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Brief Report

The Mitochondrial Genome of *Littoraria melanostoma* Reveals a Phylogenetic Relationship within Littorinimorpha

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Abstract: *Littoraria melanostoma* (Gray, 1839) is one of the most common species of gastropods in mangroves. They quickly respond during the early stage of mangrove restoration and usually form a dominant community within a certain period. We characterized the complete mitochondrial genome of this species. The whole mitogenome of *L. melanostoma* was 16,149 bp in length and its nucleotide composition showed a high AT content of 64.16%. It had 37 genes, including 13 protein-coding genes, 2 ribosomal RNA genes, and 22 transfer RNA genes, and 1 control region between tRNA-Phe and *COX3*. The A/T composition in the control region was 74.7%, and is much higher than the overall A/T composition of the mitochondrial genomes. The amino acid composition and codon usage of the mitochondrial genomes from seven superfamilies of Littorinimorpha were analyzed, and the results showed that CUU (Leu), GCU (Ala), AUU (Ile), UCU (Ser), UUA (Leu), GUU (Gly), and UUU (Phe) are the commonly used codons. The maximum likelihood phylogenetic tree reconstructed using 62 species of Littorinimorpha presented consistency between the molecular and morphological classifications, which provide a basis to understand the phylogeny and evolution of this order. In the phylogenetic tree, *L. melanostoma* is located within Littorinoidea and is closely related to *L. sinensis*, a rock-dwelling species that is widespread in the coastal intertidal zone of China.

Keywords: mangrove; gastropods; phylogeny; evolution; Littorinimorpha

1. Introduction

Mangroves are woody plant communities that become established on the intertidal flats of tropical and subtropical coasts. The harsh natural conditions of the coastal intertidal zone and the unique advantages of the transition zone between the land and sea have produced the unique biome of the mangrove ecosystem. In addition, the resulting extremely rich biodiversity also plays a crucial role in the ecosystem. As one mollusk group in mangroves, gastropods play an important role in the detritus cycle and consume a large amount of plant tissue and humus [1].

Littorinidae (Children, 1834), one of the major groups of arboreal gastropods in mangrove forests, adapt to the special environment of the coastal intertidal zone through numerous physiological and ecological manners, including their multiple reproductive means, which are known as a variable reproductive strategy [2]; complex food composition [3]; vertical climbing ability [4–6]; and variable shell color and shape [7]. As gastropods have a limited ability to move, they only generally move on the same tree without external interference [8], and it is difficult for them to migrate from the intertidal zone to land. Consequently, they have a relatively fixed pattern of spatial distribution [9,10]. Simultaneous studies have shown that species of Littorinidae respond quickly to the restoration of mangrove vegetation, and that they can form a dominant community in the early stage of vegetation restoration, as exemplified by *Littoraria melanostoma* [11]. Most of the Littorinidae in mangroves live on mangrove and salt marsh plants, driftwood, and stakes.

