The complete mitochondrial genome of *Harpago chiragra* and *Lambis lambis* (Gastropoda: Stromboidea): implications on the Littorinimorpha phylogeny

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The complete mitochondrial genomes of *Harpago chiragra* and *Lambis lambis* (Strombidae) were determined with the size of 15,460 bp and 15,481 bp, respectively, and both sequences contained 13 protein-coding genes, 22 tRNAs, and two rRNAs. *H. chiragra* and *L. lambis* have similar mitochondrial features, corresponding to typical gastropod mitochondrial genomes, such as the conserved gene order, a high A+T content (66.22% for *H. chiragra* and 66.10% for *L. lambis*), and preference for A+T-rich codons. The start or termination codon of same protein-coding gene in *H. chiragra* was consistent with that in *L. lambis*, except for the termination codon of cox1 gene (TAG for *H. chiragra* and TAA for *L. lambis*) and the start codon of nad4 (GTG for *H. chiragra* and ATG for *L. lambis*). Pairwise sequence alignments detected different degrees of variations in *H. chiragra* and *L. lambis* mitochondrial genomes; and the two species had lower levels of genetic distance (0.202 for nucleotide sequence) and closest relationships as compared to *Strombus gigas* and *Oncomelania hupensis*. The 13 partitioned nucleotide sequences of protein coding genes of *H. chiragra* and *L. lambis* were aligned with representatives of the main lineages of gastropods and their phylogenetic relationships were inferred. *H. chiragra* and *L. lambis* share the same gene order as Littorinimorpha species, except Vermetoidea, which demonstrate a gene rearrangement in species. The reconstructed phylogeny supports three major clades within Littorinimorpha: 1) Stromboidea, Tonnoidea, Littorinoidea, and Naticoidea, 2) Rissooidea and Truncatelloidea, and 3) Vermetoidea. In addition, a relaxed molecular clock calibrated with fossils dated the diversification of Strombidae near 112 (44–206) Mya and a possible radiation is detected to occur between 45–75 Mya, providing implications to understand the Cenozoic replacement event (65–135 Mya) of Aporrhaidae by Strombidae.

Molecular phylogenetic analyses provided a different approach compared with traditional morphological methods to estimate the relationships among species based on the topological hypotheses¹⁻³. The mitochondrial DNA has a high rate of base substitution and lacks of recombination during inheritance; besides it possesses an unique transmission mode named doubly uniparental inheritance (DUI) in molluscs⁴⁻⁵. Hence mitochondrial genomic analyses was proved as a valid molecular tool in constructing phylogenies, and has been used for phylogenetic analyses in various taxa⁶⁻⁷.

The derived phylogenetic relationships based on molecular data may disagree with the evolutionary hypothesis proposed using morphological data⁸⁻¹¹. Neogastropoda was widely accepted as a monophyletic group based on morphological characters; however, Tonnoidea was placed into Neogastropoda based on the molecular analyses⁸⁻¹¹, which contradicted the monophyletic status of Neogastropoda. Among Littorinimorpha, Vermetidae is a peculiar snail family that shows a high rate of gene rearrangement¹². Based on the molecular phylogenetic analyses, Vermetidae were regarded as the sister group of the other species in Caenogastropoda; however, this

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is opposite to the morphological evidence\textsuperscript{13}. Hence, the relative position of Vermetidae in the mitochondrial phylogenetic analyses has been considered spurious, although the relationship was highly supported in previous molecular phylogenetic analyses\textsuperscript{14-15}. Strombidae species are important molluscs in shallow water of tropical and subtropical areas from past time until now\textsuperscript{16-19}. Species in Stromboidae varied greatly in shell shapes, which results in high morphological diversity\textsuperscript{9,20}. Strombus (Linné,1758) and Lambis (Röding, 1798) are the two most abundant genera in Strombidae and were once regarded as the only two genera in strombids\textsuperscript{21}. However, based on the fossil record and molecular phylogenetic analyses, the genus Strombus is justified to be subdivided into several separate genera\textsuperscript{20,21}.

