Complete Mitochondrial Genomes of Babylonia Formosae and Babylonia Zeylanica (Neogastropoda: Babyloniidae) and Increased Sampling Give New Insights Into Neogastropoda Phylogeny

Hongwei Huang
Zhejiang Ocean University
huai Yang
Zhejiang Ocean University
Jiji Li
Zhejiang Ocean University
baoying Guo (guobaobao2000@126.com)
Zhejiang Ocean University
Yingying Ye
Zhejiang Ocean University https://orcid.org/0000-0003-0056-030X

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Abstract

The phylogenetic relationships of Neogastropoda, a group of highly complex predatory marine snails, have been controversial. The two newly sequenced mitogenomes of *Babylonia formosae* and *Babylonia zeylanica* (Neogastropoda: Babyloniidae) are described. The mitogenomes of *B. zeylanica* and *B. formosae* were 16, 214 bp and 16, 181 bp in length, respectively. The mitogenomes of both species contain 13 PCGs, 22 tRNA genes, and 2 ribosomal RNA genes. The sequence of genes differed from the ancestral mitochondrial gene arrangement of Caenogastropoda mitogenomes. Also, 63 Neogastropoda species were analyzed for the genome organization of seventeen major lineage of Neogastropoda, five types of mitochondrial genome arrangement were identified. Bayesian Inference phylogenetic trees and Maximum likehood of Neogastropoda were established according to complete mitogenome. The monophyly of Neogastropoda families is strongly supported by this study, in contrast to previous molecular studies. Our results shed light on gene sequence distribution/arrangement characteristics of Neogastropoda mitogenomes, provide fundamental information for further phylogenetic studies on Neogastropoda.

Introduction

Metazoan Mitogenome (mtDNA) is a small (~14 to 20 kb) circular genome, containing 37 genes, which were divided into three parts, including 2 ribosomal RNA genes (rRNA), 2 transfer RNA genes (tRNA), and 13 protein-coding genes (PCGs) [1]. The gene arrangement was first thought to be generally conserved [2-3], can provide crucial information regarding phylogenetic relationships in Metazoa [4-6].

Generally, gene rearrangements are thought to be more common in invertebrate mitogenomes, such as in sea cucumbers [7], cephalopods [8], bivalves [9], insects [10], and crabs [11]. Because gene order of closely related species is assumed to be relatively conserved, it has been increasingly used as a valuable molecular marker to infer evolutionary lineage relationships [12-13]. Indeed, in Caenogastropoda, one of the four vital lineages of gastropod that accounts for presumably 60% of the total known living marine gastropod species [14], three studies of mtDNA rearrangement have been showed in previous study [15-16]. Namely, a translocation between the $tRNA^{Val}$ genes and $tRNA^{Ser}$ (UCN) [16], and four different vermetid species shows, a translocation of the $tRNA^{Lys}$ gene, and a translocation between a fragment of $tRNA^{Pro}$ and subunit 6 of NADH dehydrogenase [17].Considering the increased sampling give new insights into the mitochondrial genome organization of Neogastropoda, during the development of the gastropod system, comprehensive sequencing in mitochondrial genomes is needed for figuring out their gene arrangements.

Four important scientific hypotheses have been advanced in order to interpret gene rearrangement events. Recombination model characterized by DNA fragmentation and reconnection DNA breakage and rejoining was the first proposed hypothesis. As a mechanism of mitochondrial gene rearrangement, it has been proposed in frog, bird, and mussels [18-20]. Another well-known hypothesis is tandem duplication and random loss (TDRL) model. In this model, it is assumed that the rearrangements occur in both tandem and segmental duplications of some genes in the mitogenome, which are subsequently followed by random removal of duplicates [7,21]. Most of whole genome rearrangements in vertebrate species were predated and well-explained by this model [22-24]. It has been proposed that tandem duplication and non-random (TDNL) model could explain gene arrangements observed in two millipede mitogenomes [25]. Finally, the new double replications and random loss (DRRL) model has been recently proposed to explain the large-scale genome rearrangements diffusely in the flatfish *Samariscus latus* (Samaridae) [26].

Neogastropoda was widely considered as a monophyletic group among morphologists However, the evolutionary relationships among Neogastropoda families based solely on morphological characters are precarious. Current research, based on molecular studies, usually do not support this classification system of monophyly of Neogastropoda [27-32]. For example, (a research presents a novel classification model that divides Neogastropoda into six superfamilies: Muricoidea, Pseudolivoidea, Buccinoidea, Olivoidea, Cancellarioidea, and Conoidea. Another study by Harasewych *et al.* reports that Neogastropoda is not a monophyletic group based on the analysis of partial COI and 16S rRNA sequences.

Some studies have shown that mitogenomic rearrangements can provide significant information about the origin and evolution [33-35]. In the current research, we have sequenced and identified the whole mitogenomes of *B. formosae* and *B. zeylanica*, to uncover the mitogenomic rearrangements and the evolution within Neogastropoda. The order of genes in *B. formosae* and *B. zeylanica* mitogenomes were compared with other Neogastropoda mitogenomes, and underlying rearrangement mechanisms were analyzed and dissected. The mitogenome sequences of 63 species representing 17 families of Neogastropoda were downloaded from the GenBank database to structure the phylogenetic tree. Our findings shed light on the gene arrangement features of Neogastropoda mitogenomes and provide fundamental information for further evolutionary studies on Neogastropoda.

Materials And Methods

Sampling, DNA Extraction, PCR Amplification and Sequencing

Babylonia formosae and *B. zeylanica* used in this study were obtained from Hainan, China (20°03'N; 110°32'E) and Guangdong, China (23°35'N; 116°68'E) respectively and immediately preserved in 95% ethanol. Genomic DNA was extracted from adductor musculature using the SQ Tissue DNA Kit (OMEGA) following the manufacturer's protocol and stored at −20℃ before sequencing.Complete mitogenome sequences were amplified in each sample and the results were compared with the existing sequences in GenBank database for final identification of the species.

