BMC Genomics

RESEARCH

Open Access

Phylogenetic analysis, gene rearrangement and divergence time estimation of Patellogastropoda (Mollusca, Gastropoda) based on complete mitochondrial genomes

Wanting Liu[1], Alexei V. Chernyshev[2], Jiji Li[1], Yingying Ye[1*] and Xiangli Dong[1*]

Abstract

Background Patellogastropoda, or true limpets, is a major taxonomic group within the Gastropoda, Mollusca. The complete mitochondrial genomes have been widely used to study the phylogenetic relationships of Patellogastropoda. To further enhance the understanding of the phylogenetic relationships among families within this subclass, we obtained the complete mitochondrial genome sequences of five species using third-generation sequencing technology: *Limalepeta lima*, *Lepeta kuragiensis*, *Lottia versicolor*, *Lottia instabilis*, and *Lottia tenuisculpta*. We examined mitochondrial genome structure, nucleotide composition and skew, as well as amino acid content and codon usage. Additionally, we assessed selective pressures on protein-coding genes in Lepetidae and Lottiidae species, constructed a phylogenetic tree for Patellogastropoda, analyzed gene arrangement, and estimated the divergence times within Patellogastropoda.

Results The mitochondrial genomes of these five species all encoded 38 genes. They exhibited a higher AT base content, and the protein-coding genes were under purifying selection. The Patellogastropoda was divided into two clades: Clade 1 included a part of the Lottioidea superfamily and the Patelloidea superfamily, whereas the Lottiidae family within the Lottioidea superfamily formed a separate clade (Clade 2). The mitochondrial genomes of the Patellogastropoda exhibited extensive gene rearrangements, particularly those in Lottiidae, which were the most complex. The Patellogastropoda lineage was estimated to have originated in the Paleozoic Permian era, with active differentiation occurring in the Mesozoic Cretaceous and the Cenozoic era.

Conclusions Our research results contribute to a better understanding of the phylogenetic relationships within the Patellogastropoda, and reveal the extensive gene rearrangements, providing further insights into the evolution and genetics of gastropods.

Keywords Mitochondrial genome, Patellogastropoda, Phylogeny, Gene arrangement, Divergence time

*Correspondence:
Yingying Ye
yeyy@zjou.edu.cn
Xiangli Dong
dongxiangli@zjou.edu.cn
[1] National Engineering Research Center for Marine Aquaculture, Zhejiang Ocean University, Zhoushan 316022, China
[2] A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, Vladivostok 690041, Russia

Background

The subclass Patellogastropoda belongs to the Mollusca phylum and the Gastropoda class. Patellogastropods exhibit a broad global distribution, with their habitats typically concentrated within the intertidal zone [1]. As primary herbivores in the intertidal zone, Patellogastropods have cap-shaped shells that can reach a maximum length of over 20 cm [2]. In recent years,

research on Patellogastropoda has encompassed multiple subdisciplines within the biological sciences, such as aquaculture [3], morphology [4], biogeography [5], reproductive biology [6], biomechanics [7], and developmental biology [8–10], among others.

Historically, the classification of Patellogastropoda was based on morphological features such as shell shape [2]. Although the external shell morphology is a key feature for the classification of Patellogastropoda species, this characteristic exhibits a high degree of variability [11, 12]. The extensive intraspecific morphological variation, coupled with specific ecotypes exhibited by some species, easily leads to misclassification [13]. Other morphological characters, such as the radula and gills, have been similarly problematic due to convergent evolution among limpets, leading to ongoing debates regarding the phylogenetic relationships and composition of Patellogastropoda [14]. Morphological analyses traditionally have placed Patellogastropoda in a basal position within Gastropoda, as the sister group to all other gastropods [15, 16]. Regarding familial classification within Patellogastropoda, the taxon was previously recognized as comprising three families, i.e., Acmaeidae, Patellidae, and Lepetidae [17]. Patellogastropoda has subsequently been reclassified into six families, i.e., Acmaeidae, Lepetidae, Lottiidae, Nacellidae, Neolepetopsidae, and Patellidae [11].

In recent years, the application of molecular phylogenetics has greatly advanced the understanding of Patellogastropoda's taxonomy and phylogenetic relationships. Molecular research on Patellogastropoda primarily focused on partial sequences of a few individual genes, such as *cox1*, *12S*, *16S*, and *18S* [18–20]. Among these, the molecular phylogenetic study with the most extensive sample coverage was conducted by Nakano and Ozawa, who established a detailed molecular phylogenetic tree based on partial sequences of three mitochondrial genes (*12S rRNA*, *16S rRNA*, and *COI*) [14]. Their findings led to several conclusions: first, Lottiidae and Acmaeidae were considered synonyms (a view later refuted [21]); second, Pectinodontinae was elevated to the family level as Pectinodontidae; and third, a new family, Eoacmaeidae, was established. Consequently, Patellogastropoda was classified into six families, i.e., Eoacmaeidae, Patellidae, Nacellidae, Pectinodontidae, Lepetidae, and Lottiidae. According to the latest records in the World Register of Marine Species (WoRMS) (https://www.marinespecies.org/), there are currently nine valid families of Patellogastropoda classified under two superfamilies (Lottioidea and Patelloidea). Lottioidea includes seven families, i.e., Acmaeidae, Eoacmaeidae, Lepetidae, Lottiidae, Neolepetopsidae, Pectinodontidae, and Rhodopetalidae; and Patelloidea includes two families, i.e., Nacellidae and Patellidae [14, 21–25].

With the rapid development of complete mitochondrial genome sequencing and amplification techniques, mitochondrial genomes have been widely utilized in studying the phylogenetic relationships of numerous gastropod species [26–29]. When inferring evolutionary relationships, the complete mitochondrial genome offers a significantly larger amount of information compared to short sequences of individual genes. In this study, we sequenced the complete mitochondrial genomes of five Patellogastropoda species and analyzed their basic structural features, base content and bias, amino acid content, and codon usage. We also calculated the selective pressure values of protein-coding genes (PCGs) in species from the families Lepetidae and Lottiidae. Additionally, by incorporating other mitochondrial genome data of Patellogastropoda from the NCBI database, we constructed a phylogenetic tree for the subclass Patellogastropoda, compared the arrangement of mitochondrial genes within this group, and estimated the divergence times. The five new species we sequenced all belong to the superfamily Lottioidea. Among them, the complete mitochondrial genome sequences of the genera *Limalepeta* and *Lepeta* are not clear in the phylogenetic position within Patellogastropoda. The three complete mitochondrial genome sequences of the genus *Lottia* enrich our understanding of the mitochondrial genome diversity of the family Lottiidae. This study provides further supplementation and refinement for the phylogenetic research of Patellogastropoda.

Materials and methods

Sample collection and DNA extraction

The samples of the five research species were collected from the Vostok Bay and the Ussuri Bay in the Sea of Japan (Table 1). After morphological identification, muscle tissues from the samples were stored in absolute ethanol. Total genomic DNA was extracted using the salting-out method [30] and its quality was assessed by 1% agarose gel electrophoresis. The extracted DNA was stored at −20 °C.

Mitogenomes sequencing, assembly, and annotation

For PacBio library construction and sequencing, more than 5 μg of sheared and concentrated DNA was applied to size selection by the Blue Pippin (Sage Science, Beverly MA, USA). Approximately 10 kb SMRTbell libraries were prepared according to the manufacturer's instructions (PacBio, Menlo Park, CA, USA). One SMRT cell and 10 Gb of data were

Table 1 Sampling information for five Patellogastropoda species

Superfamily	Family	Species	Sampling date	Location	Accession no.
Lottioidea	Lepetidae	*Limalepeta lima*	2022-10-18	Vostok Bay, Sea of Japan	OR574472
Lottioidea	Lepetidae	*Lepeta kuragiensis*	2022-10-18	Vostok Bay, Sea of Japan	OR574473
Lottioidea	Lottiidae	*Lottia versicolor*	2022-11-08	Ussuriisky Bay, Sea of Japan	OR574474
Lottioidea	Lottiidae	*Lottia instabilis*	2022-11-08	Ussuriisky Bay, Sea of Japan	OR574475
Lottioidea	Lottiidae	*Lottia tenuisculpta*	2022-10-18	Vostok Bay, Sea of Japan	OR574476

sequenced on the PacBio Sequel II platform. Prior to assembly, raw reads were filtered by Trimmomatic v0.39 [31]. This filtering step was performed to remove the reads with adaptors, the reads showing a quality score below 20 (Q < 20), the reads containing a percentage of uncalled bases ("N" characters) equal to or greater than 10% and the duplicated sequences. The mitochondrial genome was reconstructed using a combination of de novo and reference-guided assemblies, and the following three steps were used to assemble the mitogenome. First, the filtered reads were assembled into contigs using MitoZ v2.3 [32], potential mitochondrial contigs were extracted by aligning against the NCBI mitogenome database. Second, the potential mitochondrial contigs were aligned to the reference mitogenomes using BLAST v2.8.1 + [33], and aligned contigs (> 80% query coverage) were ordered and connected manually according to the reference mitogenomes. Finally, MUMmer v3.23 [34] was used to check whether these contigs were circular. The mitochondrion genes were annotated using the online MITOS tool [35], using default parameters to predict protein-coding genes, transfer RNA (tRNA) genes, and ribosome RNA (rRNA) genes. The position of each coding gene was determined using BLAST searches against reference mitochondrial genes. Manual corrections of genes for start/stop codons were performed in SnapGene Viewer (https://www.snapgene.com/) by referencing the reference mitochondrial genome.

Sequence analysis

Circular maps of the mitochondrial genomes for five species within the Lottioidea superfamily were generated using the Proksee server [36]. The base composition of the mitochondrial genomes, as well as the amino acid content and the relative synonymous codon usage (RSCU) for each protein-coding gene, were calculated using MEGA 11 [37]. The formulas for calculating the AT and GC base skewness are AT-skew = (A - T)/(A + T) and GC-skew = (G - C)/(G + C) [38]. The ratio of nonsynonymous substitutions (Ka) to synonymous substitutions (Ks) was calculated using DnaSP 6 [39].