Based on the paleontological studies, Strombidae probably originated from Aporrhaidae during Cenomanian-Turonian, and evolved at low diversities during the rest of the Cretaceous\textsuperscript{19}. During the course of evolution, whereas Aporrhaidae species underwent K/T mass extinction in late Cretaceous, a major genera and species radiation in Strombidae occurred during the early Cenozoic and continued to the Pliocene\textsuperscript{18,22,23}.

In the present study, we determined the complete mitochondrial genomes of *H. chiragra* and *L. lambis*, and analyzed the genomic features of the two species, including their structural characters and nucleotide composition. In early taxonomical studies, *H. chiragra* shared close relationships with *L. lambis* in Strombidae, yet this was mainly based on their similar tissue anatomies (e.g. egg masses, and radulae)\textsuperscript{18}, regardless of the great morphological difference in adults. To valid the taxonomy relation between *H. chiragra* and *L. lambis*, we attempt to determine their phylogenetic relationships based on the mitochondrial genomes. Thus, a robust phylogeny based on the concatenated 13 protein coding genes of 13 Littorinimorpha species was constructed. These data provides a framework for further evolutionary studies among Littorinimorpha.

**Materials and Methods**

**Specimen and mitochondrial DNA extraction.** Individuals of *H. chiragra* and *L. lambis* were collected from the coastal waters of Quanfu Island, South China Sea. Total genomic DNA was extracted from the foot muscle using a modified standard phenol-chloroform procedure\textsuperscript{22} and then stored at $-20$ °C.

**Determination of partial sequences.** Short fragments of cox1 gene were PCR amplified using the universal primers LCO-1490/HCO-2198\textsuperscript{25}. Based on the reference genome of *S. gigas*\textsuperscript{22}, primers were designed using Primer Premier 5\textsuperscript{27} to amplify short fragments of atp6, cox3, and cyt b (Supplementary Table S1). Long PCR primers were designed to amplify the regions between the genes based on the partial sequences obtained.

**PCR amplification and sequencing.** PCR was performed in a 30 μL reaction mixture containing 3 μL of dNTPs (2.5 mM each), 3 μL of 10 × LA buffer (Mg\textsuperscript{2+}), 1 μL of template DNA (100 ng/μL), 1 μL of each forward and reverse primer, and 0.5 μL of TaKaRa LA-Taq DNA polymerase. The thermal cycling conditions are: 94 °C for 3 min followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 68°C for 5 min, with a final extension step of 72°C for 10 min. PCR products were purified using an EZ-10 Spin Column DNA Gel Extraction Kit (Sangon Biotech), and then directly sequenced using the primer walking method. DNA sequencing was performed on an ABI PRISM 3730 (Applied Biosystems) automatic sequencer.

**Genome annotation and sequence analysis.** Sequence assembly was performed using the Seqman program, DNASTAR (http://www.DNASTAR.com). The annotations of protein-coding genes were conducted using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/) with invertebrate mitochondrial genetic code. The transfer RNA (tRNA) genes were identified using ARWEN (http://130.235.46.10/ARWEN/) and MITOS web servers (http://mitos.bioinf.uni-leipzig.de/index.py) using the chloroplast or invertebrate gene code and default search code.

The gene annotation of *rrnL* and *rrnS* were conducted using BLAST searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) by identifying their similarity to gene sequences of *S. gigas* and *Conomurex luhaanus*. The A + T content values were computed using MEGA 6.06\textsuperscript{28} and GC and AT skews were calculated according to the formula described before\textsuperscript{28}, AT skew = (A − T)/(A + T); GC skew = (G − C)/(G + C), where A, T, G, and C are the occurrences of the four nucleotides. The relative synonymous codon usage (RSCU) values of each protein coding gene were calculated using MEGA 6.06\textsuperscript{28}. The number of base substitutions per site between *H. chiragra L. lambis*, *S. gigas*, and *C. luhaanus* were calculated in MEGA 6.06\textsuperscript{28} using the Kimura 2-parameter model\textsuperscript{29}.

