom of the phylogenetic tree as the sister group of all gastropods [43, 44]. Molecular analysis has also repeatedly classified Patellogastropoda as the sister group of other gastropods [20, 45, 46], but the position of these groups varies in molecular analysis. Cunha and Giribet [47] inferred that Vetigastropoda diverged earlier than Patellogastropoda based on amino acid sequence, while Feng et al. [48] reconstructed the phylogenetic relationship of Gastropoda using PCGs and found that Heterobranchia was at the root, followed by Patellogastropoda. Putri et al. [40] added the family Acmaeidae to their phylogenetic analysis and used 13 PCGs to construct the tree. The relationship between each family is consistent with our analysis results. The phylogenetic tree constructed according to our analysis results also meet the requirements of *Nipponacmea* and *Lottia* as one branch, followed by reuniting Patelloida into one branch. The results of our PCGs analysis are consistent with most molecular analyses and support the early morphological classification Patellogastropoda as being the earliest group isolated from Gastropoda. Previous studies have shown that the phenomenon of LBA has occurred multiple times in Patellogastropoda [20, 49, 50]. We reconstructed the phylogenetic tree of gastropods by omitting the third codon position, reducing the noise or false signals caused by the high mutation rate of the third position, thus obtaining a more accurate evolutionary branch. However, the Lottiidae family is still the most unique existence in Patellogastropoda. After data optimization, they still tend to the outermost end of the entire evolutionary tree and show incredible changes in gene rearrangement analysis.

The adaptive evolution of limpets

Fossil data provide critical calibration points for molecular phylogenetic studies. By using dated fossils, researchers can calibrate molecular clocks to estimate the timing of divergence events within Patellogastropoda [51]. This can increase the resolution and accuracy of phylogenetic trees, leading to a better understanding of the relationships between different Patellogastropod species and lineages [52]. According to records, the oldest limpet fossils can be traced back to the Middle Ordovician period [53]. Fossils of Patellidae found in California date back to approximately 75 MYA in the late Cretaceous [54]. Initially, Nakano and Ozawa [55] used *12 S rRNA* and *16 S rRNA* to estimate the divergence of Patellogastropoda to the Cretaceous period of 132–154 MYA. Later, the *COI* gene was added to the analysis, and it was found that the limpet originated in the Jurassic period, and its main branches formed from the Mesozoic to the Cenozoic Era [56]. At present, there is no unified divergence time for Patellogastropoda. Putri et al. [40] estimated that the earliest divergence time of Patellogastropoda was 337.2 MYA, while Qi et al. [57] estimated that the earliest divergence was 247.88 MYA. Based on the analysis of

13 PCGs, we obtained a similar result for the time estimation of Patellogastropoda divergence. We have tested the reliability of the final calculation result based on fossil records.

During the transition from the Paleozoic to the Mesozoic Era, the landmasses of earth gradually converged into the supercontinent Pangea, which later split into Laurasia in the north and Gondwana in the south. Crame [58] proposed three historical stages of anti-tropical distribution, the oldest tied to the Jurassic and Cretaceous periods after the breakup of Pangea. In the early Miocene, cold-water fauna was interrupted by global warming, and the latest stage occurred during the Pleistocene-Pliocene glacial period, influencing global gastropod distribution patterns. The evolutionary history of Patellogastropoda, particularly in relation to the divergence of major lineages such as *Nacella* and *Cellana*, is complex and shaped by significant geological and climatic events [59, 60]. Fossil evidence from the Upper Eocene *Cellana ampla* in Oregon provides a basis for dating the split between *Nacella* and *Cellana* at around 38 MYA, aligning with the opening of the Southern Ocean and the onset of Antarctic glaciation. This period of ocean cooling likely promoted the divergence of these genera, with *Nacella* adapting to colder waters and *Cellana* to warmer tropical and subtropical regions. The genus Patelloida of Lottiidae also has a long evolutionary history, with fossils dating back to the Cretaceous in California [55]. Its distribution expanded westward, likely driven by global currents during the Cretaceous period. The fossil record of *Niveotectura* from Miocene to Pleistocene sediments in the northern Pacific further supports the evolutionary timelines, with the minimum of divergence estimated at 15 MYA.

Recent molecular studies of Patellogastropoda suggest that while some earlier estimates were based on fossil evidence and single-gene analysis, newer approaches using multiple gene datasets confirm the timing of these divergence events. These studies highlight the limited dispersal abilities of some species, such as the Patelloida profunda group, which is restricted to tectonically uplifted islands in the Pacific [61]. The shorter larval duration of these limpets enhances geographic structuring and genetic differentiation, as seen in phylogeographic patterns. Several geological events during the Cenozoic also played critical roles in shaping modern Patellogastropod distribution. The closing of the Panamic portal around 3.5 MYA and the opening of the Bering Strait during the Pliocene facilitated migrations between the Pacific and Atlantic basins, evidenced by the presence of *Lottia* species in both regions [56].