Phylogenetic analysis

In order to analyze the phylogenetic relationships of Patellogastropoda, we downloaded the complete mitochondrial genomes of 38 species from GenBank (https://www.ncbi.nlm.nih.gov/genbank/), including 36 existing species of Patellogastropoda and two species of Vetigastropoda. These two Vetigastropoda species, *Tegula lividomaculata* (KT207826) and *Bolma rugosa* (KT207824), were used as outgroups [40]. Additionally, five newly sequenced species of Lottioidea were included, making a total of 43 species used to construct a phylogenetic tree based on Patellogastropoda (Table S1).

The software DAMBE 7 [41] was used to identify the nucleotide sequences of PCGs for each sequence and concatenate them in the same order to form a dataset. The sequences were then aligned and trimmed using MEGA 11 [37]. The nucleotide substitution saturation was calculated using DAMBE 7 [41] to assess the suitability of these sequences for constructing a phylogenetic tree. In this study, two methods were used for phylogenetic analysis: Bayesian inference (BI) using MrBayes 3.2.7a [42] and maximum likelihood (ML) using IQ-tree 2.1.3 [43]. For the BI method, the file format was first converted using the software PAUP 4 [44]. Then, MrMTgui, along with PAUP 4 [44], Modeltest 3.7 [45], and MrModeltest 2.3 [46], were used to select the best-fit substitution model (GTR + I + G) according to the Akaike information criterion. The Markov chain Monte Carlo (MCMC) was run simultaneously with three heated chains and one cold chain for 2 million generations, with a sampling frequency of every 1000 generations and a burn-in rate of 25%. The convergence of independent runs was evaluated by the average standard deviation of split frequencies (< 0.01). We also constructed phylogenetic trees using PhyloBayes 3 [47]: using the CAT-GTR model, running two chains independently, each for 20,000 generations, sampling every 10 generations, and discarding the first 25% of iterations from each chain as burn-in. After the runs

were completed, we used the program tracecomp in PhyloBayes to check for convergence of parameters between the chains. The maximum potential scale reduction factor (PSRF) for all parameters was < 1.01, and the effective sample size (ESS) was > 200, indicating sufficient sampling. We used bpcomp to combine the posterior tree samples from the two chains, calculated a 50% majority rule consensus tree, and node support was expressed as posterior probability (PP); the topological differences between the chains were evaluated using the maxdiff value of bpcomp (with a threshold set at < 0.3), confirming the consistency of the results. For the ML method, ModelFinder [48] was used to select the best nucleotide substitution model (TVM + F + R9) and the best amino acid substitution model (Q.bird + F + R6). Bootstrap values were set to 1000 to reconstruct the consensus tree. Node support was expressed as bootstrap proportions in percentage (BP). Finally, the software Figtree 1.4.4 [49] was used to view and edit the phylogenetic tree.

Gene arrangement

In order to study the gene rearrangement characteristics of Patellogastropoda, we utilized the newly sequenced genomes of five Lottioidea species and retrieved the complete mitochondrial genomes of 36 Patellogastropoda species from GenBank. Using the *COI* gene as a starting point, the mitochondrial genes of these 41 species were linearly arranged and compared with the hypothetical ancestral gene order of Gastropoda [40]. To ensure the accuracy of gene arrangement, the Galaxy platform's MITOS2 tool [50] was utilized to re-annotate the mitochondrial genomes of some species.

Divergence time estimation

The divergence times for 41 species in Patellogastropoda were estimated using a nucleotide dataset of 13 protein-coding genes. The analysis used BEAST v1.10.4 [51], with a Bayesian tree topology as the framework. The molecular clock model used an uncorrelated relaxed clock with a lognormal distribution. The tree prior model used the Yule process and selected a random starting tree. Fossil calibration points were assigned normal prior distributions with a standard deviation of 2.0. Four calibration points were used (Table S2). The first calibration point was set at the divergence of *Bathyacmaea* and *Pectinodonta*, based on the oldest known fossil, *Pectinodonta palaeoxylodia*, which dates from the Rupelian to Chattian stage (23.0–33.9 MYA, million years ago; mean: 28.5) and was discovered in the USA (Washington) [52]. The second calibration point, set between *Cellana* and *Nacella* at the divergence of Nacellidae, was based on a fossil of *Cellana* from the Ypresian stage (47.8–56.0 MYA; mean: 51.9) in Mexico (Baja California Sur) [53]. The third calibration point was set at the divergence between Patellidae and the remaining families of Clade 1 (Lepetidae + Neolepetopsidae + Acmaeidae + Pectinodontidae + Nacellidae), based on the *Patella* fossil from the Ladinian to Carnian stages (227.3–241.5 MYA; mean: 234.4), which was discovered in New Zealand [54]. The fourth calibration point was the fossil record of Lottiidae (237.0–246.7 MYA; mean: 241.9) [55]. All data came from the fossil records in The Paleobiology Database (https://paleobiodb.org/#/). The Markov Chain Monte Carlo (MCMC) ran for four times, each for 20 million generations, with a sample every 1,000 generations. LogCombiner v1.10.4 [51] from the BEAST suite was used to combine the results of the four runs. Tracer v1.7.2 [56] was used to check the convergence of the chains, ensuring that the ESS for all parameters were greater than 200. Then, TreeAnnotator v1.10.4 [51] from the BEAST suite was used to discard the first 10% of the samples as burn in. Finally, FigTree v1.4.4 [49] was used to view the generated tree, displaying the 95% highest posterior density (95% HPD), node ages, and scale axes representing time points.

Results

Structural characteristics of five mitogenomes

The complete mitochondrial genome sequences of the five species within the superfamily Lottioidea had the following lengths: *Limalepeta lima* was 20,655 bp, *Lepeta kuragiensis* was 20,348 bp, *Lottia versicolor* was 20,213 bp, *Lottia instabilis* was 18,926 bp, and *Lottia tenuisculpta* was 25,738 bp (Fig. 1). The mitochondrial genomes of these five species encoded 38 genes (Table S3), including 13 protein-coding genes (PCGs), 23 transfer RNA genes (tRNAs), and 2 ribosomal RNA genes (rRNAs). Among them, *L. lima* and *L. kuragiensis* in the family Lepetidae exhibited a duplication of *trnL1*, whereas *L. versicolor*, *L. instabilis,* and *L. tenuisculpta* in the family Lottiidae showed a duplication of *trnM*.

Both *L. lima* and *L. kuragiensis* had the same distribution of gene coding on the heavy (H) strand and light (L) strand; each encoded 6 PCGs, 12 tRNAs, and 2 rRNAs on the H strand, and 7 PCGs and 11 tRNAs on the L strand. Similarly, *L. versicolor* and *L. tenuisculpta* also exhibited identical gene coding distributions, encoding 9 PCGs, 18 tRNAs, and 2 rRNAs on the H strand, and 4 PCGs and 5 tRNAs on the L strand. However, although the mitochondrial genome of *L. instabilis* had the same number and types of genes on the H and L strands as those of *L. lima* and *L. kuragiensis*, there were differences in the specific PCGs and tRNAs they encoded.

Among the mitochondrial genomes of the five species, the longest PCG was *nad5*, with a length of approximately 1740 bp, coding for about 580 amino acids. The

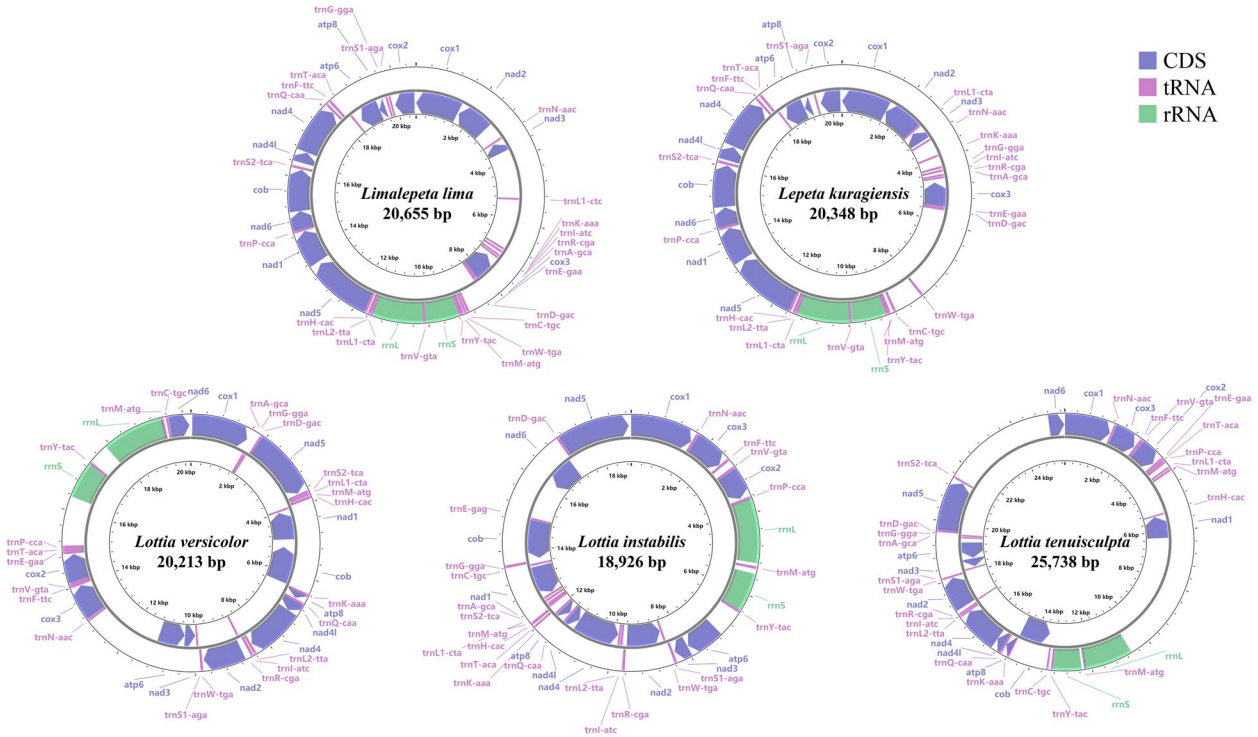

Fig. 1 Circular maps of mitochondrial genomes from five species

cox1, *nad2*, *cob*, and *nad4* genes all exceeded 1000 bp in length. In contrast, *atp8* was the shortest PCG, ranging from 160 to 252 bp. Additionally, most tRNAs had lengths between 66 and 69 bp, *12S rRNA* ranged from 868 to 930 bp, while *16S rRNA* exceeded 1300 bp in length.