**Phylogenetic analyses.** The phylogenetic analyses were based on the concatenated nucleotide and amino acid alignments of thirteen protein-coding genes in seventeen complete mitochondrial genomes, including *H. chiragra*, *L. lambis*, and 13 other available mitochondrial genomes of Littorinimorpha (Supplementary Table S2). Besides, *Tegula lividomacula* and *Tegula brunnea* from the order Trochidae served as outgroup. The thirteen-partitioned nucleotide and amino acid sequences of the protein-coding genes were aligned using MAFFT\textsuperscript{30} with automatic selection of alignment algorithm. Then the alignments were treated with Gblocks\textsuperscript{31} using default parameters, and the ambiguously aligned regions were removed from the analyses. Multiple gene alignments were concatenated using PhyloSuite\textsuperscript{34}. Then we evaluated the saturation in the codon-based data sets of 13 protein coding genes in DAMBE7\textsuperscript{35}, and the results showed that the DNA sequences were un saturated in 1st, 2nd, and 3rd codon sites. The best-fit partition schemes of amino acid and nucleotide sequences were selected using PartitionFinder 2.1.1\textsuperscript{36}. Two methods were used to perform the phylogenetic analyses: Maximum Likelihood (ML) and Bayesian inference (BI). ML analysis was conducted using RAxML\textsuperscript{37} web server on the CIPRES Science Gateway V3.3 (http://www.phylo.org/index.php/) based on the partitioned nucleotide alignments, with GTR + G substitution model and 1,000 bootstraps for node reliability estimation. Bayesian analyses were conducted in MrBayes\textsuperscript{38} for 200 million generations (sampling every 1000 generations) based on the partitioned nucleotide and amino acid alignments. All parameters were checked using Tracer v1.5\textsuperscript{39}. The first 50,000 trees were discarded as burnin, and the remaining sampled trees were used to estimate the Bayesian posterior probabilities.
Estimate of divergence time. The estimation of divergence time of the major Littorinimorpha lineages were conducted using BEAST v.1.7.539 based on the partitioned amino acid sequences of 13 protein coding genes. A lognormal relaxed-clock model was selected as the molecular clock model. A Yule process of speciation was chosen for the tree prior. The final Markov chain was set to 100 million generations, sampling every 10,000 generations. The effective sample size of all parameters was above 200. The convergence of the chains was checked with Tracer v.1.539, and the first 1,000 generations sampled were discarded as part of the burn-in process.

The posterior distribution of the estimated divergence times was specified based on the prior fossil knowledge. Two calibration points were selected, using a normal distribution of prior probability: 342.8 Mya was used as prior divergence time for Vermetoidea based on the Paleocene fossil collection in Belgium and the United Kingdom40, and the prior divergence time of Truncatellidae was set as 66.04 Mya according to the oldest fossil record of Paleocene in Belgium41–43. Besides, the divergence time of Tegula was 85 Mya based on the Cretaceous fossil record in United States41, and this point was used to cross-validate the accuracy of the dated tree.

Results and Discussion

Genome organization of H. chiragra and L. lambis. The complete mitochondrial genome sequences of H. chiragra and L. lambis are 15,460 bp and 15,481 bp, respectively (Tables 1, 2), and both contain 13 protein coding genes (PCGs), 22 tRNAs and two rRNAs (Fig. 1). This (+) strand encodes for trnD, trnV, trnL, trnL, trnP, trnS, trnH, trnF and the cluster KARNI (trnK, trnA, trnR, trnN, and trnI) and trnS. The (−) strand encodes for the cluster MYCWQGE (trnM, trnY, trnC, trnW, trnQ, trnG, and trnE) and trnT (Fig. 1). Four overlaps between