The family Littorinidae comprises more than 200 species that are common members of marine intertidal communities around the world, and most of them only live on mangrove plants [12]. Species of Littorinidae living on mangroves that have been reported in China include *L. melanostoma*, *L. ardouiniana*, *L. intermedia*, *L. pallescens*, *L. articulata*, and *L. scabra*, etc. [13–15]. *L. melanostoma* is often found in the branches and leaves of mangroves (Figure 1).
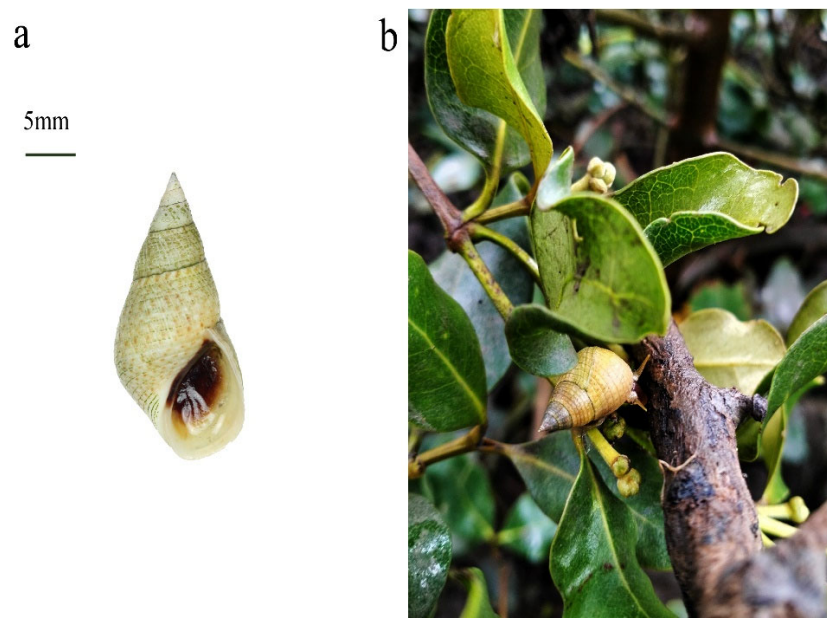
Figure 1. (**a**) Specimen image of *L. melanostoma*. (**b**) *L. melanostoma* inhabited on the leaves of *Avicennia marina* (a common species of mangrove in China).

Morphological differences make the identification of gastropods confusing, and the classification information is constantly adjusted. In recent years, molecular systematic studies were used to analyze the evolution and phylogeny of gastropods. Reid et al. used nuclear 28S rRNA, mitochondrial 12S rRNA, and COI to construct the phylogeny of the family Littorinidae [12] and the genus *Littoraria* [16]. Li et al. used complete mitochondrial genome sequences to analyze the phylogenic relationship between the genera *Littorina* and *Littoraria*, but only four species were used in this study [17]. The phylogeny of Stromboidea, a superfamily in Littorinimorpha, was studied based on 13 mitochondrial protein-coding genes [18]. A phylogenetic study based on the mt genomes of deep-sea gastropods from Abyssochrysoidea indicated that this superfamily belonged to Littorinimorpha [19,20], but up to now, in the MolluscaBase (www.molluscabase.org), (accessed on 5 September 2023) their taxonomic status of order level remains uncertain [21].

Through searching in Genbank, we found that the number of gastropods with available mitogenomes has increased. Up to August 2023, the complete or partial mitochondrial genome of ~100 Littorinimorpha species were available in the National Center of Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov), but there were few studies on the phylogenetic analyses that investigate relationships across the Littorinimorpha.

In this study, *L. melanostoma*, one of the most common Gastropoda species in the mangrove wetlands of China, was studied for its molecular evolution and phylogeny. The mitochondrial genomes of *L. melanostoma* was sequenced, and the genome structure, base composition, codon usage, intergenic region, and codon preference of the mitochondrial genomes were analyzed. In addition, a phylogenetic tree of 62 species from 9 superfamilies of Littorinimorpha based on 13 protein-coding genes (PCGs) was constructed using the maximum likelihood (ML) method. This study may increase our understanding of the phylogeny and evolution of Littorinimorpha.

2. Materials and Methods

2.1. Sample Collection and DNA Extraction

Specimens of *L. melanostoma* was obtained from mangrove wetlands in Beihai, Guangxi, China (21.57° N, 109.16° E), and vouchered in the specimen room of the Guangxi Mangrove Research Center (Accession numbers LM#11-20, respectively). Since this species consists of unprotected invertebrates, no specific permission was required to collect samples from these locations. Total genomic DNA was obtained from the muscles of individuals using a QIAamp DNA Micro Kit (Qiagen, Hilden, Germany).