Base content and skewness

The base composition of the mitochondrial genomes of the five species was as follows: A: 27.59% to 41.17%, T: 25.19% to 30.24%, G: 10.09% to 22.39%, C: 17.88% to 25.25% (Table S4). The AT content of the mitochondrial genomes (including PCGs, tRNAs, and rRNAs) in these species was higher than the GC content, showing an AT bias. According to the calculation formula for AT and GC skewness, if the content of A bases is higher than that of T bases and the content of G bases is higher than that of C bases, the calculation result is positive, and vice versa. The skewness values calculated for the five species showed that the range of AT skewness values in these genomes was from −0.01 to 0.23, while the range of GC skewness values was from −0.38 to 0.11. Specifically, except for the positive GC skewness value of the PCGs in *L. instabilis*, the AT and GC skewness values of the PCGs in these five species were negative. The GC skewness values of the tRNAs and the AT skewness values of the rRNAs in these five species were positive.

Amino acid content and codon usage

The amino acid content and the relative synonymous codon usage in the mitochondrial genomes of the five species were compared and analyzed (Fig. S1). The results showed that in Lepetidae, *L. lima* and *L. kuragiensis*, the two amino acids with the highest usage frequency were Lys and Thr, with contents exceeding 9.20%; whereas the two amino acids with the lowest usage frequency were Asp and Cys. In Lottiidae, *L. versicolor*, *L. instabilis*, and *L. tenuisculpta*, one of the two amino acids with the highest usage frequency was Leu1, and the other was Pro or Ser1, with contents exceeding 7.20%; while one of the two amino acids with the lowest usage frequency was Asp, and the other was Cys, His, or Arg.

Relative Synonymous Codon Usage (RSCU) refers to the relative probability among synonymous codons encoding a specific amino acid, which eliminates the influence of amino acid composition on codon usage. RSCU is used to measure the bias in codon usage: when RSCU = 1, it indicates no preference in codon usage; RSCU > 1 indicates a higher usage of the codon; conversely, RSCU < 1 indicates a lower usage of the codon. Comparing the relative synonymous codon usage rates of the five species, the results revealed that codon preferences exhibited different patterns among these species. Specifically, in *L. lima*, the codon with the highest usage frequency was CUA (Leu1), and the lowest was

GCG (Ala). In *L. kuragiensis*, the codon with the highest usage frequency was UUA (Leu2), and the lowest was ACG (Thr). In *L. versicolor*, the codon with the highest usage frequency was UCU (Ser2), and the lowest was UCG (Ser2). In *L. instabilis*, the codon with the highest usage frequency was AGA (Ser1), and the lowest was UCG (Ser2). In *L. tenuisculpta*, the codon with the highest usage frequency was UCU (Ser2), and the lowest was GCG (Ala). Overall, each of the five species had between 31 and 34 preferred codons (RSCU > 1).

By comparing the start and stop codons of PCGs in the five species, six start codons and two stop codons were identified (Table 2). The majority of PCGs in the five mitochondrial genomes initiated with ATG and ATT, accounting for 80%, with a few initiating with ATA, GTG, ATC, and TTG. All five species used TAA or TAG as stop codons.

Selective pressure analysis

The ratio of nonsynonymous substitutions (Ka) to synonymous substitutions (Ks), denoted as Ka/Ks, indicates whether selective pressure is acting on a protein-coding gene. A Ka/Ks ratio greater than 1 suggests positive selection; a ratio of 1 indicates neutral evolution; and a ratio less than 1 indicates purifying selection. In this study, selective pressure analysis was conducted on 13 PCGs for species from Lepetidae (*L. lima* and *L. kuragiensis*) and Lottiidae (*L. versicolor*, *L. instabilis*, and *L. tenuisculpta*), along with other species from the same families in the GenBank database. The results showed that the Ka/Ks ratios of the 13 PCGs in both Lepetidae and Lottiidae were less than 1 (Fig. 2), indicating that these genes were primarily subjected to purifying selection during evolution. In Lepetidae, the Ka/Ks value of *cox1* was the lowest (0.107), suggesting it experienced the strongest purifying selection pressure. In contrast, the Ka/Ks value of *atp8* was the highest (0.534), followed by *nad2* (0.367), *nad6* (0.240), and *nad4l* (0.201). These genes had higher Ka/Ks values, indicating that they underwent weaker purifying selection compared to other PCGs. Similarly, in Lottiidae, the Ka/Ks value of *cox1* was also the lowest (0.119), indicating strong purifying selection pressure, while the Ka/Ks value of *atp8* was the highest (0.720), followed by *nad6* (0.563), *nad2* (0.497), and *nad4l* (0.447). These genes with higher Ka/Ks values suggest they experienced relatively weaker purifying selection compared to other PCGs.

Table 2 Start and stop codons of PCGs in five species

gene	Start/Stop Codon				
	L. lima	*L. kuragiensis*	*L. versicolor*	*L. instabilis*	*L. tenuisculpta*
cox1	TTG/TAG	ATT/TAG	ATT/TAA	GTG/TAG	GTG/TAG
cox2	ATG/TAA	ATG/TAG	ATG/TAA	ATG/TAA	ATG/TAG
atp8	ATT/TAG	ATT/TAG	ATG/TAA	ATG/TAA	ATG/TAA
atp6	ATT/TAG	ATG/TAG	ATG/TAG	ATG/TAA	ATT/TAA
nad4	ATT/TAA	ATT/TAA	ATG/TAG	ATG/TAG	ATG/TAA
nad4l	ATG/TAA	ATA/TAA	ATT/TAA	ATG/TAA	ATG/TAA
cob	ATT/TAA	ATG/TAA	ATG/TAG	ATG/TAG	ATG/TAG
nad6	ATC/TAA	ATC/TAA	ATA/TAA	ATA/TAA	ATG/TAG
nad1	ATG/TAA	ATG/TAA	ATG/TAG	ATG/TAA	ATT/TAG
nad5	ATG/TAA	ATG/TAA	ATG/TAA	GTG/TAA	ATG/TAA
cox3	ATG/TAG	GTG/TAA	ATG/TAG	ATA/TAG	ATG/TAG
nad3	GTG/TAA	ATG/TAG	ATG/TAG	ATG/TAG	ATG/TAG
nad2	ATG/TAG	ATG/TAG	ATG/TAA	ATA/TAA	ATG/TAA

Phylogenetic analysis

The study conducted a phylogenetic analysis using both nucleotide and amino acid sequences of 13 PCGs from 41 species across seven families within two superfamilies (Lottioidea and Patelloidea) of the Patellogastropoda, along with two Vetigastropoda species as an outgroup. The phylogenetic tree of Patellogastropoda was constructed using both Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The ML tree and BI tree constructed based on the nucleotide sequence (PCGnt) presented an identical topology, and most nodes showed high support (Fig. 3). The ML tree and BI tree based on the amino acid sequence (PCGaa) displayed a slightly different topology (Fig. 4).

The analysis revealed that the Patellogastropoda was divided into two clades, with Lottioidea being identified as non-monophyletic. In the phylogenetic tree based on the PCGnt sequence, Clade 1 included the lineage formed by Lepetidae, Neolepetopsidae, and Acmaeidae, which was found to be sister to Pectinodontidae. This group then clustered with Nacellidae, followed by Patellidae. Clade 2 consisted of Lottiidae. The branching pattern within Patellogastropoda was thus reconstructed as ((((Lepetidae + Neolepetopsidae + Acmaeidae) + Pectinodontidae) + Nacellidae) + Patellidae) + Lottiidae. The topology of most families in the ML tree based on the PCGaa sequence was consistent with that of the PCGnt sequence, but differences occurred in the relationships among Lepetidae, Neolepetopsidae, Pectinodontidae, and Acmaeidae. The ML tree based on the PCGaa sequence showed that the clade formed by Lepetidae and Neolepetopsidae was most closely related to Pectinodontidae, and then formed a sister group with the clade formed by Neolepetopsidae and Acmaeidae. The BI tree based on the PCGaa sequence differed from the ML tree in the branching of Lepetidae and Neolepetopsidae and