| Gene   | From | To   | Size (nts) | Size (aa) | Intergenic nucleotides | Start codon | Termination codon |
|--------|------|------|------------|-----------|------------------------|-------------|-------------------|
| cox1   | 1    | 1536 | 1536       | 511       | 4                      | ATG         | TAA               |
| cox2   | 1556 | 2242 | 687        | 228       | 19                     | ATG         | TAA               |
| trnD(gac) | 2241 | 2308 | 68         | 2         |                        | ATG         | TAA               |
| ap8    | 2309 | 2467 | 159        | 52        | 0                      | ATG         | TAA               |
| ap8t   | 2472 | 3167 | 696        | 232       | 4                      | ATG         | TAA               |
| trnM(agt) | 3204 | 3271 | 68         | 36        |                        | ATG         | TAA               |
| trnT(tac) | 3290 | 3355 | 66         | 18        |                        | ATG         | TAA               |
| trnC(tgc) | 3358 | 3422 | 65         | 2         |                        | ATG         | TAA               |
| trnW(aga) | 3424 | 3490 | 67         | 1         |                        | ATG         | TAA               |
| trnQ(caa) | 3492 | 3553 | 62         | 1         |                        | ATG         | TAA               |
| trnC(gga) | 3566 | 3632 | 67         | 12        |                        | ATG         | TAA               |
| trnE(gaa) | 3634 | 3703 | 70         | 1         |                        | ATG         | TAA               |
| trnS    | 3708 | 4693 | 986        | 4         |                        | ATG         | TAA               |
| trnW(gta) | 4694 | 4760 | 67         | 0         |                        | ATG         | TAA               |
| rnl     | 4753 | 6147 | 1395       | −8        |                        | ATG         | TAA               |
| trnL1(tca) | 6150 | 6218 | 69         | 2         |                        | ATG         | TAA               |
| trnL2(tta) | 6226 | 6294 | 69         | 7         |                        | ATG         | TAA               |
| nd1    | 6296 | 7237 | 942        | 313       | 1                      | ATG         | TAA               |
| trnP(cca) | 7247 | 7315 | 69         | 9         |                        | ATG         | TAA               |
| nad6   | 7320 | 7826 | 507        | 168       | 4                      | ATG         | TAA               |
| cytB   | 7836 | 8975 | 1140       | 379       | 9                      | ATG         | TAA               |
| trnS2(tca) | 8990 | 9055 | 66         | 14        |                        | ATG         | TAA               |
| trnT(tca) | 9076 | 9141 | 66         | 20        |                        | ATG         | TAA               |
| nad4l  | 9150 | 9446 | 297        | 98        | 8                      | ATG         | TAA               |
| nad4   | 9440 | 10813| 1374       | 457       | −7                     | ATG         | TAA               |
| trnH(cac) | 10818| 10884| 67         | 4         |                        | ATG         | TAA               |
| nad5   | 10885| 12612| 1728       | 575       | 0                      | ATG         | TAA               |
| trnF(ttc) | 12654| 12725| 72         | 41        |                        | ATG         | TAA               |
| co3    | 12780| 13559| 780        | 259       | 54                     | ATG         | TAA               |
| trnK(aaa) | 13597| 13666| 70         | 37        |                        | ATG         | TAA               |
| trnA(gca) | 13693| 13763| 71         | 26        |                        | ATG         | TAA               |
| trnR(cga) | 13774| 13842| 69         | 10        |                        | ATG         | TAA               |
| trnN(aac) | 13854| 13923| 70         | 11        |                        | ATG         | TAA               |
| trnL(tac) | 13925| 13992| 68         | 1         |                        | ATG         | TAA               |
| nad3   | 13994| 14347| 354        | 117       | 1                      | ATG         | TAA               |
| trnS1(agg) | 14351| 14418| 68         | 3         |                        | ATG         | TAA               |
| nad2   | 14407| 15477| 1071       | 356       | −12                    | ATG         | TAA               |

Table 1. Organization of the mitochondrial genome of Lambis lambis (15,481 bp).
adjacent genes were detected in *H. chiragra* and *L. lambis*, in addition, another region between *atp8* and *trnV* was found only in *H. chiragra*, but not in *L. lambis* (Fig. 1). The lengths of genes (including PCGs, tRNAs and rRNAs) and intergenic nucleotides are 15129 bp, 331 bp for *H. chiragra* and 15146 bp, 335 bp for *L. lambis*, respectively (Tables 1, 2), in which the gene length of the overlapping nucleotides was counted once.

The organization of the *H. chiragra* and *L. lambis* mitochondrial genomes was compared with that of other species in Littorinimorpha (Fig. 2). Among the gastropod species, mitochondrial genomes are estimated to show high rates of gene rearrangement between major lineages44. However, the gene orders of the two newly sequenced mitochondrial genomes were similar to the consensus gene order shared by most previously published species from Littorinimorpha26,45 (Fig. 2).