Sequence Assembly, Annotation and Analysis

The sequences were assembled and analyzed via Illumina genetic analyzer by Origingene Bio-pharm Technology Co., Ltd., (Shanghai, China). NOVOPlasty (https://github.com/ndierckx/NOVOPlasty) stitching software was used to join together multiple iterations of sequenced fragments to obtain optimal assembly results. All assembled *B. formosae* and *B. zeylanica* mitochondrial genes were aligned with *Babylonia lutosa* (KF897830.1) identified by BLAST,

after verification of sequences in the NCBI database. In addition, relevant information about mitogenome was annotated by Sequin software (version 15.10 http://www.ncbi.nlm.nih.gov/Sequin/). The boundaries of the 13 protein coding genes (PCGs) and 2 ribosomal RNA (rRNA) genes were verified using NCBI-BLAST (http://blast.ncbi.nlm.nih.gov). The secondary structure of tRNA genes were predicted using both the MITOS Web Server [36] and tRNAscan-SE 1.21 [37]. The final mitogenome map was produced using CGView [38]. The base composition of mitogenomes was acquired using MEGA X [39]. To examine strand asymmetry, the AT-skew and GC-skew values were calculated using the formulas: AT-skew = (A−T)/(A+T); GC-skew = (G−C)/(G+C) [40].

Phylogenetic Analyses

Sixty-three Neogastropoda mitogenomes were downloaded from the GenBank (https://www.ncbi.nlm.nih.gov/genbank/) for phylogenetic analysis (Table.1). Lepetellida and Patellogastropoda have been thought to be closely related to Neogastropoda, hence, one Patellogastropoda and two Lepetellida species, *Lottia digitalis*, *Haliotis rufescens* and *Haliotis rubra*, were chosen as the outgroup. Fasta files containing nucleotide sequence for all 13 PCG genes were retrieved from GenBank using PhyloSuite [41]. Nucleotide sequence alignments were performed using MAFFT [42] in the default configuration, and were manually checked with BioEdit [43]. Sequences containing ambiguous bases were systematically discarded using Gblock [44]. Subsequently, all alignments were concatenated into single fasta and nexus format files for phylogenetic analyses. Phylogenetic analyses were inferred using Maximum Likelihood (ML) and Bayesian Inference (BI). ML phylogenetic inference was performed using IQ-TREE [45], using 1000 rapid bootstrap replications under the GTR+F+R6 model. BI analysis was conducted in MrBayes 3.2.6 [46], with the best-fit GTR+I+G models selected from 24 models using MrModelTest 2.3 [47]. BI analyses were implemented using general Lset values (e.g., nst and rates) allowing the program to converge on the best estimates of the model parameters. BI analyses were run for 2,000,000 generations, sampling trees every 100 generations, for a total of 20,000 trees sampled. Four chains, three heated (temperature = 0.5) and one cold, were simultaneously run using Metropolis-coupled Markov chain Monte Carlo (MCMCMC) to enhance the mixing capabilities of the Markov chains. To guarantee the stationary had been reached, the average standard deviation of split frequencies was set below 0.01.

Results

Genomic Characteristics

The complete mitogenomes of *B. formosae* and *B. zeylanica* were 16, 214 bp and 16, 181 bp in length, respectively (GenBank accession number MK577482 and MN604402). This is close to the size of the other Babylonia mitogenomes that have previously been published (15, 346 bp for *B. lutosa* (KF897830.1) and 15, 445 bp for *Babylonia areolata* (HQ416443.1)) (Table 1). All of these mitogenomes comprised 22 tRNAs, 13 PCGs, and two rRNAs (Fig.1A-B, Table 2). The total length for the 13 PCGs of *B. formosae* and *B. zeylanica* were 10, 806 bp and 11, 247 bp, respectively. Most of genes are encoded by the H-strand except for the tRNA genes trnC, trnE, trnG, trnM, trnQ, trnT, trnW, and trnY, encoded by the L-strand. The PCGs ranged in size from 264 bp (ND4L) to 1671 bp (ND5) in *B. formosae*, and from 159 bp (ATP8) to 1731 bp (ND5) in *B. zeylanica* (Table 2). Ten PCGs (ND1, ND2, ND3, ND4, ND4L, COI, COII, ATP8, ATP6, and Cytb) use the typical ATG start codon, with the exception of ND5, ND6, and COIII genes in the *B. formosae* mitochondrial genome. And ND6, ND4, ND5, and ND2 in *B. zeylanica*, starting with the alternative start codon ATT. The termination codons in PCGs were either TAA or TAG in these two mt genomes (Table 3).

Skewnes and Composition

Three values (GC-skew, AT-skew, and A+T content) are usually used to assess the whole DNA sequences composition. These statistics were calculated for the newly sequenced PCGs in *B. formosae* and *B. zeylanica* mitogenomes. The A+T content of ATP8 (70.52%) is the highest and A+T content of COX3 (59.82%) is the lowest ATP8 (70.52%) and the lowest in COX3 (59.82%). The GC-skews are from-0.227 (ND5) to 0.222 (ND3) in the *B. formosae* genome, and the GC-skews are from -0.232 (ND6) to 0.256 (ND3) in the *B. zeylanica* mitogenome. All AT-skews are negative in both mitogenomes (Table 2). For *B. formosae* and *B. zeylanica*, the mtDNA A+T% was 65.41% and 66.32%, respectively, showing a noticeable AT bias. Coding regions accounted for 66.6% and 65.52% in these respective mtDNA, whereas non-coding region lengths were respectively 3,764 and 1,175 bp, distributed in 24 regions of the mitochondrial genome, accounting for 23.2% and 7% of total length.