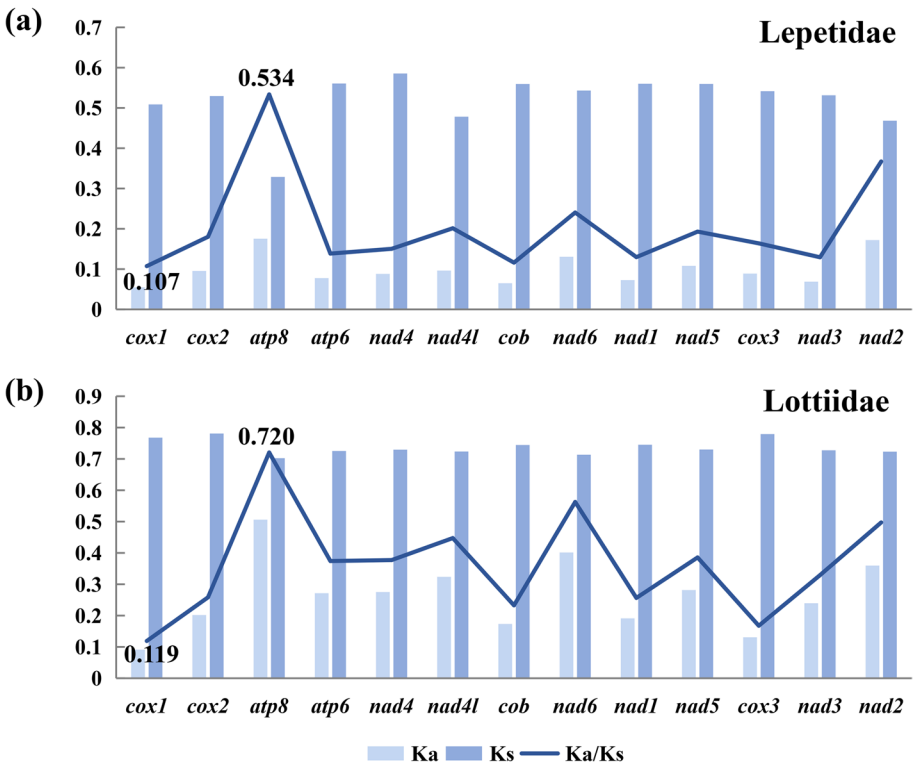

Fig. 2 Selective pressure analysis of 13 PCGs. **a** Lepetidae family, **b** Lottiidae family. The list of species used for the analysis is provided in Table S1. The light blue and medium blue bars represent the number of nonsynonymous substitutions per nonsynonymous site (Ka) and the number of synonymous substitutions per synonymous site (Ks), respectively. The dark blue line represents the Ka/Ks ratio

the positional change of Nacellidae. The phylogenetic results based on nucleotide sequences had higher support values than those based on amino acid sequences.

Within Lepetidae, the newly sequenced species from the genera *Limalepeta* and *Lepeta*, specifically *L. lima* and *L. kuragiensis*, were found to be closely related with strong support (PCGnt: PP = 1, BP = 100%; PCGaa: PP = 1, BP = 94%). Next in relation were species of the genera *Sagamilepeta* and *Iothia*, with *Paralepetopsis* from Neolepetopsidae inserted among them, suggesting that Neolepetopsidae may be a polyphyletic group. In Lottiidae, the other three newly sequenced species (*L. versicolor*, *L. instabilis*, and *L. tenuisculpta*) appeared within the *Lottia* genus of the phylogenetic tree. A species from the *Discurria* genus and another from the *Scurria* genus were inserted into *Lottia*, and when taken into account, this rendered *Lottia* as a paraphyletic group. Additionally, a clade formed by species of *Lottia*, *Discurria*, and *Scurria* was sister to species of *Nipponacmea*, followed by the monophyletic genus *Patelloida*, with high branch support values (PCGnt: PP = 1, BP = 100%; PCGaa: PP = 1, BP = 100%).

Gene arrangement

By comparing the arrangement of mitochondrial PCGs in Patellogastropoda, we found that the PCG arrangement in Lepetidae and Neolepetopsidae was more similar to that of the hypothetical ancestral Gastropoda, with only 1–2 protein-coding genes having changed positions (Fig. 5). The PCG arrangement in Acmaeidae, Pectinodontidae, and Nacellidae remained consistent with the hypothetical ancestral gene order. In Patellidae, compared to the gastropod ancestral gene order, the *cox2* gene moved from downstream of *cox1* to downstream of *cob*; the gene segment *nad6-nad1-cox3* moved from downstream of *cob* to downstream of *cox1* and was inverted; and *nad3* and *nad2* exchanged positions. In contrast, Lottiidae exhibited a large number of gene rearrangements.

Furthermore, a more detailed comparison of the mitochondrial gene arrangement revealed that, compared to the hypothetical ancestral gene order of Gastropoda, Lepetidae and Neolepetopsidae showed more pronounced differences in the arrangement of tRNAs (Fig. 6). Lepetidae retained two gene segments that were consistent with the hypothetical ancestral Gastropoda:

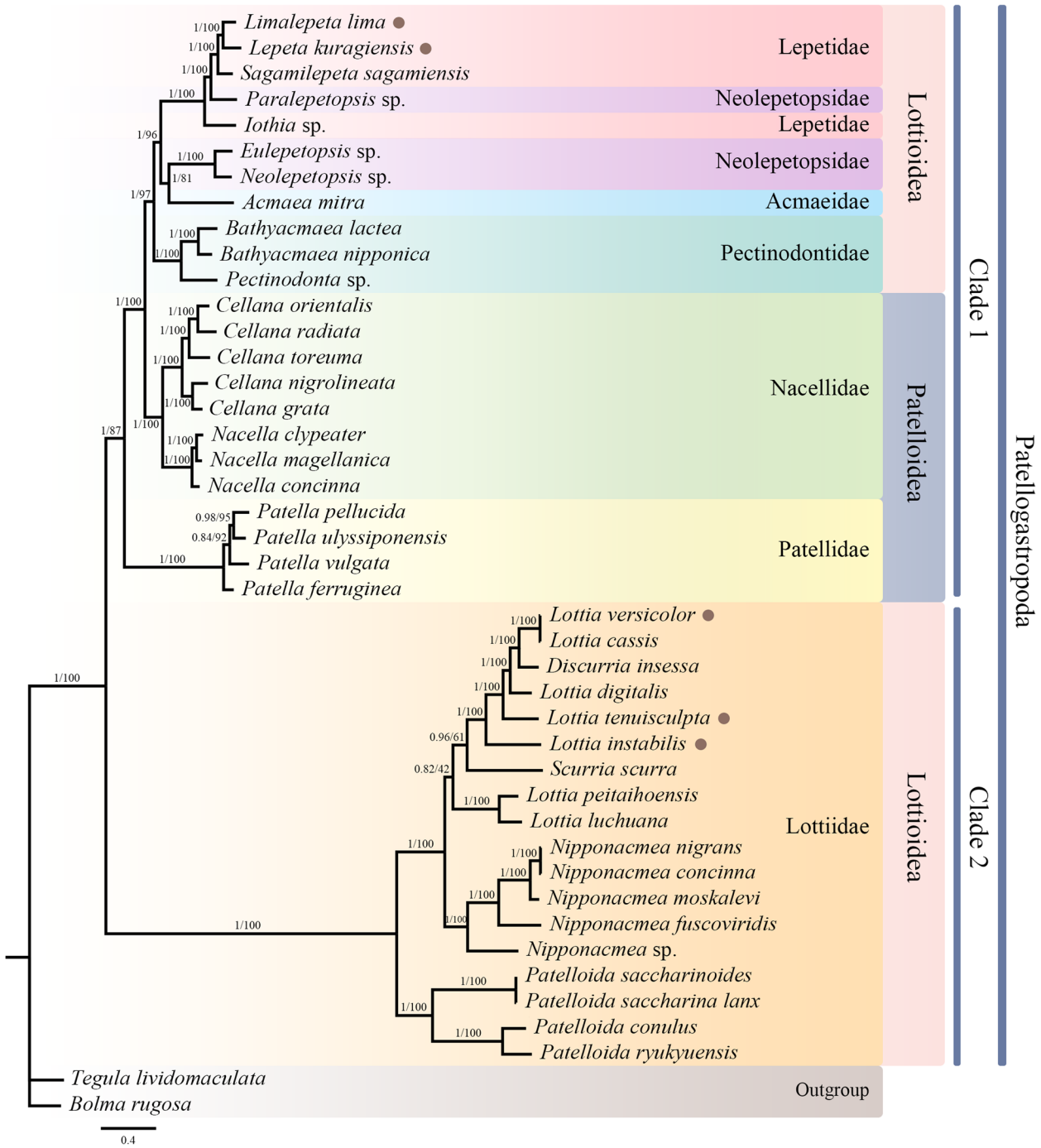

Fig. 3 Phylogenetic tree based on the nucleotide sequences of 13 PCGs from Patellogastropoda. The numbers located before each node represent the support values of the BI (posterior probabilities)/ML (bootstrap proportions in percentage) tree. The newly determined mtDNA sequences in this study are marked with brown circular patterns

trnS-cob-nad6-trnP-nad1 and *trnL-trnL-rrnL-trnV-rrnS*. In Lottiidae, we identified 12 types of gene rearrangement patterns, indicating significant gene rearrangements. Among the seven *Lottia* species, five species shared the same gene segment (*trnK-atp8-trnQ-nad4l-nad4-trnL-trnI-trnR-nad2-trnW-trnS-nad3-atp6*); similarly, in all *Nipponacmea* species, a common gene segment was identified (*cox1-trnL-cox3-trnM-nad4*).