Nucleotide composition. The overall base compositions of the mitochondrial genomes on the (+) strand were both biased toward A and T. For *H. chiragra*, the nucleotide content was found to be A = 28.26%, T = 37.6%, C = 16.57%, and G = 17.21%. For *L. lambis*, the nucleotide content was A = 28.61%, T = 37.49%, C = 16.50%, and G = 17.40% (Table 3). For the entire mitochondrial genomes, the AT and GC-skews on the (+) strand were −0.128 and 0.019 for *H. chiragra* and −0.134 and 0.026 for *L. lambis*, respectively (Table 3).

The nucleotide composition of the single gene region of *H. chiragra* and *L. lambis* were calculated. The A + T content of protein coding genes (PCGs), tRNA, rRNA, and non-coding regions (NCRs) is similar between *H. chiragra* and *L. lambis* (Table 3). For single genes, similar A + T content was only detected in *cox3* (60%), *nad2* (68%) and *nad4* (66%).

| Gene | From | To  | Size (nts) | Size (aa) | Intergenic nucleotides | Start codon | Termination codon |
|------|------|-----|------------|----------|------------------------|-------------|-------------------|
| cox1 | 1    | 1536| 1536      | 511      | 5                      | ATG         | TAG               |
| cox2 | 1556 | 2242| 687       | 228      | 19                     | ATG         | TAA               |
| trnD(gac) | 2241 | 2308 | 68       | 2        |                        |             |                   |
| atp8 | 2309 | 2467| 159       | 52       | 0                      | ATG         | TAA               |
| atp6 | 2470 | 3165| 696       | 231      | 2                      | ATG         | TAA               |
| trnM(atg) | 3202 | 3269 | 68      | 36       |                        |             |                   |
| trnT(tac) | 3288 | 3353 | 66      | 18       |                        |             |                   |
| trnC(tgc) | 3356 | 3420 | 65      | 2        |                        |             |                   |
| trnW(ga) | 3422 | 3488 | 67      | 1        |                        |             |                   |
| trnQ(caa) | 3490 | 3551 | 62      | 1        |                        |             |                   |
| trnG(gga) | 3564 | 3630 | 67      | 12       |                        |             |                   |
| trnE(gaa) | 3632 | 3701 | 70      | 1        |                        |             |                   |
| rnsS | 3707 | 4684 | 978     | 5        |                        |             |                   |
| trnV(gta) | 4685 | 4751 | 67      | 0        |                        |             |                   |
| rnl | 4737 | 6127 | 1391    | –15      |                        |             |                   |
| trnL1(ta) | 6134 | 6202 | 69      | 6        |                        |             |                   |
| trnL2(ta) | 6212 | 6280 | 69      | 9        |                        |             |                   |
| nd1 | 6282 | 7223 | 942     | 313      | 1                      | ATG         | TAG               |
| trnP(cca) | 7234 | 7301 | 68      | 10       |                        |             |                   |
| nad6 | 7303 | 7809 | 507     | 168      | 1                      | ATG         | TAA               |
| cytb | 7821 | 8960 | 1140    | 379      | 11                     | ATG         | TAA               |
| trnS2(aca) | 8975 | 9040 | 66      | 14       |                        |             |                   |
| trnT(aca) | 9059 | 9125 | 67      | 18       |                        |             |                   |
| nad4l | 9135 | 9431 | 297     | 98       | 9                      | ATG         | TAG               |
| nad4 | 9425 | 10798 | 1374   | 457      | –7                     | GTC         | TAA               |
| trnF(cac) | 10808 | 10874 | 67    | 9        |                        |             |                   |
| nad5 | 10875 | 12602 | 1728  | 575      | 0                      | ATG         | TAA               |
| trnP(ttc) | 12634 | 12702 | 69    | 31       |                        |             |                   |
| cox3 | 12756 | 13535 | 780   | 259      | 53                     | ATG         | TAA               |
| trnK(aaa) | 13574 | 13643 | 70    | 38       |                        |             |                   |
| trnA(gea) | 13671 | 13742 | 72    | 27       |                        |             |                   |
| trnR(cga) | 13754 | 13822 | 69    | 11       |                        |             |                   |
| trnN(aac) | 13835 | 13901 | 67    | 12       |                        |             |                   |
| trnA(tca) | 13904 | 13971 | 68    | 2        |                        |             |                   |
| nad3 | 13973 | 14326 | 354   | 117      | 1                      | ATG         | TAA               |
| trnS1(age) | 14329 | 14396 | 68    | 2        |                        |             |                   |
| nad2 | 14385 | 15455 | 1071  | 356      | –12                    | ATC         | TAG               |