In conclusion, the evolutionary and biogeographical history of Patellogastropoda is shaped by a combination of tectonic shifts, ocean currents, and climatic changes. The use of both fossil records and molecular data allows for more precise estimations of divergence times, shedding light on the processes that have driven the diversification and distribution of this marine group. Further discoveries in the fossil record and a deeper understanding of their larval biology and dispersal capabilities will continue to refine our knowledge of their historical biogeography.

Conclusion

To summarize, the study focused on the mitochondrial genomes of five Lottiidae species using next-generation sequencing technology. The analysis revealed variations in gene composition and structural characteristics among the species. Additionally, selection pressure analysis suggested purifying selection acting on all protein-coding genes. Furthermore, our study provided insights into the phylogenetic relationships within the Patellogastropoda, indicating Lottiidae is the earliest monophyletic group to differentiate within the subclass Patellogastropoda. It is also the family with the greatest genetic rearrangement in this subclass, which is unique among the relatively conservative gastropod species. These findings contribute to enriching the mitogenome database of Patellogastropoda and enhance our understanding of the genetic characteristics and phylogenetic relationships within the gastropod taxa.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10904-z.

Supplementary Material 1

Author contributions

J.F. and J.M. conducted the experiments, analyzed the data, and wrote the original draft. J.L. contributed analysis software, revised the manuscript. Y.Y. conceived and designed the experiments, revised the manuscript. All authors have read and approved the final manuscript.

Funding

This work was supported by the Project of Bureau of Science and Technology of Zhoushan (No. 2021C21017) and the National Key R&D Program of China (2019YFD0901204).

Data availability

The data that support the findings of this study are available from the corresponding author. The mitogenome sequences used in this project were available in GenBank with accession numbers: MZ362261, OP537520, MZ329339, OL944392 and OL944393, respectively.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]National Engineering Research Center for Marine Aquaculture, Zhejiang Ocean University, Haida South Road 1, Zhoushan 316022, China
[2]Department of Veterinary Sciences, University of Pisa, Viale delle Piagge 2, Pisa 56124, Italy
[3]Italian Institute for Environmental Protection and Research, ISPRA, Via del Cedro 38, Livorno 57123, Italy

Received: 3 June 2024 / Accepted: 15 October 2024
Published online: 07 November 2024