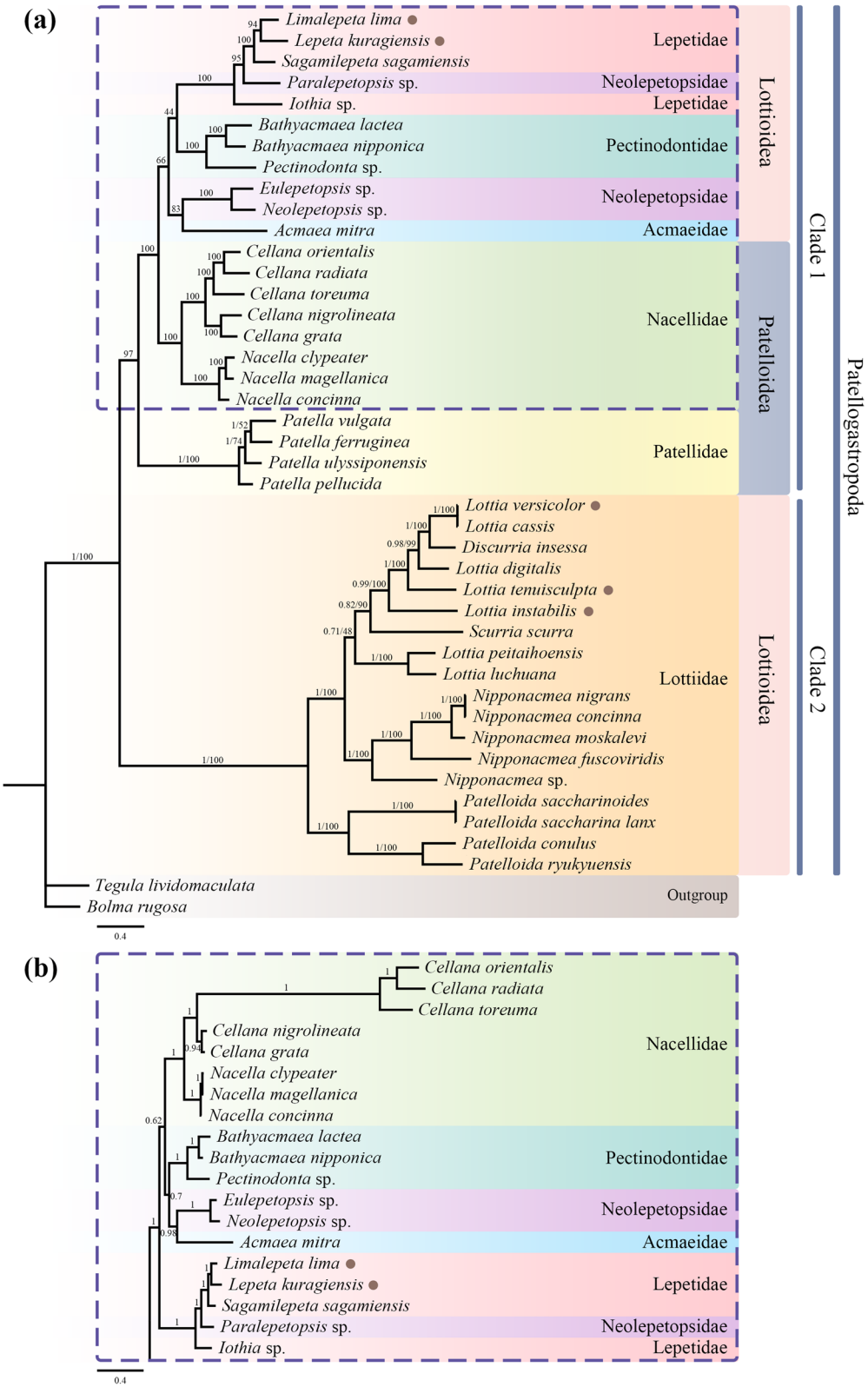

Fig. 4 Phylogenetic tree based on the amino acid sequences of 13 PCGs from Patellogastropoda. The numbers located before each node represent the support values of the BI (posterior probabilities)/ML (bootstrap proportions in percentage) tree. The newly determined mtDNA sequences in this study are marked with brown circular patterns. **a** shows the combined topology of the ML tree and BI tree, with differences between ML and BI results highlighted by purple dashed boxes. **b** displays the different results of the BI tree

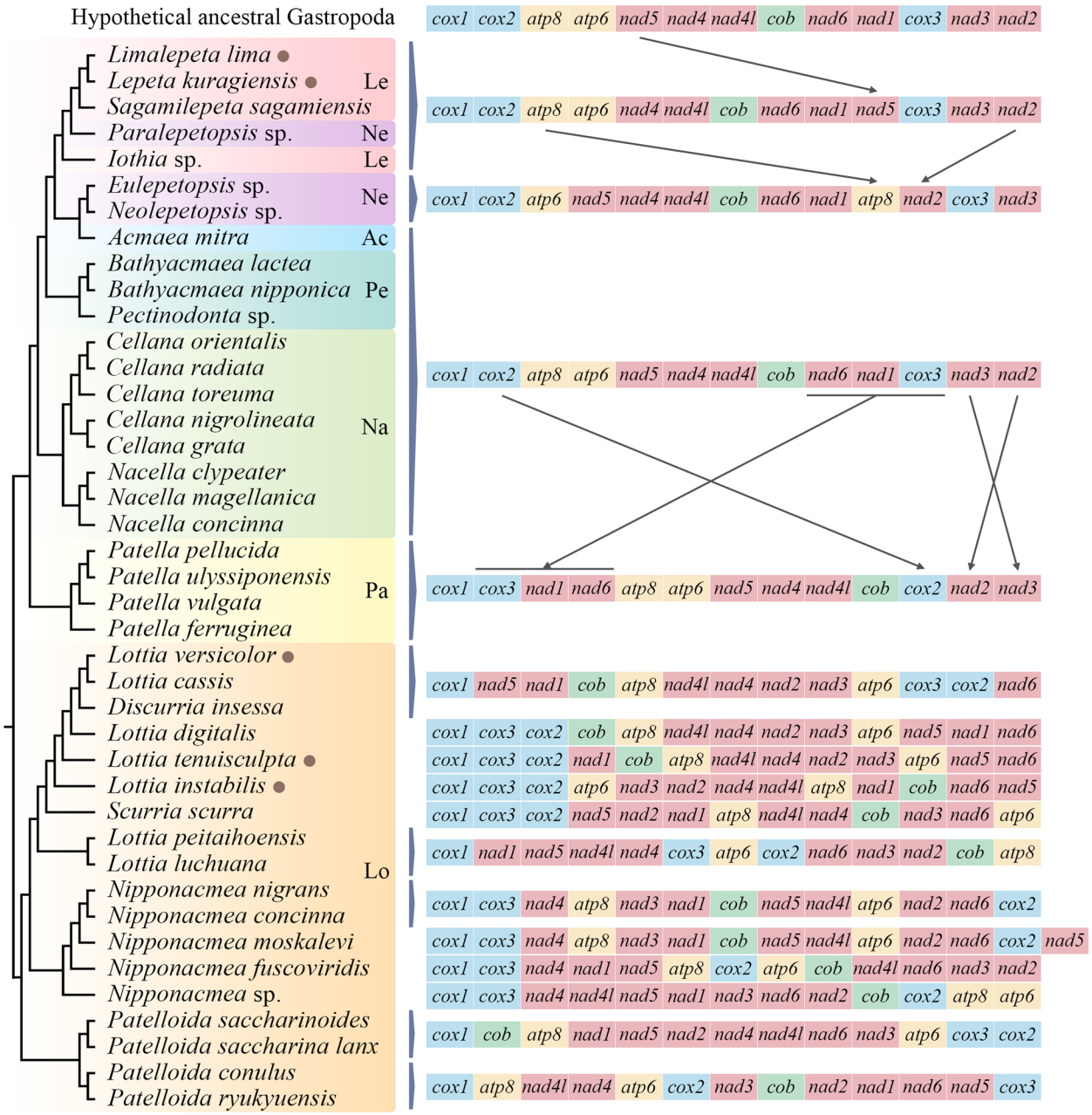

Fig. 5 Linear arrangement of mitochondrial PCGs in Patellogastropoda. The abbreviations for families in this figure are as follows: Le: Lepetidae; Ne: Neolepetopsidae; Ac: Acmaeidae; Pe: Pectinodontidae; Na: Nacellidae; Pa: Patellidae; Lo: Lottiidae. The newly determined mtDNA sequences in this study are marked with brown circular patterns

Divergence time estimation

Divergence time estimation of Patellogastropoda was conducted using 13 PCGs from the mitochondrial genome (Fig. 7). The Patellogastropoda lineage was inferred to have originated in the Paleozoic Permian period, approximately 278.02 MYA (95% HPD: 250.50–320.06 MYA). Subsequently, two major clades within Patellogastropoda diverged in the Mesozoic Triassic period, around 234.71 MYA (95% HPD: 230.819–238.657 MYA) and 241.80 MYA (95% HPD: 237.872–245.697 MYA), respectively. The majority of genus-level divergences occurred during the Mesozoic Cretaceous and Cenozoic periods. The newly sequenced species *L. lima* and *L. kuragiensis* diverged from *Sagamilepeta sagamiensis* in the Cenozoic Neogene, approximately 9.26 MYA (95% HPD: 6.37–12.32 MYA). The divergence between