2.2. Sequencing, Assembling and Analysis

The *cox1* gene was amplified using the universal primers LCO1490 and HCO2198 via the standard PCR method [22]. The extracted DNA was sequenced using a NovaSeq 6000 platform (Illumina, San Diego, CA, USA), and the mitogenome was assembled with NOVOPlasty v2.7.0 [23] and annotated with MitoZ v2.4 [24]. PCGs were determined through determining the open reading frames (ORFs) based on the invertebrate mitochondrial genetic code, and rRNAs and tRNAs were identified using the MITOS Web Server (http://mitos2.bioinf.uni-leipzig.de/index.py) [25]. The codon usage was calculated using MEGA 7.0 [26]. Strand bias was calculated using the following formulae: AT skew = (A − T)/(A + T), and GC skew = (G − C)/(G + C) [27]. The circular maps of the mitochondrial genomes were drawn using the online mitochondrial visualization tool Organellar Genome DRAW [28]. The nucleotide composition, codon usage, and comparative mitogenomic architecture tables for the mitogenomes and data that were used to plot the relative synonymous codon usage (RSCU) figures were all calculated/created using PhyloSuite [29].

2.3. Phylogenetic Analysis

The phylogenetic analyses were based on the concatenated nucleotide and amino acid alignments of 13 PCGs in 62 complete mt genomes, including *L. melanostoma*, and 61 species from 9 superfamilies of Littorinimorpha (Table 1). The nucleotide sequences of the complete mt genomes were downloaded from GenBank. The total quantity of complete mt genomes and amino acid sequences of 13 PCGs were aligned using MAFFT v.7.215 [30]. Following this, MACSE (multiple alignment of coding sequences) was used to account for frameshifts and stop codons with the score matrix 'BLOSUM62', which were then trimmed with Gblocks [31], with the minimum length of a block being '10' and the maximum number of contiguous non-conserved positions being '8', respectively. The phylogenetic tree was reconstructed using IQ-TREE v2 [32] based on ML with the partitioning method [33], and the parameter used was Bootstrap: ultrafast, num of bootstrap: 10,000, max of iteration: 1000, and minimum correlation coefficient: 0.9. The phylogenetic tree was visualized using the Interactive Tree Of Life (iTOL) v5 (https://itol.embl.de) [34].

Table 1. Classification and origins of the mitogenomic sequences used in this study.

Taxonomy Superfamily	Species	bp	Accession Number
	Littoraria melanostoma (Gray, 1839)	16,149	NC064398
	Littoraria ardouiniana (Heude, 1885)	16,261	ON920192
	Littoraria sinensis (R. A. Philippi, 1847)	16,420	MN496138
Littorinoidea	*Littorina brevicula* (R. A. Philippi, 1844)	16,356	MT362562
	Littorina saxatilis (Olivi, 1792)	16,887	KU952094
	Littorina fabalis (W. Turton, 1825)	16,418	KU952092
	Melarhaphe neritoides (Linnaeus, 1758)	15,676	MH119311