References

1. Qu J, Shi B, Xu Y, Li R, Sun X, Guo X, Cui Y, Wang S, Wang X. Phylogeography of *Patelloida pygmaea* along the China coast. Marine Biodivers. 2021;51:1–13.
2. Zhengang F. The bending and habitat environment of limpet shells. Ocean Sci. 1982;04:29.
3. Jiping L. Study in classification of true limpets and cryptic diversity of *Circe scripta* along coast of China. Ocean University of China; 2015.
4. Liu Y, Li Y, Wang M, Bao Z, Wang S. The complete mitochondrial genome and phylogenetic analysis of the deep-sea limpet *Bathyacmaea lactea*. Mitochondrial DNA B Resour. 2021;6:2090–1.
5. Zhang S, Zhang J, Zhang S. A new species of *Bathyacmaea* (Gastropoda: Pectinodontidae) from a methane seep area in the South China Sea. Nautilus. 2016;130:1–4.
6. Zhang S, Zhang S. *BathyaBecki becki*, a new species of pectinodontid limpet (Gastropoda: Pectinodontidae) from a hydrothermal vent of the Manus Back-Arc Basin. Nautilus. 2017;131:217–25.
7. Zhang M. Research on bionic cutting tool based on structural characteristics and biological materials of limpet's teeth. Harbin University of Science and Technology; 2022. (in Chinese).
8. Yu X, Wang M, Cao X, Wu Z. The oogenesis and female gonadal histology of *Cellana grata* (Gould). Acta Hydrobiologitic Sinica. 2010;34:9. (in Chinese).
9. Wang Q. A study of *hox* gene expression patterns in limpet and chiton early development. Institute of Oceanology, Chinese Academy of Sciences; 2019. (in Chinese).
10. Xu T, Wang Y, Sun J, Chen C, Watanabe HK, Chen J, Qian P, Qiu J. Hidden historical habitat-linked population divergence and contemporary gene flow of a deep-sea patellogastropod limpet. Mol Biol Evol. 2021;38:5640–54.
11. Li X, Shan W, Yang Y, Joralmon D, Zhu Y, Chen Y, Yuan Y, Xu H, Rong J, Dai R. Limpet tooth-inspired painless microneedles fabricated by magnetic field-assisted 3D printing. Adv Funct Mater. 2021;31:2003725.
12. Goldstien SJ, Gemmell NJ, Schiel DR. Molecular phylogenetics and biogeography of the nacellid limpets of New Zealand (Mollusca: Patellogastropoda). Mol Phylogenet Evol. 2006;38:261–5.
13. Powell AWB. New Zealand Mollusca: marine, land and freshwater shells. Collins, 1979.
14. Ridgway SA, Reid DG, Taylor JD, Branch GM, Hodgson AN. A cladistic phylogeny of the family Patellidae (Mollusca: Gastropoda). Philosophical transactions of the royal society of London. Ser B: Biol Sci. 1998;353:1645–71.
15. Mclean JH. Neolepetopsidae, a new docoglossate limpet family from hydrothermal vents and its relevance to patellogastropod evolution. J Zool. 1990;222:485–528.
16. Nakayama R, Sasaki T, Nakano T. Molecular analysis reveals a new cryptic species in a limpet *Lottia Kogamogai* (Patellogastropoda: Lottiidae) from Japan. Zootaxa. 2017;4277:237–51.
17. Nakano T, Ozawa T. Systematic revision of *Patelloida Pygmaea* (Dunker, 1860) (Gastropoda:Lottiidae), with a description of a new species. J Molluscan Stud. 2005;71:357–70.
18. Nakano T, Spencer HG. Simultaneous polyphenism and cryptic species in an intertidal limpet from New Zealand. Mol Phylogenet Evol. 2007;45:470–9.
19. Ballard JWO, Whitlock MC. The incomplete natural history of mitochondria. Mol Ecol. 2004;13:729–44.
20. Feng J, Guo Y, Yan C, Ye Y, Lü Z. Comparative analysis of the complete mitochondrial genomes in two limpets from Lottiidae (Gastropoda: Patellogastropoda): rare irregular gene rearrangement within Gastropoda. Sci Rep. 2020;10:1–14.
21. Kong L, Li Y, Kocot KM, Yang Y, Qi L, Li Q, Halanych KM. Mitogenomics reveals phylogenetic relationships of Arcoida (Mollusca, Bivalvia) and multiple independent expansions and contractions in mitochondrial genome size. Mol Phylogenet Evol. 2020;150:106857.
22. Feng J. The complete mitochondrial genomes sequence analysis and phylogenetic study of 4 genera 13 species of the family Neritidae. Zhejiang Ocean University; 2021. (in Chinese).
23. Sevigny JL, Kirouac LE, Thomas WK, Ramsdell JS, Lawlor KE, Sharifi O, Grewal S, Baysdorfer C, Curr K, Naimie AA. The mitochondrial genomes of the nudibranch mollusks, melibe leonina and tritonia diomedea, and their impact on gastropod phylogeny. PLoS ONE. 2015;10:e0127519.
24. Gu M. Atlas of the famous shell in the world for identification and appreciation, 2005. (in Chinese).
25. Zhang S. China marine shellfish Atlas, 2008. (in Chinese).
26. Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality gnomic DNA for PCR-based techniques. Nucleic Acids Res. 1997;25:4692–3.
27. Jin J, Yu W, Yang J, Song Y, Li D. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biol. 2020;21:1–31.
28. Bernt M, Donath A, Juhling F, Externbrink F, Florentz C, Fritzsch G, Putz J, Middendorf M, Stadler PF. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 2013;69:313–9.
29. Xia X. DAMBE7: New and improved tools for data analysis in molecular biology and evolution. Mol Biol Evol. 2018;35:1550–2.
30. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35:1547–9.
31. Julio R, Albert FM, Juan CS-D, Sara G-R, Pablo L, Sebastián E-R, Sánchez-Gracia A, Alejandro S-G. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol. 2017;34:3299–302.
32. Hassanin A, Leger 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;54:277–98.
33. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012;61:539–42.
34. Minh BQ, Schmidt H, Chernomor O, Schrempf D, Lanfear R. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2019;37:1530–4.
35. Swofford DL. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4.0b10. 4 ed, 2002.
36. Posada D, Modeltest. A tool to select the best-fit model of nucleotide substitution. Version 3.7. Available from: darwin.uvigo.es. 2005.
37. Nylander JAA. MrModeltest 2.3. Program distributed by the author evolutionary biology centre. Uppsala University; 2004.
38. Rambaut A, FigTree. Version 1.4.3. http://tree.bio.ed.ac.uk/software/figtree/ 2016.
39. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol. 2012;29:1969–73.
40. Rambaut A, Suchard M, Xie D, Drummond A. Tracer v1. 6. 2014, 2015.
41. Putri ET, Lee D, Kwak H, Kim YK, Nakano T, Park JK. Complete mitochondrial genomes of the Acmaeidae limpets provide new insights into the internal phylogeny of the Patellogastropoda (Mollusca: Gastropoda). Front Mar Sci. 2022;10:577.
42. Saenz-Agudelo P. The complete mitochondrial genome of the true limpet *Scurria scurra* (Lottiidae). Mitochondrial DNA Part B. 2022;7:642–3.
43. Ponder WF, Lindberg DR. Towards a phylogeny of gastropod molluscs: analysis using morphological characters. Zool J Linn Soc. 1997;119:83–265.
44. Haszprunar G. On the origin and evolution of major gastropod groups, with special reference to the Streptoneura. J Molluscan Stud. 1988;54:367–441.
45. Colgan DJ, Ponder WF, Eggler PE. Gastropod evolutionary rates and phylogenetic relationships assessed using partial 28S rDNA and histone H3 sequences. Zoolog Scr. 2000;29:29–63.
46. McArthur AG, Harasewych MG. Molecular systematics of the major lineages of the Gastropoda. In: Lydeard C, Lindberg DR, editors. Molecular Systematics and Phylogeography of mollusks. Washington, D.C.: Smithsonian Books; 2003. pp. 1–22.
47. Cunha TJ, Giribet G. A congruent topology for deep gastropod relationships. Proc Royal Soc B. 2019;286:20182776.