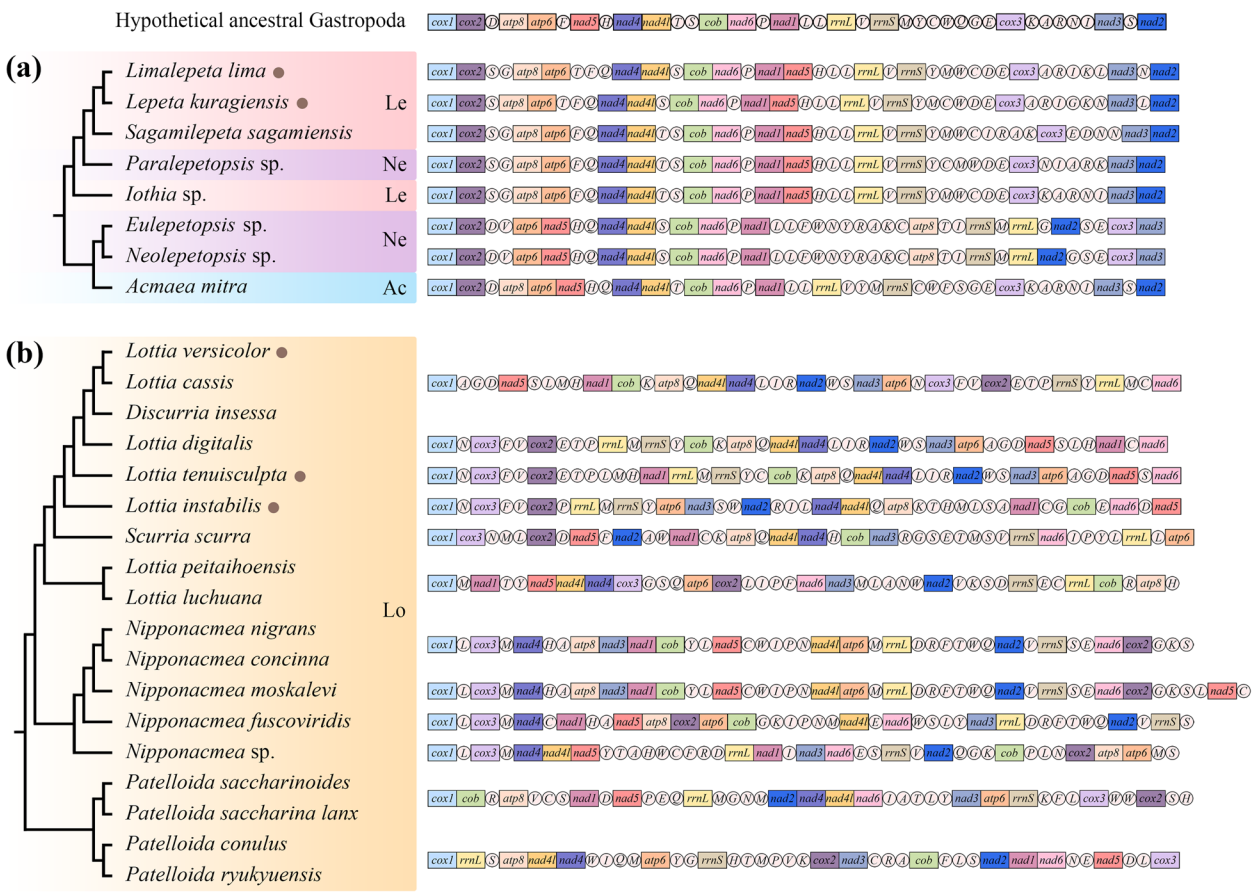

Fig. 6 Linear arrangement of mitochondrial genes in Patellogastropoda. tRNA genes are marked with amino acid abbreviations. The abbreviations for families in this figure are as follows: Le: Lepetidae; Ne: Neolepetopsidae; Ac: Acmaeidae; Lo: Lottiidae. The newly determined mtDNA sequences from this study are highlighted with brown circular patterns

Lottia and *Nipponacmea* took place in the Mesozoic Cretaceous, around 123.81 MYA (95% HPD: 73.495–172.517 MYA), followed by further diversification of *Lottia*. The three *Lottia* species newly sequenced in this study diverged at 63.37 MYA, 45.40 MYA, and 0.24 MYA, respectively.

Discussion

The structural characteristics of the mitochondrial genomes of the five Lottioidea species were analyzed, revealing that they encode 23 tRNA genes, with duplications of *trnL1* in the two Lepetidae species and *trnM* in the three Lottiidae species. The presence of 23 or 24 tRNA genes has also been reported in other Patellogastropoda species [57–61]. In the mitochondrial genomes of these five species, the longest PCG is *nad5*, while *atp8* is the shortest PCG; the content of AT bases is higher than that of GC bases, which are characteristics similar to other Patellogastropoda species [57–62]. The AT and GC skewness of species within the same genus *Lottia* exhibit similar characteristics [63]. A comparison of amino acid content and codon usage among the five species shows that species within the same family have similar amino acid content, but there are differences in codon usage bias among different species. The majority of PCGs in the five mitochondrial genomes use AT(N) as the start codon, and all PCGs employ TA(N) as the stop codon, which is largely consistent with other Patellogastropoda species [57–59, 61–66]. Additionally, Miao et al. [59] and Feng et al. [61] have conducted selection pressure analysis on Lottiidae, and their results are consistent with this study, with PCGs having Ka/Ks values less than 1, indicating purifying selection.

Putri et al. [60] conducted a phylogenetic analysis of Patellogastropoda using 13 protein-coding genes, and their results revealed a robust sister-group relationship between Acmaeidae and Pectinodontidae, followed by Nacellidae, with Patellidae at the base of these families. Our findings are consistent with theirs. However, it is noteworthy that we included species from the Lepetidae and Neolepetopsidae families in our study, and found that Neolepetopsidae may be a polyphyletic group. Chen et al.

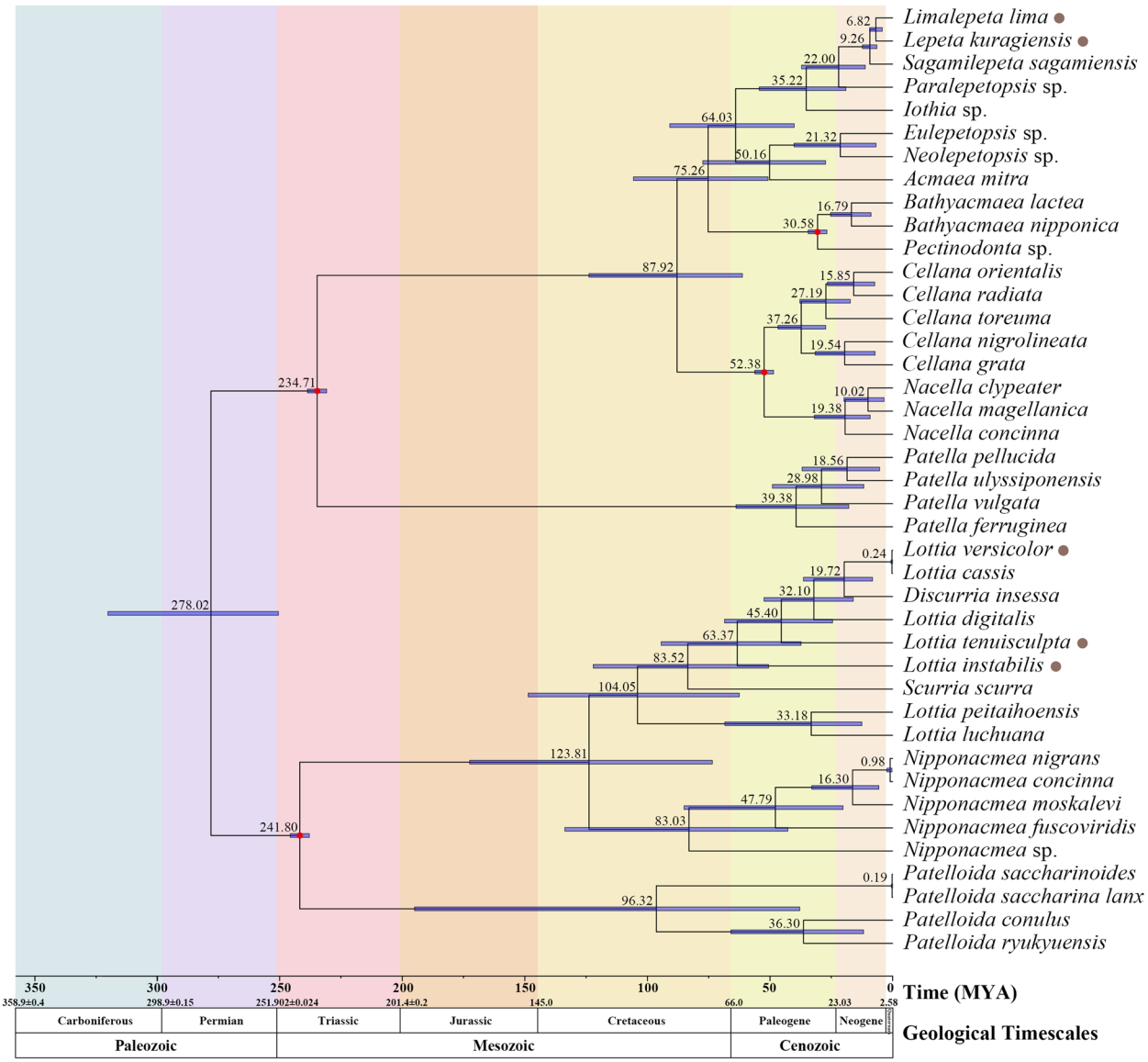

Fig. 7 Estimation of divergence times based on 13 PCGs of Patellogastropoda. Calibration points are marked with red dots. The newly determined mtDNA sequences in this study are marked with brown circular patterns

[67] constructed the phylogenetic relationships of Patellogastropoda using the mitochondrial *COI* gene, which also revealed that *Paralepetopsis* from Neolepetopsidae is nested within Lepetidae. However, in terms of the morphological features of the radula, the *Paralepetopsis polita* they studied was similar to other *Paralepetopsis* species within Neolepetopsidae. Therefore, as Chen et al. [67] suggested, future research should utilize sequence data from the type species of *Paralepetopsis*, *Paralepetopsis floridensis*, to refine the phylogenetic reconstruction of Neolepetopsidae. Our study showed that within Lepetidae, *Limalepeta* and *Lepeta* are sister groups, which is in agreement with the findings of Waren et al. [68]. Harasewych and McArthur [18] used partial *18S rDNA* sequences in an early study and found the closest relationship to be between Acmaeidae and Neolepetopsidae, followed by Lepetidae. In contrast, Chen et al. [67, 69] recently used the *COI* gene to restore the sister relationship between Lepetidae and Neolepetopsidae. Our phylogenetic tree results based on nucleotide sequences only show that Acmaeidae, Lepetidae, and Neolepetopsidae are grouped together. Our ML tree based on amino acid sequences shows that the four families Neolepetopsidae, Lepetidae, Pectinodontidae, and Acmaeidae form

a monophyletic group. This topology is consistent with a recent study based on nucleotide sequences of 13 PCGs [61]. Therefore, more species with complete mitochondrial genomes are needed for further evaluation in the future.

Lottiidae is the most diverse within Patellogastropoda, and its monophyly has been confirmed in earlier studies [14, 19, 20]. Feng et al. [57] previously constructed a phylogenetic tree for Gastropoda species based on 13 PCGs of the mitochondrial genome. The results indicated that Lottiidae forms an independent branch within Patellogastropoda. Subsequently, the mitochondrial genome database of Lottiidae has gradually become more comprehensive. In this study, we included 18 Lottiidae species and also concluded that Lottiidae is a monophyletic group and is the sister group to the other families within Patellogastropoda, which aligns with the findings of Saenz-Agudelo [58], Miao et al. [59], and Feng et al. [61]. Additionally, at the genus level within Lottiidae, our phylogenetic relationships revealed that *Patelloida* is the sister group to the clade formed by *Lottia* and *Nipponacmea*, consistent with recent research results [59, 60, 63]. The phylogenetic tree constructed in this study showed that a species of *Discurria* and a species of *Scurria* are nested within *Lottia*, thus indicating that *Lottia* is a paraphyletic group. Similar phenomena have also been observed in the phylogenetic trees constructed by other researchers [14, 60, 63].