Table 1. *Cont.*

Taxonomy Superfamily	Species	bp	Accession Number
Stromboidea	*Harpago chiragra* (Linnaeus, 1758)	16,404	MN885884
	Lambis lambis (Linnaeus, 1758)	15,481	MH115428
	Conomurex luhuanus (Linnaeus, 1758)	15,799	KY853669
	Tibia fusus (Linnaeus, 1758)	16,083	MZ359282
	Xenophora japonica Kuroda & Habe, 1971	15,684	MW244823
	Laevistrombus canarium (Linnaeus, 1758)	15,626	MT937083
	Varicospira cancellate (Lamarck, 1816)	15,864	MW244822
	Tridentarius dentatus (Linnaeus, 1758)	15,500	MW244820
	Terebellum terebellum (Linnaeus, 1758)	15,478	MW244821
	Struthiolaria papulose (Martyn, 1784)	15,475	MW244818
	Aporrhais serresiana (Michaud, 1828)	15,455	MW244817
	Ministrombus variabilis (Swainson, 1820)	15,292	MW244824
	Onustus exutus (Reeve, 1842)	16,043	MK327366
Cypraeoidea	*Cypraea tigris* Linnaeus, 1758	16,177	MK783263
	Purpuradusta gracilis (Gaskoin, 1849)	16,240	OP723877
	Mauritia arabica (Linnaeus, 1758)	16,926	MZ667219
	Lyncina vitellus (Linnaeus, 1758)	16,269	OP714183
	Naria erosa (Linnaeus, 1758)	16,020	OP738004
	Erronea caurica (Linnaeus, 1758)	15,857	OP714186
	Monetaria annulus (Linnaeus, 1758)	16,087	LC469295
Naticoidea	*Paratectonatica tigrine* (Röding, 1798)	16,148	MN419027
	Naticarius hebraeus (Martyn, 1786)	15,384	KP716634
	Polinices sagamiensis Pilsbry, 1904	15,383	MK478018
	Mammilla mammata (Röding, 1798)	15,319	MK433194
	Cryptonatica andoi (Nomura, 1935)	15,302	MK433195
	Euspira gilva (R. A. Philippi, 1851)	16,119	MN419026
	Neverita didyma (Röding, 1798)	15,252	MK548644
	Notocochlis gualteriana (Récluz, 1844)	15,176	MK500872
	Glossaulax reiniana (Dunker, 1877)	15,254	MH543334
	Tanea lineata (Röding, 1798)	15,156	MK507894
Vermetoidea	*Dendropoma gregarium* M. G. Hadfield & Kay, 1972	15,641	HM174252
	Ceraesignum maximum (G. B. Sowerby I, 1825)	15,578	HM174253
	Thylacodes squamigerus (P. P. Carpenter, 1857)	15,544	HM174255
	Eualetes tulipa (Rousseau, 1843)	15,078	NC_014585
Tonnoidea	*Charonia lampas* (Linnaeus, 1758)	15,405	MG181942
	Tonna galea (Linnaeus, 1758)	17,504	OR282483
	Ficus variegate Röding, 1798	15,736	MW376482
	Bursa rhodostoma (G. B. Sowerby II, 1835) [#]	15,393	MW316791
	Tutufa rubeta (Linnaeus, 1758)	15,397	MW316790
	Galeodea echinophora (Linnaeus, 1758)	15,388	KP716635
	Monoplex parthenopeus (Salis Marschlins, 1793)	15,270	EU827200
Truncatelloidea	*Oncomelania hupensis* Gredler, 1881	15,191	EU079378
	Stenothyra glabra A. Adams, 1861	15,704	MZ196218
	Tricula hortensis Attwood & D. S. Brown, 2003	15,179	EU440735
	Potamopyrgus antipodarum (J. E. Gray, 1843)	16,846	OQ161206
	Bithynia leachii (Sheppard, 1823)	15,682	MT410857
	Godlewskia godlewskii (W. Dybowski, 1875)	15,224	KY697387
	Baicalia turriformis (W. Dybowski, 1875)	15,127	KY697386
	Korotnewia korotnewi (Lindholm, 1909) [##]	15,171	KY697389
	Maackia herderiana (Lindholm, 1909)	15,154	KY697388
Calyptraeoidea	*Desmaulus extinctorium* (Lamarck, 1822)	16,608	OQ511529

Table 1. *Cont.*

Taxonomy Superfamily	Species	bp	Accession Number
Abyssochrysoidea	*Alviniconcha adamantis* S. B. Johnson, Warén, Tunnicliffe, Van Dover, Wheat, T. F. Schultz & Vrijenhoek, 2014	16,342	OL351262
	Desbruyeresia armata C. Chen, H. K. Watanabe & Sasaki, 2019	15,825	OM063153
	Provanna clathrate Sasaki, Ogura, H. K. Watanabe & Fujikura, 2016	15,344	OK632464
	Ifremeria nautilei Bouchet & Warén, 1991	15,664	KC757644

\# *Bursa rhodostoma* (G. B. Sowerby II, 1835) is now accepted as *Lampasopsis rhodostoma* (G. B. Sowerby II, 1835). \#\# The species name *Korotnewia korotnewi* in NCBI is incorrect; it should be *Korotnewia korotnevi* [21].