Table 2. Organization of the mitochondrial genome of *Harpago chiragra* (15,460 bp).
Among the different types of genes, the tRNA genes and rRNA genes of *H. chiragra* and *L. lambis* show positive AT skews, whereas all types of protein coding genes show negative AT skews. Both the tRNA and rRNA genes of *H. chiragra* and *L. lambis* show positive GC skews. Some protein coding genes (*atp6, cob, nad4, nad5, and nad6* in *H. chiragra* and *L. lambis* and *atp8* in *L. lambis*) show negative GC skews, while the other types of protein coding genes and RNA genes (tRNA genes and *rrnL, rrmS*) show positive GC skews.

Figure 1. Linear comparison of the gene organization of *H. chiragra* and *L. lambis* mitochondrial genomes. The blue lines indicated genes coded by the minor strand. Positive numbers mean the length in bp of non-coding regions between genes and negative numbers represent overlapping nucleotides between genes.

Figure 2. Linear comparisons of the organization of the mitochondrial genomes of Littorinimorpha.

Table 3. AT-content, AT-skew, and GC-skew for mitochondrial genes of *H. chiragra* and *L. lambis*.
Protein coding genes (PCGs). Excluding the termination codons, the mitochondrial genomes of *H. chiragra* and *L. lambis* encode 3,744 and 3,745 amino acids, respectively. Comparison between species of start and termination codons of protein coding genes showed that only 2 PCGs initiate or stop with different codons, which were detected in *nad4* gene (initiated with GTG in *H. chiragra* and ATG in *L. lambis*) and *cox1* gene (stopped with TAG in *H. chiragra* and TAA in *L. lambis*). In addition, 12 PCGs contain the same start (*nad2*: ATC; ATG...
paraphyletic. However, when the cladistics analyses of species in *H. chiragra* were based solely on morphological characters, the results clustered one species between *L. lambis* and *H. chiragra* (Fig. 3). The RSCU also reflected the synonymous substitutions in protein-coding genes were more frequent than nonsynonymous substitutions.

The codon usage of the mitogenomes of *H. chiragra* and *L. lambis* was similar to that of other Strombidae species. All codons were used in the mitogenomes of these two species, however the codon frequencies varied between each other. Amino acids encoded by A + T-rich codons are more common than those encoded by G + C-rich codons. The ratio of A + T/G + C-rich codons was 2.61 in *L. lambis*, which was lower than what was found for *H. chiragra* (2.70). The relative synonymous codon usage (RSCU) is different between *H. chiragra* and *L. lambis*, implying a relatively distant relationship. Compared with the nucleotide divergence among the four Strombidae mitogenomes, the pairwise divergence values calculated using the amino acid sequences were lower, indicating that synonymous substitutions in protein-coding genes were more frequent than nonsynonymous substitutions.

Pairwise divergence among four Strombidae mitochondrial genomes was calculated based on separate and concatenated protein-coding genes (Supplementary Fig. S2). The nucleotide divergence between *H. chiragra* and *L. lambis* was 0.151, which was the lowest genetic divergence measured here, confirming the close relationship between *H. chiragra* and *L. lambis*. *Strombus gigas* and *C. luhuanus* have a nucleotide divergence of 0.351, indicating a relatively distant relationship. Compared with the nucleotide divergence among the four Strombidae mitogenomes, the pairwise divergence values calculated using the amino acid sequences were lower, indicating that synonymous substitutions in protein-coding genes were more frequent than nonsynonymous substitutions.