tRNA Secondary Structure

In all Babylonia mt genomes, as in *B. formosae* and *B. zeylanica*, the tRNAs ranged from 65 to 70 bp in length (in length ranged from 65 bp to 70bp). Most of the predicted tRNAs conform to the expected structure with the canonical cloverleaf structures (Fig. 2). Departing from the canonical structure, trnS1 and trnS2 did not have a dihydrouridine (DHU) stem and the DHU arm simply formed a loop, and in trnF, the TψC arm did not form a loop. A similar DHU stem-loss phenomenon was generally observed in stoneflies and mt genomes of other insects [48-49]. The secondary structures presented a high degree of structural synteny in the two Babylonia mt genomes, trnD was identical in the two genomes and most tRNAs (fourteen out of 22) had fewer than five nucleotide differences between the two genomes. In contrast, the trnS2 and trnT had the highest number of variation, with more than 10 indels or substitutions in trnT, and nucleotide insertion-deletion in trnS2 and trnT (Fig. 2). The anticodon arm of these tRNAs was the most highly conserved region while the DHU arm and the TΨC arm variable loop had the greater degree of variability in nucleotide substitutions or indels.

Gene Arrangement of Major Lineage of Neogastropoda

The gene sequence of Caenogastropoda consensus is quire resembles two reported in Neritimorphs and Vetigastropods, the latter of which had the closest to the ancestral mollusc gene order in gastropods. Herein, the gene arrangements of 63 taxa in 17 Neogastropoda families were compared with the hypothetical ancestral Caenogastropoda (Fig.3). The gene orders of the newly sequenced mt genomes were consisted with the most common Neogastropoda mt genomes (Type I). Within Neogastropoda, minor differences of *Fusiturris similis* and *Oxymeris dimidiate* regarding the consensus gene order were founded. The former differs in the relative position of the trnS (Type III), and the latter differs in the relative position of the trnV (Type IV). The most notable finding was the essential

difference between *Clavatula tripartita* of Clavatulidae and *Profundiconus teramachii* of Conidae, which the trnF gene was lost in the two mitochondrial genomes (Type II and V). Moreover, the relative position of the trnS in *C. tripartita* mt genome was also altered in compared to the consensus gene order.

Phylogenetic Analysis

To investigate the monophyly of major lineage Neogastropoda families, and analyze the phylogenetic position of *B. formosae* and *B. zeylanica* within Neogastropoda, both BI and ML trees were structured, using 63 species of Neogastropoda belonging to 17 families, and (using 63 species of Neogastropoda) as outgroup, two Littorinimorpha and one Lepetellida species (Fig.4). The tree results displayed that the phylogenetic patterns between the BI and ML trees were highly congruent. And the BI tree had most valuable data (Fig.4). Both phylogenetic trees obviously demonstrated that *B. formosae* and *B. zeylanica* have the closest relationship with *B. lutosa* and *Babylonia areolata*, and all four Babylonia species formed a Babyloiidae clade. Our results suggest that Babyloiidae was closely associated with Costellariidae and Volutidae. Of the 17 families included in this phylogenetic tree and each of them formed a monophyletic group. At the level of surperfamilies, each surperfamilies also formed a monophyletic group.

Discussion

Mitochondrial Gene Arrangements

To dissect the gene arrangement of seventeen major lineage of Neogastropoda, we compared the gene order of 63 taxa of 17 Neogastropoda families, including Babayloniidae, Drilliidae, Turridae, Cancellariidae, Costellariidae, Buccinidae, Melongenidae, Nassariidae, Muricidae, Volutidae, Fasciolariidae, Ancillariidae, Conidae, Columbellidae, Terebridae, Fusiturridae and Clavatulidae (Fig.3). Compared with the putative Caenogastropoda ancestor mitochondrial genome Caenogastropoda, our results show that 59 species of 14 Neogastropoda families have the same gene order, which shows a relatively stable gene order, where the genes were located on the heavy chain, only eight genes that are in the light (minus) chain are included in the cluster of tRNAs MYCWQGE and tRNA-Thr (T). Except *P. teramachii*, specie of Conidae, which trnF gene was lost. However, compared with the most common rearrangement within Neogastropoda, a slight difference in the order of shared genes was found, in *F. similis, O. dimidiate,* and *C. tripartite* of the Fusiturridae, Terebridae and Clavatulidae families, only certain tRNA sequences exhibit translocation (trnV), inversion (trnS) and deletion (trnF), The first two gene arrangements are as previously reported [16,50]. Also, the comparisons of genomes within major lineage of Neogastropoda, in this study, we compared the 63 gene arrangement patterns in Neogastropoda, and found that protein-coding and rRNA gene rearrangements were not be known. These results were in accordance with the findings reported in bees [51]. Herein, 63 Neogastropoda species were analyzed for the genome organization of seventeen major lineage of Neogastropoda, five types of mitochondrial genome arrangement were identified, which shed a more systematic understanding for Neogastropoda.

Monophyly of Neogastropoda

Within the megadiverse phylum Mollusca, the Gastropoda is among the most widespread and abundant, surpassing 10,000 living species. The monophyly of the group is becoming more generally accepted among morphologists because its members share several critical morphological traits [52-53]. The majority of the molecular studies were concentrated on all gastropod species [29-30,32] or caenogastropoda [31] phylogeny, and only included a limited number of Neogastropods. Previous Neogatropod phylogeny studies were only based on certain gene segments. The monophyly of Neogastropoda remains unset in both molecular-based phylogenies and morphological. Cunha *et al.* (2009) suggested that shared morphological features of Neogastropoda are homoplasious, and molecular datasets analysis often do not contain adequate information to address the current phylogenetic question.