3. Results and Discussion

3.1. Genome Structure and Organization

The complete *L. melanostoma* mitochondrial genome was 16,149 bp long, and it was uploaded to GenBank after annotation (ACCESSION ID: NC064398). The gene compositions of *L. melanostoma* were the same as most of the mitochondrial genomes of gastropods that have been published, and contained 37 genes, including 13 protein-coding, 2 rRNA, and 22 tRNA genes [24,35] (Figure 2). According to the difference in G+T content, the two strands of mitochondrial DNA could be separated into a heavy strand (H strand) and a light strand (L strand). The 13 protein-coding genes in the mitochondrial genomes of *L. melanostoma* are located on the H strand, which is consistent with the findings of previous studies that showed that the mitochondrial genomes of Littorinidae, such as *Littorina fabalis*, *Littorina obtusata*, and *Littorinasaxatilis*, harbor protein-encoding genes on the H strand [35]. Most genes were located on the H strand, except for the eight tRNAs that were located on the L strand, and include *trnM* (CAU), *trnY* (GUA), *trnC* (GCA), *trnW* (UCA), *trnQ* (UUG), *trnG* (UCC), *trnE* (UUC), and *trnT* (UGU). The thirteen PCGs include seven NADH dehydrogenase genes (complex I)—*ND1*, *ND2*, *ND3*, *ND4*, *ND4L*, *ND5*, and *ND6*; three cytochrome c oxidase genes (complex IV)—*COX1*, *COX2*, and *COX3*; two ATPase subunits (*ATP6* and *ATP8*); and one cytochrome b gene.

Table 2 summarizes the proportions of gene bases and protein-coding gene sequence bases in the complete mitochondrial genome sequences of *L. melanostoma*. The base composition of the *L. melanostoma* mitochondrial genome was 29.79% A, 34.37% T, 14.66% G, and 21.18% C. The A+T content (64.16%) of its mitochondrial genes was higher than the G+C content (35.84%), and the A+T content of the protein-coding genes was 62.39%. These results indicate that *L. melanostoma* genomes display an obvious nucleotide composition that is biased to A+T, which is consistent with the other genomes of Littorinidae species that have been reported [35]. The base composition bias is usually reflected by the AT skew and GC skew. The calculated AT skew and GC skew of the *L. melanostoma* mitochondrial genome were 0.071 and −0.182, respectively. These data indicate that the bases T and C appear more frequently than A and G in the mitochondrial genomes of *L. melanostoma*.

Table 2. Composition and base content of the *L. melanostoma* protein-coding genes.

	Littoraria melanostoma							
	A%	T%	G%	C%	AT%	GC%	AT Skew	GC Skew
Mitogenome	29.79	34.37	14.66	21.18	64.16	35.84	−0.071	−0.182
All PCGs	27.77	34.62	15.15	22.46	62.39	37.61	−0.110	−0.194
COX1	26.43	34.18	17.71	21.68	60.61	39.39	−0.128	−0.101
COX2	28.97	30.57	17.76	22.71	59.53	40.47	−0.027	−0.122
ATP8	32.70	36.48	10.69	20.13	69.18	30.82	−0.055	−0.306
ATP6	26.44	36.06	13.22	24.28	62.50	37.50	−0.154	−0.295
ND1	26.09	34.50	15.23	24.17	60.60	39.40	−0.139	−0.227
ND6	27.31	34.94	13.05	24.70	62.25	37.75	−0.123	−0.309
CYTB	25.70	33.07	15.09	26.14	58.77	41.23	−0.125	−0.268

Table 2. *Cont.*

| | *Littoraria melanostoma* | | | | | | | |
	A%	T%	G%	C%	AT%	GC%	AT Skew	GC Skew
ND4L	25.70	33.07	15.09	26.14	68.01	31.99	−0.125	−0.268
ND4	28.56	37.21	13.57	20.66	65.77	34.23	−0.132	−0.207
ND5	30.15	33.13	13.35	23.36	63.29	36.71	−0.047	−0.273
COX3	25.90	32.18	19.36	22.56	58.08	41.92	−0.108	−0.076
ND3	27.35	39.32	15.10	18.23	66.67	33.33	−0.180	−0.094
ND2	28.89	37.38	14.39	19.33	66.27	33.73	−0.128	−0.147

Figure 2. Circular maps of the mitogenomes of *L. melanostoma*.