**Non-coding regions.** There were 34 non-coding regions distributed in the *H. chiragra* and *L. lambis* genomes, 403 bp for *H. chiragra* and 393 bp for *L. lambis* (Fig. 1). The non-coding sequences were generally characterized by short nucleotide fragments, ranging from 1 bp to 53 bp in *H. chiragra* and 1 bp to 54 bp in *L. lambis* among every non-coding fragment. The largest non-coding region was found between the gene *cox3* and *trnF* (53 bp for *H. chiragra* and 54 bp for *L. lambis*). This location was proposed as a candidate to contain the control region in other gastropod mitochondrial genomes. Among the non-coding regions, there were 20 regions with different lengths between *H. chiragra* and *L. lambis* and 14 intervals with same length.

**Phylogenetic analyses.** The selected partition schemes for phylogenetic analyses were listed in Supplementary Tables S3, S4. The topological structure of the trees inferred by two different methods (ML and BI) was essentially uniform (Fig. 4). All nodes in the BI tree were near 100% supported and the nodes in the ML tree were also highly supported. Within Stromboidea, the phylogenetic tree shows that Stromboidea form an independent branch as (**S. gigas + (C. luhuanus + (L. lambis and H. chiragra))**, *L. lambis* is the closest extant relative of *H. chiragra*, and this clade clustered with *S. gigas* and *C. luhuanus*. Research derived from combined phylogenetic analyses of molecular and morphological data has revealed that *Lambis* was monophyletic and *Strombus* was paraphyletic. However, when the cladistics analyses of species in *Lambis* were based solely on morphological characters, the results clustered one *Lambis* species (*L. crocata*) into the outgroups of species, suggesting that *Strombus* is polyphyletic and the *Lambis* is paraphyletic. *Lambis crocata* was not included in the present study since there is no complete mitochondrial genome available for this species. Although lacking a sufficient number of species for a robust phylogenetic analysis, our phylogeny is statistically supported and aims to provide a reasonable framework for further phylogenetic research within Stromboidea.
Within Littorinimorpha, Stromboidea and Tonnoidae clustered together in the same clade, which was then clustered with (Littorinimorpha + Naticoidea), as derived by the BI method. Stromboidea, Tonnoidae, Littorinimorpha, and Naticoidea form a well-supported clade based on both ML and BI, confirming their close relationship within Littorinimorpha. Rissooidea was sister to Truncatelloidea, which together formed the second major clade. Veneroidae formed the third independent well-supported clade within Littorinimorpha.

Estimate of divergence time. To test the accuracy of the dated tree derived from BEAST, we made a cross-validation using the calibration point of genus Tegula. The oldest fossil record of Tegula was stated as 85 Mya and the documented time was coincident with the divergence time of T. lividomaculata and T. brunnea (13–309 Mya) in present study. Research documented that the number of species increased from five species within the genus Strombus during the Eocene (53 Mya) to 40 species till the Late Oligocene and the Miocene (23–36 Mya), indicating that the species diversification within Strombidae accelerated in the last 36–53 Mya. According to the present dated tree (Fig. 5) the diversification of Strombidae species occurred around 112 (44–206) Mya, and a radiation pattern (accelerated rates of diversification) is detected to occur between 45–75 Mya, which is in agreement with the fossil record in Strombidae. Besides, the diversification pattern of Strombidae species occur between the late Cretaceous and early Paleocene (65–135 Mya), and this might provide implications to understand the Cenozoic replacement event of Aporrhaidae by Strombidae. Furthermore, to better resolve the phylogenetic relationships and understand the replacement event, more Strombidae and Aporrhaidae mitochondrial genomes should be inserted into the phylogenetic analyses.

Data availability
Data is available at Genbank (accession number MH115428, MH122656).

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Author contributions
Dianheng Jiang performed the experiments, analyzed the data, wrote the paper, and prepared the figures and tables; Qi Li and Lingfeng Kong provided reagents and directed the experiment; Xiaqi Zeng collected the samples; Xiaodong Zheng contributed materials/analysis tools, supervised the work.

Competing interests
The authors declare no competing interests.

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