In this study, the PCGs arrangement order in Lepetidae and Neolepetopsidae altered by 1–2 protein-coding genes compared to the hypothetical ancestral Gastropoda, and both families preserved a segment of the same gene fragment (*trnS-cob-nad6-trnP-nad1*). Meanwhile, Acmaeidae, Pectinodontidae, and Nacellidae shared the same PCGs arrangement order, which was entirely consistent with that of the hypothetical ancestral Gastropoda, exhibiting high conservation, as reported by Putri et al. [60] and Feng et al. [61]. This study revealed that Lottiidae exhibits 12 different gene rearrangements, and the high rate of gene rearrangement in Lottiidae has been reported multiple times [57, 59–61, 63, 64]. A high rate of mitochondrial gene rearrangement is associated with a high rate of sequence evolution [70, 71].

This study inferred that the Patellogastropoda lineage likely originated during the Paleozoic Permian era, approximately 278.02 MYA, which falls within the 95% HPD range reported by Nakano and Ozawa [14], Xu et al. [63], and Feng et al. [61]. Subsequently, during the Mesozoic Triassic, the two major clades within Patellogastropoda diverged. The Permian–Triassic boundary (PTB) marks the largest mass extinction event in the Phanerozoic era, a pivotal point in the evolution of marine animals signifying the end of the Paleozoic fauna's dominance and the rise of the modern marine fauna [72, 73]. The Permian–Triassic extinction destroyed the existing ecosystems, making space for the establishment of new ecosystems. The extinction event also exhibited a clear biological selectivity, which was related to the organisms' tolerance to environmental factors [74]. Marine animals with limited physiological buffering capacity against environmental changes were more likely to be affected first [75]. The environment around the PTB and the early Triassic recovery period was characterized by water temperature fluctuations and marine hypoxia [76, 77]. Mollusks such as gastropods, which are tolerant of both high temperatures and hypoxia, were less affected and became a dominant biological type in the early Triassic marine ecosystem [78]. As mentioned by Vermeij [79], the limpet shape is not particularly effective against predators, suggesting that it might have arisen during times when species were secluded in refugia. The Permian–Triassic extinction event likely created such refugia, where species with the limpet shape could have thrived due to reduced predation pressure. The shell structure of limpets is compact, possibly providing a larger respiratory surface area, making them more suitable for survival in low-oxygen deep-sea environments.

The diversification of most genera within Patellogastropoda occurred during the Mesozoic Cretaceous and the Cenozoic era. Neubauer and Harzhauser [80] found that the increased rate of species formation around the Cenomanian (~85 Myr ago) was related to rising global sea levels. They suggest that the peak of gastropod species diversification coincided with the late phase of the Cretaceous terrestrial revolution, when angiosperms underwent radiative expansion; before diatoms entered freshwater ecosystems, the radiation and expansion of angiosperms may have provided an important basis for the diversification of freshwater gastropods. The global paleoenvironmental history of the Cenozoic era primarily experienced two stages: the greenhouse warm period from the Paleocene to the Eocene, which was a continuation of the Cretaceous greenhouse world and exhibited the warmest global climate of the Cenozoic; followed by the icehouse period from the Oligocene to the present, characterized by a general cooling of the global climate, with brief warm periods interspersed [81]. These climatic changes may have triggered a differentiation event within Patellogastropoda.

Conclusions

In this study, we obtained the complete mitochondrial genome sequences of five Patellogastropoda species, increasing the mitochondrial genome database for the Lepetidae and Lottiidae species. The mitochondrial genomes of these five species had a total length

ranging from 18 to 26 kbp, consistently containing 38 genes, including 13 PCGs, 23 tRNAs, and 2 rRNAs. They exhibited a higher AT base content, and the PCGs were under purifying selection. The Patellogastropoda was divided into two clades: Clade 1 included part of the Lottioidea superfamily and the Patelloidea superfamily, whereas the Lottiidae family within the Lottioidea superfamily formed a separate clade (Clade 2). Apart from Neolepetopsidae, all families within Patellogastropoda were monophyletic; at the genus level, we found *Lottia* to be a paraphyletic group. The mitochondrial genes of the Patellogastropoda exhibited extensive gene rearrangements, particularly those in Lottiidae, which were the most complex. The Patellogastropoda lineage was estimated to have originated in the Paleozoic Permian era, with active differentiation occurring in the Mesozoic Cretaceous and the Cenozoic era.

Abbreviations

PCGs	Protein-coding genes
tRNA	Transfer RNA
rRNA	Ribosomal RNA
RSCU	Relative synonymous codon usage
Ka	Nonsynonymous substitutions
Ks	Synonymous substitutions
BI	Bayesian inference
ML	Maximum likelihood
MCMC	Markov chain Monte Carlo
ESS	Effective sample sizes
PP	Posterior probability
BP	Bootstrap proportions in percentage
95% HPD	95% Highest posterior density
H	Heavy
L	Light
PTB	Permian-Triassic boundary
mtDNA	Mitochondrial genome

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11681-z.

Supplementary Material 1.

Acknowledgements
We thank the Editor and the reviewers for their insightful comments and suggestions on the manuscript.

Authors' contributions
LWT conducted the experiments, analyzed the data, visualized the results, and wrote the original draft. AVC conducted the survey and provided the samples. LJJ revised the manuscript and was responsible for funding acquisition. YYY and DXL designed the experiments, revised the manuscript, and were also involved in funding acquisition. All authors read and approved the final manuscript.

Funding
This study was financially supported by the NSFC Projects of International Cooperation and Exchanges (42020104009), the General scientific research projects of the Zhejiang Provincial Education Department (Y202353900), 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 datasets used and analysed during the current study are available from the corresponding author on reasonable request. The mitogenome sequences used in this project were available in GenBank with accession numbers: OR574472 to OR574476.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 10 November 2024 Accepted: 7 May 2025
Published online: 01 July 2025

References

1. Ferrari SM. Patellogastropoda and Vetigastropoda (Mollusca, Gastropoda) from the marine Jurassic of Patagonia, Argentina. Hist Biol. 2014;26(5):563–81.
2. Kristof A, de Oliveira AL, Kolbin KG, Wanninger A. A putative species complex in the Sea of Japan revealed by DNA sequence data: a study on Lottia cf. kogamogai (Gastropoda: Patellogastropoda). J Zool Syst Evol Res. 2016;54(3):177–81.
3. Castejón D, García L, Nogueira N, Andrade CA. Improving efficiency of methods for hatchery production of the limpet *Patella candei* (Patellogastropoda: Patellidae). J World Aquacult Soc. 2023;54(4):945–64.
4. Fuchigami T, Sasaki T. The shell structure of the recent Patellogastropoda (Mollusca: Gastropoda). Paleontol Res. 2005;9(2):143–68.
5. González-Wevar CA, Hüne M, Segovia NI, Nakano T, Spencer HG, Chown SL, et al. Following the Antarctic Circumpolar Current: patterns and processes in the biogeography of the limpet *Nacella* (Mollusca: Patellogastropoda) across the Southern Ocean. J Biogeogr. 2017;44(4):861–74.
6. Hodgson AN, Eckelbarger KJ. Ultrastructure of the ovary and oogenesis in six species of patellid limpets (Gastropoda: Patellogastropoda) from South Africa. Invertebr Biol. 2000;119(3):265–77.
7. Guralnick R, Smith K. Historical and biomechanical analysis of integration and dissociation in molluscan feeding, with special emphasis on the true limpets (Patellogastropoda: Gastropoda). J Morphol. 1999;241(2):175–95.
8. Wanninger A, Ruthensteiner B, Lobenwein S, Salvenmoser W, Dictus WJ, Haszprunar G. Development of the musculature in the limpet *Patella* (Mollusca, Patellogastropoda). Dev Genes Evol. 1999;209:226–38.
9. Wanninger A, Ruthensteiner B, Haszprunar G. Torsion in *Patella caerulea* (Mollusca, Patellogastropoda): ontogenetic process, timing, and mechanisms. Invertebr Biol. 2000;119(2):177–87.
10. Kristof A, de Oliveira AL, Kolbin KG, Wanninger A. Neuromuscular development in Patellogastropoda (Mollusca: Gastropoda) and its importance for reconstructing ancestral gastropod bodyplan features. J Zool Syst Evol Res. 2016;54(1):22–39.
11. Ridgway S, Reid D, Taylor J, Branch G, Hodgson A. A cladistic phylogeny of the family Patellidae (Mollusca: Gastropoda). Philos Trans R Soc Lond B Biol Sci. 1998;353(1375):1645–71.
12. Mauro A, Arculeo M, Parrinello N. Morphological and molecular tools in identifying the Mediterranean limpets *Patella caerulea*, *Patella aspera* and *Patella rustica*. J Exp Mar Biol Ecol. 2003;295(2):131–43.
13. 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(2):237–51.
14. Nakano T, Ozawa T. Worldwide phylogeography of limpets of the order Patellogastropoda: molecular, morphological and palaeontological evidence. J Molluscan Stud. 2007;73(1):79–99.