To further explore the monophyly of seventeen major lineage Neogastropoda families and the phylogenetic position of *B. formosae* and *B. zeylanica* within Neogastropoda, we generated two well-supported phylogenetic trees (Bayesian Inference phylogenetic trees and Maximum likehood). The two trees shown all Neogastropoda species clustered into the 17 families included in this phylogenetic analysis, each family in the phylogenetic tree forming a strongly supported monophyletic group with high bootstrap value or the Bayesian posterior probability, supporting monophyletic origins. Therefore, our research results demonstrated that these families were monophyletic. Our phylogenetic analysis based on complete mitochondrial genome sequence and increased neogastropod sampling within Nogastropoda lineages confirms the monophyly of Neogastropoda. This directly refutes previous molecular analyses [16,29,31-32] and confirms the correctness of morphological homology that generally support Neogastropoda as monophyletic [14,53]. Our phylogenetic framework within Nogastropoda outlined by a large number of ingroups is more suitable to test the monophyly of Neogastropoda.

Phylogenetic Relationships

The superfamily classification in this study follows Cunha *et al.* (2009). Here the two representative mitochondrial genomes from the unassigned superfamily (Neogastropoda: Babyloniidae) are obtained using next-generation sequencing. For this study, Babyloniidae (including *B. lutosa*, *B. areoiata, B. formosae* and *B. zeylanica*) together with a basal position relative to the remaining Neogastropods in both BI and ML nucleic acid analyses. Moreover, all the ML and BI analyses strongly supported the monophyly of Volutoidea, Olivoidea, Turbinelloidea, Muricoidea, Buccinoidea and Conoidea. Recent phylogenetic molecular analyses have proven that Conoidea is also a monophyletic group. Muricoidea was not found to be a monophyletic group in previous studies based on a combination of morphological and molecular data [16,31,54]. It forms two major monophyletic clades, sometimes together with Volutoidea and Olivoidea, with high support within Neogastropoda. Within the major clade in ML and BI trees, a monophyletic group containing Columbellidae, Fasciolariidae and Nassariidae is recognizable. Previous morphological and molecular studies are not large enough to test the monophyly of Buccinoidea [16,31]. Here, Buccinoidea is recovered as monophyletic, and also forms a monophyletic group together with five superfamilies, including Turbinelloidea, Volutoidea, Olivoidea, Conoidea and Muricoidea. Our results are nearly consistent with previous reports on Oliverio and Modica [55]. The classification of each family within Neogastropoda is strongly supported in all phylogenetic analyses at a lower taxonomic level. The evolutionary relationshisps among members of the Neogastropoda families remain quite blurry [56]. In Hayashi and Oliverio and Modica, Buccinidae is also found as paraphyletic or polyphyletic. However, seventeen major lineage of Neogastropoda are recovered as monophyly in our analyses. As a highly diversified group of predatory marine snails,

Neogastropoda, has often been contradicted in molecular phylogenetic studies. This is partly due to the limited Neogastropoda taxa, outgroups or insufficient gene sequences analysis. For the first time, we reconstructed a phylogeny of major lineage of Neogastropoda including a total of 63 mt genomes that represent the main lineages within the group. Our results can provide complementary results in previous phylogenetic analysis. Based on the complete mitochondrial genome data and increased sampling, our phylogenetic analyses also shed a new light on the major lineage of Nogastropoda phylogenetic framework and the phylogenetic relationships within Neogastropoda. Despite the uncomplete taxonomic coverage in the present analysis, it is a further research into Neogastropoda phylogenetic relationships, taking into account the complete mitochondrial genome of more than half of the family-level diversity of the Neogastropoda. However, considering the limited representatives of mitochondrial genomes in each superfamily of Neogastropoda, a more densely taxon sampling is needed in future studies. The more comprehensive sampling from these families will help elucidate these relationships.

Declarations

Data Availability

The complete mitogenomes of *Babylonia formosae* and *Babylonia zeylanica* has been submitted to GenBank under the accession number of MK577482 and MN604402.

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Compliance with ethical standards

Conflict of Interest Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Author Contributions

HH analyzed the data, wrote the paper, and prepared the figures and tables. HY and JL collected field material and processed the samples. YY conceived and designed the experiments, reviewed drafts of the paper. BG supervised and directed the work. All authors reviewed the manuscript.

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Tables

Table 1. List of 63 Neogastopoda species and 3 outgroups used in this paper.