3.2. PCGs and Codon Usage

The nucleotide lengths of the 13 PCGs of *L. melanostoma* were 11,034 bp, which encode 3678 amino acid residues in total. Most of the PCGs that were examined began with the ATN codon and ended with either the TAA or TAG codons (Table 3).

The codon usage of 13 PCGs in the *L. melanostoma* mitochondrial genomes is shown in Table 3. In the *L. melanostoma* genome, only one gene (*ND3*) used ATA as its start codon, while two used ATT as their start codon, namely *ND4* and *ND5*. The remaining 10 genes (*COX1*, *COX2*, *ATP8*, *ATP6*, *ND1*, *ND6*, *CYTB*, *ND4L*, *COX3*, and *ND2*) all used ATG as their start codon. *ND4L*, *ND5*, and *ND2* used TAG, CTT, and AAT as their stop codon, respectively, and these codons were each used by only one gene. There were 10 genes (*COX1*, *COX2*, *ATP8*, *ATP6*, *ND1*, *ND6*, *CYTB*, *ND4*, *COX3*, and *ND3*) that used TAA as their stop codon.

Table 3. Mitogenomic organization of *L. melanostoma*.

		Position		Size (bp)	Intergenic Nucleotides	Codon		Strand
	Gene	From	To			Start	Stop	
1	*COX1*	1	1536	1536		ATG	TAA	H
2	*COX2*	1575	2261	687	38	ATG	TAA	H
3	trnD(guc)	2268	2336	69	6			H
4	*ATP8*	2338	2496	159	1	ATG	TAA	H
5	*ATP6*	2512	3207	696	15	ATG	TAA	H
6	trnM(cau)	3240	3306	67	32			L
7	trnY(gua)	3310	3377	68	3			L
8	trnC(gca)	3382	3446	65	4			L
9	trnW(uca)	3448	3514	67	1			L
10	trnQ(uug)	3514	3578	65	−1			L
11	trnG(ucc)	3590	3656	67	11			L
12	trnE(uuc)	3710	3777	68	53			L
13	s-rRNA	3856	4756	901	78			H
14	trnV(uac)	4754	4822	69	−3			H
15	l-rRNA	4801	6219	1419	−22			H
16	trnL(uaa)	6210	6277	68	−10			H
17	trnL(uag)	6284	6352	69	6			H
18	*ND1*	6353	7291	939	0	ATG	TAA	H
19	trnP(ugg)	7301	7369	69	9			H
20	*ND6*	7374	7871	498	4	ATG	TAA	H
21	*CYTB*	7890	9029	1140	18	ATG	TAA	H
22	trnS(uga)	9040	9107	68	10			H
23	trnT(ugu)	9111	9178	68	3			L
24	*ND4L*	9185	9481	297	6	ATG	TAG	H
25	*ND4*	9505	10845	1341	23	ATT	TAA	H
26	trnH(gug)	10852	10918	67	6			H
27	*ND5*	10947	12624	1678	28	ATT	CTT	H
28	trnF(gaa)	12663	12732	70	38			H
	CR	12733	13505	773	0			
29	*COX3*	13506	14285	780	773	ATG	TAA	H
30	trnK(uuu)	14307	14378	72	21			H
31	trnA(ugc)	14385	14451	67	6			H
32	trnR(ucg)	14459	14527	69	7			H
33	trnN(guu)	14533	14602	70	5			H
34	trnI(gau)	14604	14671	68	1			H
35	*ND3*	14679	15029	351	7	ATA	TAA	H
36	trnS(gcu)	15029	15095	67	−1			H
37	*ND2*	15123	16053	931	27	ATG	AAT	H

Figure 3 shows the amino acid composition and codon usage of the mitochondrial genomes of eight species from seven superfamilies. The results showed that CUU (Leu), GCU (Ala), AUU (Ile), UCU (Ser), UUA (Leu), GUU (Gly), and UUU (Phe) were the mostly commonly used codons. These observations suggest that there is a strong AT bias for protein-coding genes in the mitochondrial genomes of Littorinidae animals.