48. Feng J, Xia L, Yan C, Miao J, Ye Y, Ji J, et al. Characterization of four mitochondrial genomes of family Neritidae (Gastropoda: Neritimorpha) and insight into its phylogenetic relationships. Sci Rep. 2021;11:11748.
49. Bergsten J. A review of long-branch attraction. Cladistics. 2005;21:163–93.
50. Uribe JE, Kano Y, Templado J, Zardoya R. Mitogenomics of Vetigastropoda: insights into the evolution of pallial symmetry. Zoolog Scr. 2016;45:145–59.
51. Allmon WD, Smith UE. What, if anything, can we learn from the fossil record about speciation in marine gastropods? Biological and geological considerations. Am Malacological Bull. 2011;29:247–76.
52. Benton MJ, Donoghue PC, Asher RJ. Calibrating and constraining molecular clocks. The timetree of life. Oxf Univ Press 2009;35–86.
53. Yochelson EL. Macroscenella (Mollusca) from the Middle Ordovician of Wisconsin—a reinterpretation and reassignment. J Paleontol. 1994;68:1252–6.
54. MacClintock C. Shell structure of patelloid and bellerophontoid gastropods (Mollusca). Peabody Museum Nat History Yale Univ Bull. 1967;22:1–140.
55. Nakano T, Ozawa T. Phylogeny and historical biogeography of limpets of the order Patellogastropoda based on mitochondrial DNA sequences. J Molluscan Stud. 2004;70:31–41.
56. Nakano T, Ozawa T. Worldwide phylogeography of limpets of the order Patellogastropoda: molecular, morphological and palaeontological evidence. J Molluscan Stud. 2007;73:79–99.
57. Qi Y, Zhong Z, Liu X, He X, Zhou Y, Zhang L, et al. Phylogenomic analyses reveal a single deep-water colonisation in Patellogastropoda. Mol Phylogenet Evol. 2024;190:107968.
58. Crame JA. Bipolar molluscs and their evolutionary implications. J Biogeogr. 1993;20:145–61.
59. González-Wevar CA, Nakano T, Cañete JI, Poulin E. Molecular phylogeny and historical biogeography of *Nacella* (Patellogastropoda: Nacellidae) in the Southern Ocean. Mol Phylogenet Evol. 2010;56:115–24.
60. González-Wevar CA, Chown SL, Morley S, Coria N, Saucéde T, Poulin E. Out of Antarctica: quaternary colonization of sub-antarctic Marion Island by the limpet genus *Nacella* (Patellogastropoda: Nacellidae). Polar Biol. 2016;39:77–89.
61. Kirkendale LA, Meyer CP. Phylogeography of the *Patelloida profunda* group (Gastropoda: Lottidae): diversification in a dispersal-driven marine system. Mol Ecol. 2004;13:2749–62.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.