Species	Superfamily	Family	Length(bp)	Accession No.
Aeneator elegans	Buccinoidea	Buccinidae	15254	NC_039120.1
Aeneator recens	Buccinoidea	Buccinidae	15264	NC_039122.1
Buccinulum fuscozonatum	Buccinoidea	Buccinidae	15246	NC_039121.1
Antarctoneptunea benthicola	Buccinoidea	Buccinidae	15229	NC_039119.1
Kelletia lischkei	Buccinoidea	Buccinidae	15225	NC_039123.1
Penion ormesi	Buccinoidea	Buccinidae	15237	MH198169.1
Penion sulcatus	Buccinoidea	Buccinidae	15227	NC_037185.1
Penion maximus	Buccinoidea	Buccinidae	15249	NC_037237.1
Buccinum undatum	Buccinoidea	Buccinidae	15265	NC_040940.1
Neptunea arthritica	Buccinoidea	Buccinidae	15256	KU246047.1
Siphonalia subdilatata	Buccinoidea	Buccinidae	15393	MG827217.2
Cominella adspersa	Buccinoidea	Buccinidae	15251	NC_039125.1
Fusinus longicaudus	Buccinoidea	Fasciolariidae	16319	NC_045906.1
Hemifusus tuba	Buccinoidea	Melongenidae	15483	MN462591.1
Nassarius festivus	Buccinoidea	Nassariidae	15195	NC_037607.1
Nassarius fraterculus	Buccinoidea	Nassariidae	15174	NC_037604.1
Reticunassa hiradoensis	Buccinoidea	Nassariidae	15194	NC_037887.1
Reticunassa festiva	Buccinoidea	Nassariidae	15172	MG744570.1
Nassarius variciferus	Buccinoidea	Nassariidae	15269	NC_029173.1
Nassarius glans	Buccinoidea	Nassariidae	15296	NC_049091.1
Nassarius sinarus	Buccinoidea	Nassariidae	15325	NC_041545.1
Nassarius javanus	Buccinoidea	Nassariidae	15325	NC_041547.1
Nassarius foveolatus	Buccinoidea	Nassariidae	15343	NC_041546.1
Nassarius hepaticus	Buccinoidea	Nassariidae	15732	MH885313.1
Nassarius conoidalis	Buccinoidea	Nassariidae	15332	NC_041310.1
Nassarius siquijorensis	Buccinoidea	Nassariidae	15337	NC_048962.1
Nassarius pullus	Buccinoidea	Nassariidae	15278	NC_041311.1
Tritia obsoleta	Buccinoidea	Nassariidae	15263	DQ238598.1
Columbella adansoni	Buccinoidea	Columbellidae	16272	KP716637.2
Amalda northlandica	Olivoidea	Ancillariidae	15354	GU196685.1
Babylonia formosae	Unassigned	Babayloniidae	16214	MK577482
Babylonia zeylanica	Unassigned	Babayloniidae	16181	MN604402
Babylonia lutosa	Unassigned	Babayloniidae	15346	KF897830.1
Babylonia areolata	Unassigned	Babayloniidae	15445	HQ416443.1
Conus quercinus	Conoidea	Conidae	16430	KY609509.1
Conus betulinus	Conoidea	Conidae	16240	MG924728.1
Conus gloriamaris	Conoidea	Conidae	15774	KU996360.1
Conus consors	Conoidea	Conidae	16112	KF887950.1
Lilliconus sagei	Conoidea	Conidae	15485	KX263255.1
Profundiconus teramachii	Conoidea	Conidae	15279	KX263256.1

Clavatula tripartita	Conoidea	Clavatulidae	15743	MH308391.1
Fusiturris similis	Conoidea	Fusiturridae	15595	EU827197.1
Oxymeris dimidiata	Conoidea	Terebridae	16513	NC_013239.1
Boreotrophon candelabrum	Muricoidea	Muricidae	15265	NC_046505.1
Ceratostoma burnetti	Muricoidea	Muricidae	15334	NC_046569.1
Ceratostoma rorifluum	Muricoidea	Muricidae	15338	NC_046526.1
Ocinebrellus falcatus	Muricoidea	Muricidae	15326	NC_046052.1
Ocinebrellus inornatus	Muricoidea	Muricidae	15324	NC_046577.1
Bolinus brandaris	Muricoidea	Muricidae	15380	EU827194.1
Murex trapa	Muricoidea	Muricidae	15408	MN462589.1
Chicoreus torrefactus	Muricoidea	Muricidae	15359	NC_039164.1
Concholepas concholepas	Muricoidea	Muricidae	15495	JQ446041.1
Reishia clavigera	Muricoidea	Muricidae	15285	DQ159954.1
Rapana venosa	Muricoidea	Muricidae	15271	KM213962.1
Menathais tuberosa	Muricoidea	Muricidae	15294	KU747972.1
Cymbium olla	Volutoidea	Volutidae	15375	EU827199.1
Melo melo	Volutoidea	Volutidae	15721	MN462590.1
Neptuneopsis gilchristi	Volutacea	Volutidae	15312	MN125492.1
Splendrillia sp	Conoidea	Drilliidae	15358	MH308395.1
Lophiotoma cerithiformis	Conoidea	Turridae	15380	DQ284754.1
Gemmuloborsonia moosai	Conoidea	Turridae	15541	NC_038183.1
Bivetiella cancellata	Volutoidea	Cancellariidae	16648	NC_013241.1
Costapex sp	Turbinelloidea	Costellariidae	15321	MW044625.1
Haliotis rufescens	Haliotoidea	Haliotidae	16646	NC_036928.1
Lottia digitalis	Lottioidea	Lottiidae	26835	DQ238599.1
Haliotis rubra	Haliotoidea	Haliotidae	16907	AY588938.1

Table 2. Genome composition of two newly sequenced Babayloniidae species (*Babylonia formosae* and *Babylonia zeylanica*)