3.3. Ribosomal and Transfer RNA Genes

Two rRNA genes, l-rRNA and s-rRNA, are located between *trnL* (UAA) and *trnV* (UAA), and between *trnV* (UAA) and *trE* (UUC), respectively.

In the mitochondrial genome of *L. melanostoma*, l-rRNA was 1419 bp, and s-rRNA was 901 bp (Table 3). A total of 22 tRNA genes were found in *L. melanostoma*, and its cloverleaf structures was 65–72 bp.

3.4. Intergenic Spaces and Overlapping Sequences

There were five overlapping gene regions in the mitochondrial genome of *L. melanostoma*, which ranged from 1 to 22 bp in length, and 30 intergenic regions, which ranged from 1 to 773 bp long. The longest intergenic region was located between *trnF* (GAA) and *COX3* (Table 3).
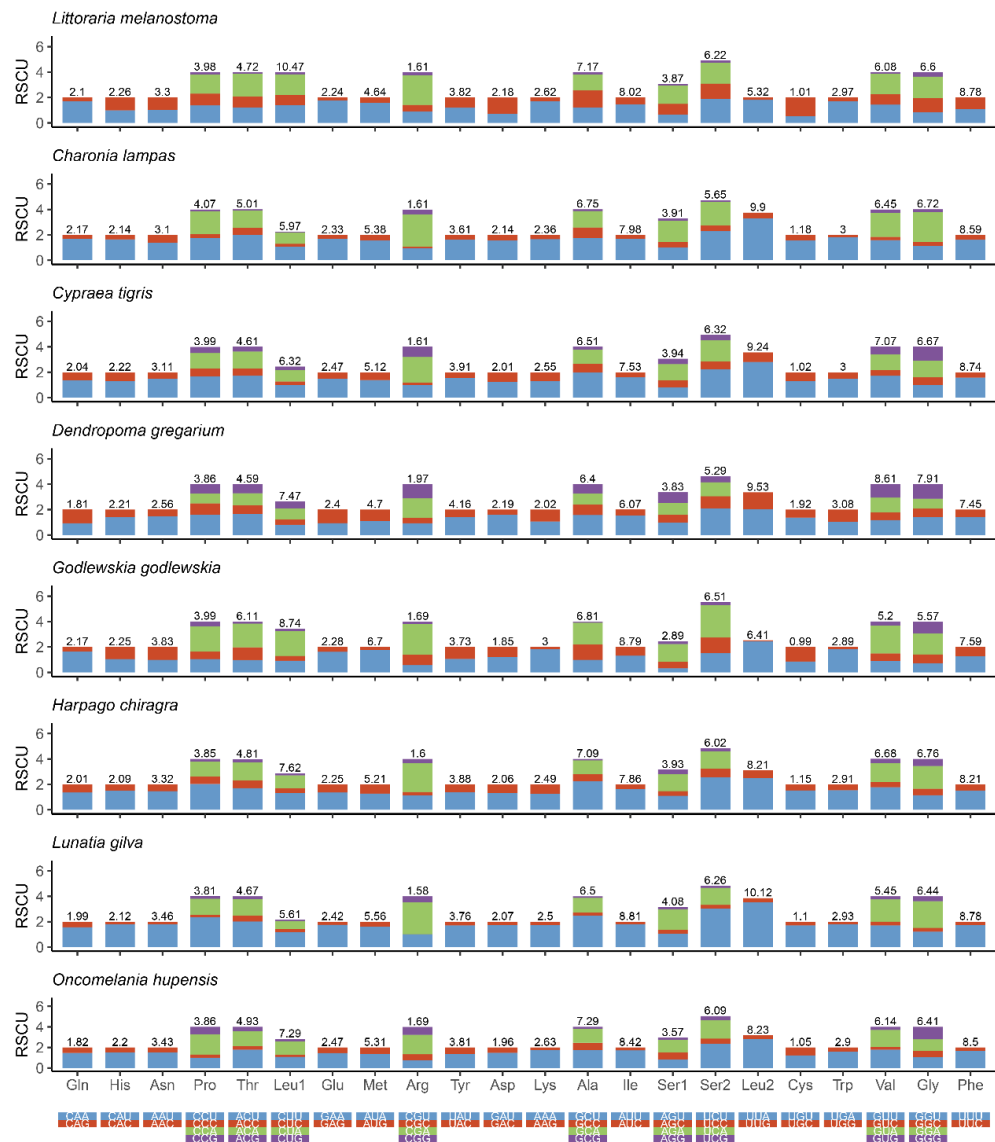
Figure 3. Relative synonymous codon usage (RSCU) in the mitogenomes of eight species of Littorinimorpha.