15. Haszprunar G. On the origin and evolution of major gastropod groups, with special reference to the Streptoneura. J Molluscan Stud. 1988;54(4):367–441.
16. Ponder WF, Lindberg DR. Towards a phylogeny of gastropod molluscs: an analysis using morphological characters. Zool J Linn Soc. 1997;119(2):83–265.
17. Powell AWB. New Zealand Mollusca: marine, land, and freshwater shells. 1979.
18. Harasewych MG, McArthur A. A molecular phylogeny of the Patellogastropoda (Mollusca: Gastropoda). Mar Biol. 2000;137:183–94.
19. Nakano T, Ozawa T. Phylogeny and historical biogeography of limpets of the order Patellogastropoda based on mitochondrial DNA sequences. J Molluscan Stud. 2004;70(1):31–41.
20. Yoon SH, Kim W. 18S ribosomal DNA sequences provide insight into the phylogeny of patellogastropod limpets (Mollusca: Gastropoda). Mol Cells. 2007;23(1):64–71.
21. Nakano T, Sasaki T. Recent advances in molecular phylogeny, systematics and evolution of patellogastropod limpets. J Molluscan Stud. 2011;77(3):203–17.
22. Bouchet P, Frýda J, Hausdorf B, Ponder W, Valdés A, Warén A. Classification and nomenclator of gastropod families. 2005.
23. Bouchet P, Rocroi J-P, Hausdorf B, Kaim A, Kano Y, Nützel A, et al. Revised classification, nomenclator and typification of gastropod and monoplacophoran families. Malacologia. 2017;61(1–2):1–526.
24. Cunha TJ, Giribet G. A congruent topology for deep gastropod relationships. Proc R Soc B. 2019;286(1898):20182776.
25. Sharina SN, Chernyshev AV. The taxonomic position of brooding limpets of the genera Erginus and Rhodopetala (Patellogastropoda). Zool Anz. 2022;299:200–6.
26. Cunha RL, Grande C, Zardoya R. Neogastropod phylogenetic relationships based on entire mitochondrial genomes. BMC Evol Biol. 2009;9:1–16.
27. Feng J, Xia L, Yan C, Miao J, Ye Y, Li J, et al. Characterization of four mitochondrial genomes of family Neritidae (Gastropoda: Neritimorpha) and insight into its phylogenetic relationships. Sci Rep. 2021;11(1):11748.
28. Grande C, Templado J, Lucas Cervera J, Zardoya R. The complete mitochondrial genome of the nudibranch *Roboastra europaea* (Mollusca: Gastropoda) supports the monophyly of opisthobranchs. Mol Biol Evol. 2002;19(10):1672–85.
29. Uribe JE, Zardoya R, Puillandre N. Phylogenetic relationships of the conoidean snails (Gastropoda: Caenogastropoda) based on mitochondrial genomes. Mol Phylogenet Evol. 2018;127:898–906.
30. Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 1997;25(22):4692–3.
31. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20.
32. Meng G, Li Y, Yang C, Liu S. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. Nucleic Acids Res. 2019;47(11):e63–e63.
33. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403–10.
34. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, et al. Versatile and open software for comparing large genomes. Genome Biol. 2004;5:1–9.
35. Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, et al. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 2013;69(2):313–9.
36. Grant JR, Enns E, Marinier E, Mandal A, Herman EK, Chen C-y, et al. Proksee: in-depth characterization and visualization of bacterial genomes. Nucleic Acids Res. 2023;51(W1):W484–92.
37. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022–7.
38. 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(2):277–98.
39. Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, et al. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol. 2017;34(12):3299–302.
40. Uribe JE, Kano Y, Templado J, Zardoya R. Mitogenomics of Vetigastropoda: insights into the evolution of pallial symmetry. Zool Scr. 2016;45(2):145–59.
41. Xia X. DAMBE7: New and improved tools for data analysis in molecular biology and evolution. Mol Biol Evol. 2018;35(6):1550–2.
42. Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012;61(3):539–42.
43. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2020;37(5):1530–4.
44. Swofford DL. PAUP*. phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland: Sinauer Associates; 1998.
45. Posada D. Modeltest: a tool to select the best-fit model of nucleotide substitution. Version 3.7. 2005.
46. Nylander J. MrModeltest 2.3. Program distributed by the author. In: Evolutionary biology centre, Uppsala University. 2004.
47. Lartillot N, Lepage T, Blanquart S. PhyloBayes 3: a Bayesian software package for phylogenetic reconstruction and molecular dating. Bioinformatics. 2009;25(17):2286–8.
48. Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Meth. 2017;14(6):587–9.
49. Rambaut A, Drummond AJ. FigTree, version 1.4.4. 2018.
50. Jalili V, Afgan E, Gu Q, Clements D, Blankenberg D, Goecks J, et al. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2020 update. Nucleic Acids Res. 2020;48(W1):W395–402.
51. Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol. 2018;4(1):vey016.
52. Squires RL, Goedert JL. A new species of the volutid gastropod *Fulgoraria* (*Musashia*) from the Oligocene of Washington. Veliger. 1994;37:400–9.
53. Squires RL, Demetrion RA. Paleontology of the Eocene Batique Formation, Baja California Sur, Mexico. Contrib Sci (Los Angel). 1992;434:1–55.
54. Trechmann CT. The Trias of New Zealand. Quarterly journal of the Geological Society. 1917;73(1–4):165–246.
55. De Toni A. Illustration of the Triassic fauna of Valpedena (Cadore). Padova: Soc. Coop. Tip; 1913.
56. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst Biol. 2018;67(5):901–4.
57. Feng J, Guo Y, Yan C, Ye Y, Li J, Guo B, et al. 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):19277.
58. Saenz-Agudelo P. The complete mitochondrial genome of the true limpet *Scurria scurra* (Lottiidae). Mitochondrial DNA Part B. 2022;7(4):642–3.
59. Miao J, Li J, Ye Y, Guo B, Xu K. Complete mitochondrial genome sequencing of *Lottia luchuana* and phylogenetic analysis of the family Lottiidae. Acta Hydrobiol Sin. 2023;47(8):1248–60.
60. Putri ET, Lee D, Kwak H, Kim Y, Nakano T, Park J-K. Complete mitochondrial genomes of the "Acmaeidae" limpets provide new insights into the internal phylogeny of the Patellogastropoda (Mollusca: Gastropoda). Front Mar Sci. 2023;10: 1134991.
61. Feng J, Miao J, Li J, Ye Y. Exploring the mitogenomic of Lottiidae (Patellogastropoda): phylogenetics, gene rearrangement and evolutionary divergence time estimations. BMC Genomics. 2024;25(1):1055.
62. Gaitán-Espitia JD, González-Wevar CA, Poulin E, Cardenas L. Antarctic and sub-Antarctic Nacella limpets reveal novel evolutionary characteristics of mitochondrial genomes in Patellogastropoda. Mol Phylogenet Evol. 2019;131:1–7.
63. Xu T, Qi L, Kong L, Li Q. Mitogenomics reveals phylogenetic relationships of Patellogastropoda (Mollusca, Gastropoda) and dynamic gene rearrangements. Zool Scr. 2022;51(2):147–60.
64. Feng J, Miao J, Ye Y, Li J, Xu K, Guo B, et al. Insights into the Deep Phylogeny and Novel Divergence Time Estimation of Patellogastropoda from Complete Mitogenomes. Genes. 2022;13(7): 1273.
65. 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 Part B. 2021;6(7):2090–1.

66. Sun J, Liu Y, Xu T, Zhang Y, Chen C, Qiu J-W, et al. The mitochondrial genome of the deep-sea limpet Bathyacmaea nipponica (Patellogastropoda: Pectinodontidae). Mitochondrial DNA Part B. 2019;4(2):3175–6.
67. Chen C, Zhong Z, Qiu J-W, Sun J. A new Paralepetopsis limpet from a South China Sea seep hints at a paraphyletic Neolepetopsidae. Zool Stud. 2023;62:62.
68. Ware´n A, Nakano T, Sellanes J. A new species of Iothia (Gastropoda: Lepetidae) from Chilean methane seeps, with comments on the accompanying gastropod fauna. Nautilus. 2011;125(1):1–14.
69. Chen C, Zhou Y, Watanabe HK, Zhang R, Wang C. Neolepetopsid true limpets (Gastropoda: Patellogastropoda) from Indian Ocean hot vents shed light on relationships among genera. Zool J Linn Soc. 2022;194(1):276–96.
70. Shao R, Dowton M, Murrell A, Barker SC. Rates of gene rearrangement and nucleotide substitution are correlated in the mitochondrial genomes of insects. Mol Biol Evol. 2003;20(10):1612–9.
71. Xu W, Jameson D, Tang B, Higgs PG. The relationship between the rate of molecular evolution and the rate of genome rearrangement in animal mitochondrial genomes. J Mol Evol. 2006;63:375–92.
72. Sepkoski JJ. A factor analytic description of the Phanerozoic marine fossil record. Paleobiology. 1981;7(1):36–53.
73. Van Valen LM. A resetting of Phanerozoic community evolution. Nature. 1984;307(5946):50–2.
74. Song H, Tong J. Mass extinction and survival during the Permian-Triassic crisis. Journal of Earth Science. 2016;41(6):901–18.
75. Payne JL, Clapham ME. End-Permian mass extinction in the oceans: an ancient analog for the twenty-first century? Annu Rev Earth Planet Sci. 2012;40(1):89–111.
76. Sun Y, Joachimski MM, Wignall PB, Yan C, Chen Y, Jiang H, et al. Lethally hot temperatures during the Early Triassic greenhouse. Science. 2012;338(6105):366–70.
77. Grasby S, Beauchamp B, Embry A, Sanei H. Recurrent early triassic ocean anoxia. Geology. 2013;41(2):175–8.
78. Song H, Wignall PB, Chu D, Tong J, Sun Y, Song H, et al. Anoxia/high temperature double whammy during the Permian-Triassic marine crisis and its aftermath. Sci Rep. 2014;4(1): 4132.
79. Vermeij GJ. The limpet form in gastropods: evolution, distribution, and implications for the comparative study of history. Biol J Linn Soc. 2017;120(1):22–37.
80. Neubauer TA, Harzhauser M. Onset of Late Cretaceous diversification in Europe's freshwater gastropod fauna links to global climatic and biotic events. Sci Rep. 2022;12(1):2684.
81. Culver SJ, Rawson PF. Biotic response to global change: the last 145 million years. Cambridge: Cambridge University Press; 2006.

Publisher's Note