	Babylonia formosae							*Babylonia zeylanica*						
Region	Size(bp)	A(%)	T(%)	G(%)	C(%)	A+T(%)	AT-skew	GC-skew	Size(bp)	A(%)	T(%)	G(%)	C(%)	A+T(%)	AT-skew
Mitogenome	16214	29	36.7	17.3	17.3	65.41	-0.122	0.002	16181	29.4	37	16.8	16.9	66.32	-0.1
cox1	1512	26	37	18.9	18.2	62.96	-0.174	0.018	1533	26	37.8	18.5	17.7	63.8	-0.18
cox2	666	28	35	19.5	17.7	62.76	-0.115	0.048	687	29.4	34.2	18.6	17.8	63.61	-0.0
atp8	156	35	35.9	12.8	16.7	70.52	-0.018	-0.130	159	35.2	36.5	13.2	15.1	71.7	-0.0
atp6	675	25	39.6	16.9	18.4	64.75	-0.222	-0.042	696	26.6	38.9	15.4	19.1	65.52	-0.18
cox3	774	21	39.2	23.8	16.4	59.82	-0.309	0.183	780	22.1	38	22.6	17.4	60	-0.2
nad3	351	22	42.2	21.9	14	64.11	-0.316	0.222	354	24	44.1	20.1	11.9	68.08	-0.2
nad1	894	23	40.8	19.1	16.6	64.32	-0.270	0.072	942	25.3	41.2	17.3	16.2	66.46	-0.2
nad5	1671	27	37.3	13.9	22	64.09	-0.163	-0.227	1731	28.3	37.3	13.5	20.9	65.57	-0.1
nad4	1347	28	37.7	14.3	20.3	65.4	-0.153	-0.176	1374	28.4	38.2	13.8	19.6	66.59	-0.1
nad4l	264	28	38.3	15.5	18.6	65.91	-0.161	-0.089	297	31.3	37.7	15.2	15.8	69.02	-0.0
nad6	447	23	47.2	13.7	16.6	69.8	-0.353	-0.096	498	25.1	44.6	11.7	18.7	69.68	-0.2
cob	1119	25	37	15.7	21.8	62.47	-0.185	-0.162	1140	25.4	38.6	16.1	20	63.95	-0.2
nad2	930	27	38.3	20.2	14.8	64.95	-0.179	0.153	1056	28.7	39.4	18.1	13.8	68.08	-0.1
tRNAs	1483	34	32.6	19.1	14.2	66.69	0.023	0.146	1485	34.2	32.7	19.2	13.9	66.87	0.02
rRNAs	2281	37	31.8	18.3	12.9	68.83	0.076	0.173	2274	37.1	32	17.9	13	69.08	0.07
PCGs	10806	26	38.4	17.3	18.6	64.05	-0.198	-0.037	11247	26.9	38.6	16.5	18	65.52	-0.1

Table 3. Start/Stop codons of protein-coding genes (PCGs) from Babayloniidae species (*Babylonia formosae* and *Babylonia zeylanica*)

Species	*Babylonia formosae*		*Babylonia zeylanica*	
PCGs	Start	Stop	Start	Stop
cox1	ATG	TAA	ATG	TAA
cox2	ATG	TAA	ATG	TAA
atp8	ATG	TAA	ATG	TAA
atp6	ATG	TAA	ATG	TAG
nad1	ATG	TAG	ATG	TAA
nad6	ATT	TAG	ATT	TAA
cob	ATG	TAA	ATG	TAA
nad4l	ATG	TAG	ATG	TAG
nad4	ATG	TAA	ATT	TAA
nad5	ATT	TAA	ATT	TAA
cox3	ATT	TAA	ATG	TAG
nad3	ATG	TAA	ATG	TAA
nad2	ATG	TAA	ATT	TAA

Figures

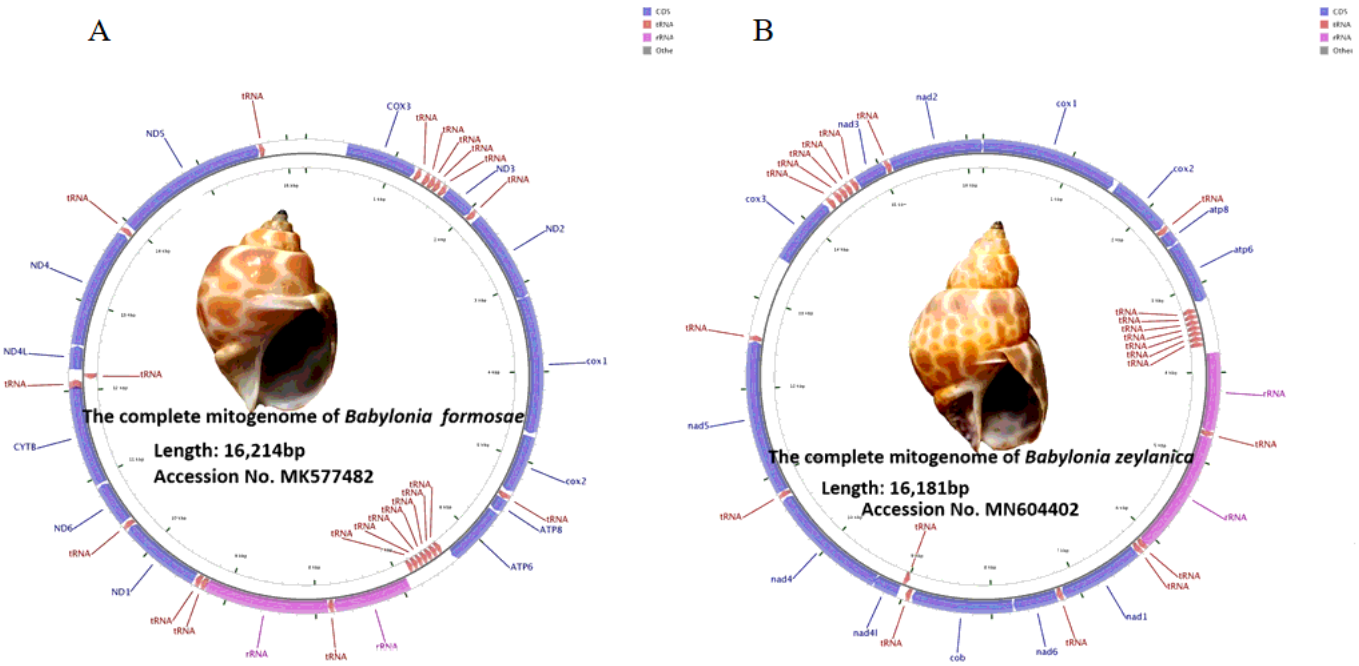

Figure 1

Maps of the mitochondrial genomes of two Babylonia species (B. formosae (A) and B. zeylanica (B))

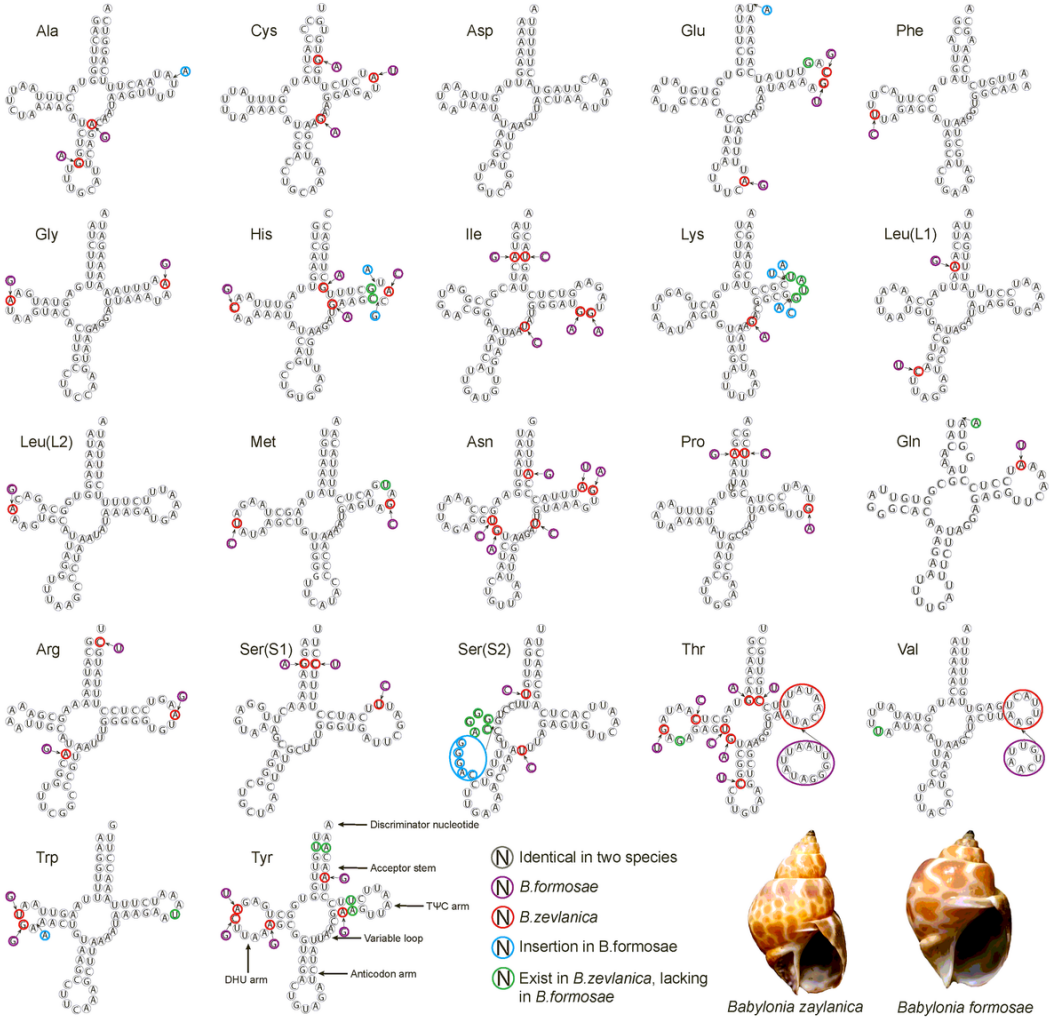

Figure 2

Secondary structure of tRNAs of B. formosae and B. zeylanica

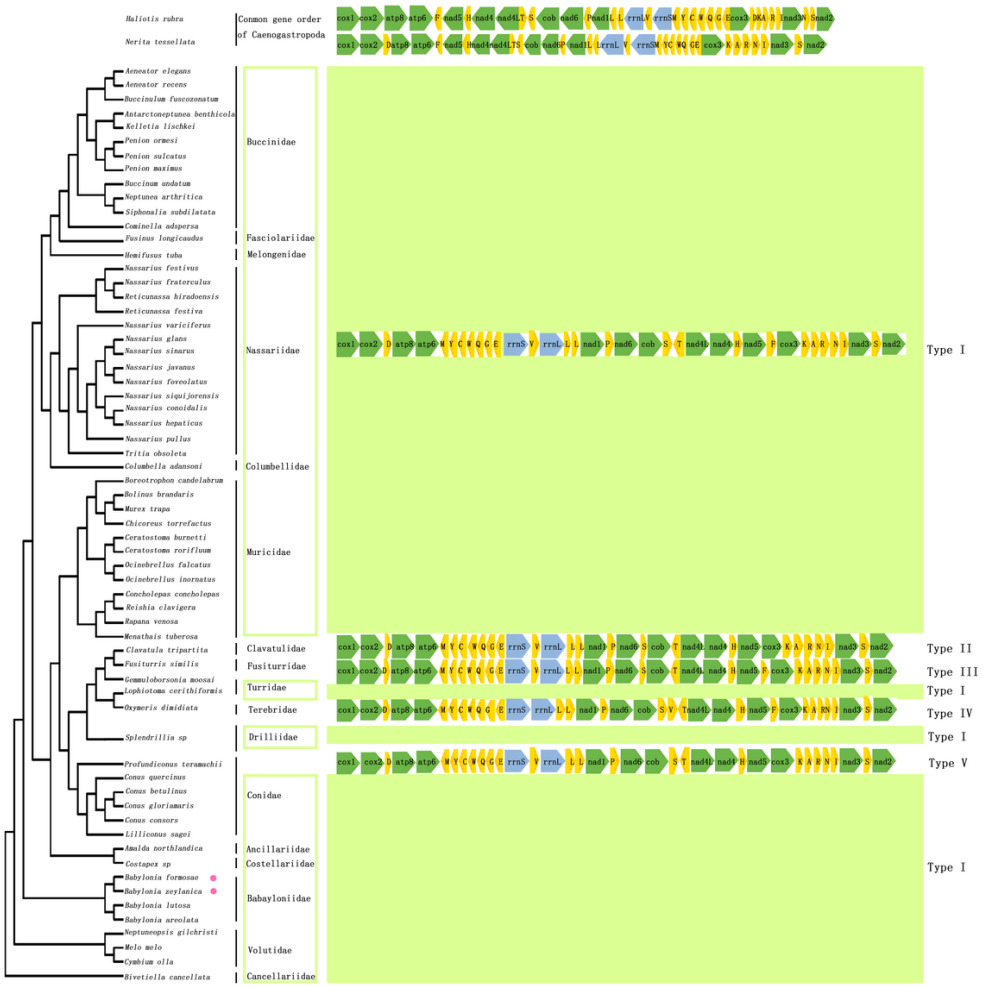

Figure 3

Mitochondrial genome organization of the two new Babyloniidae species and mitochondrial gene arrangement pattern of seventeen major lineage of Neogastropoda. The topology was generated from phylogenetic analysis. The direction of the arrows indicates the strand orientation (“+strand” to the right and “− strand” to the left; and the light green boxes on the outside indicate that the species are in the same gene order).

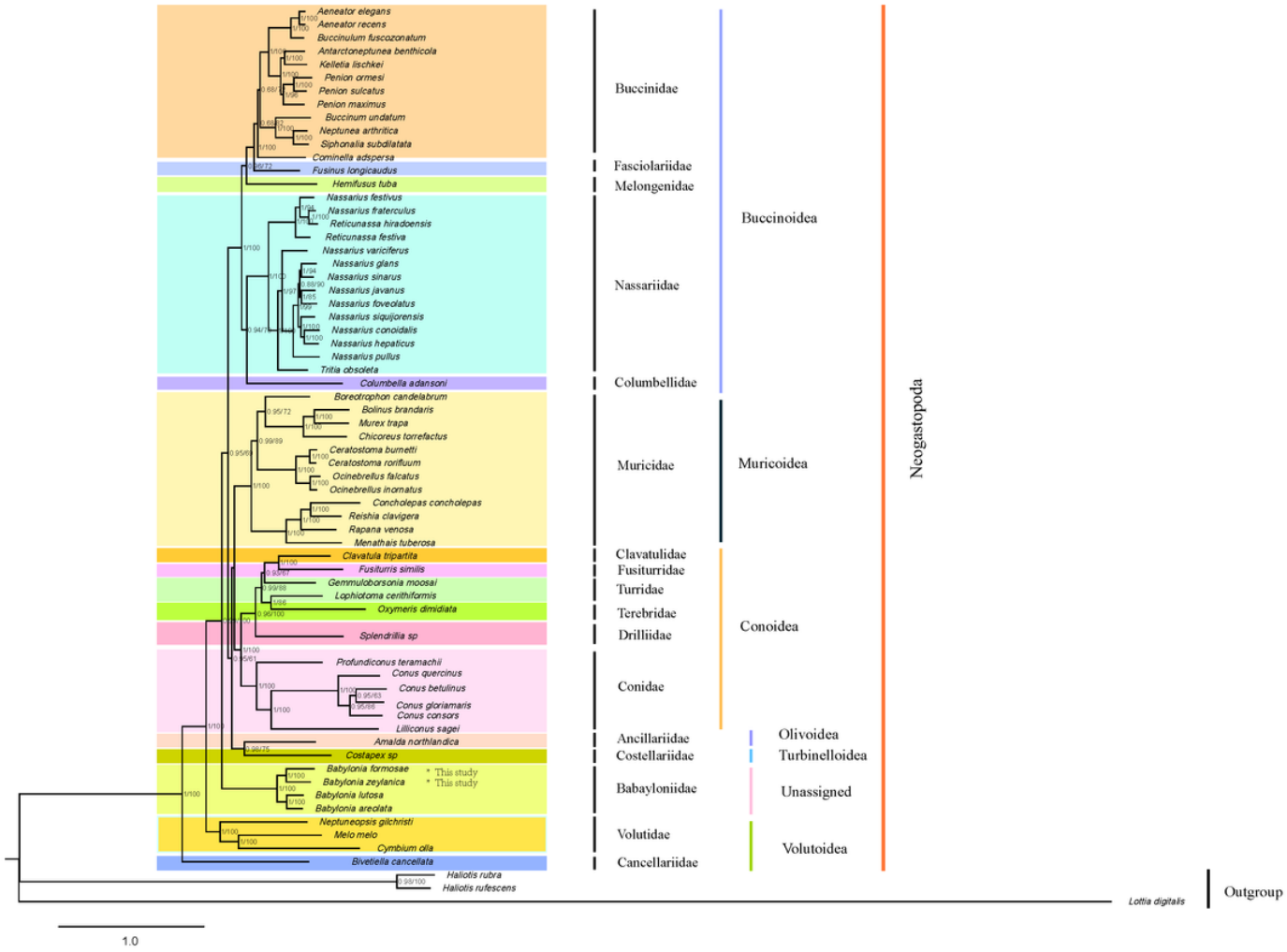

Figure 4

Phylogeny of Neogastropoda based on nucleotide sequences. The phylogenetic tree was inferred from the nucleotide sequences of 13 mitogenome PCGs using BI and ML methods. Numbers on branches indicate posterior probability. The different colored lines represent the families and superfamilies of these species. Rectangles with different background colors are used to distinguish different families.