3.5. Control Regions

The control region (CR) of mitochondrial DNA is the primary non-coding region of the mitochondrial genome of animals, also known as the D-loop region, which is a key part for the replication and transcription of the mitochondrial genome and regulates the replication and transcription of the mitochondrial genome. During the process of evolution, since the selection pressure that acts on this region is relatively non-intrusive, the CR usually displays the largest sequence and variation in length, the highest rate of evolution, and is the most polymorphic in the mitochondrial genome [36]. However, since the UTR sequences of invertebrates are poorly conserved, there is no defined CR in their mitochondrial genomes [37]. For example, Marques studied the genomes of *L. fabalis*, *L. obtusata*, and *L. saxatilis*, and found a region that contained some unique features, such as a non-coding region with a hairpin structure and a tandem repeat sequence, located between tRNA-Phe (*trnF* [GAA]) and *COX3*, and an AT content that was higher than the overall AT content in the mitochondrial genome. This region was then predicted as the CR. Similarly, we found a non-coding sequence that contained some unique features in the *L. melanostoma* genomes. It was between tRNA-Phe and *COX3*, and the AT content was 74.7%. This is much higher than the AT content of the mitochondrial genomes (64.16%).

Thus, we consider that this region is a unique non-coding region of the Littorinoidea superfamily, which may play a regulatory role in the replication and transcription of the mtDNA of this genus.

3.6. Phylogenetic Analyses

To further study the genetic background and taxonomic relationship of Littorinimorpha, the complete mitochondrial genome sequences of *L. melanostoma* were compared with the complete mitochondrial genome sequences of sixty-one other species from nine superfamilies of Littorinimorpha. A phylogenetic tree was constructed based on 13 PCGs using IQ-TREE v2 with the ML method (Figure 4). The phylogenetic relationship was found to be significantly consistent with the results using the traditional classification method. The protagonist of this study, *L. melanostoma*, was included in the Littorinoidea clade, and is closely related to *L. sinensis*, which is a rock-dwelling species that is widespread in the coastal intertidal zone of China, just as in the previous study [16]. Littorinoidea and Naticoidea were grouped into one clade, which is consistent with a previously published study [38]. A further previous study confirmed that Vermetoidea is placed at the basal position within Littorinimorpha, and our study obtained a consistent conclusion in which the four Vermetoidea species formed a sister clade of the other superfamilies [39]. The other eight superfamilies were grouped into one clade, while Calyptraeoidea presented a sister branch of the other superfamilies. The mt genome of *D. extinctorium* is the only species of Calyptraeoidea superfamily, which was uploaded in Genbank, which indicates that this superfamily lacks attention. The position of four deep-sea snails from Abyssochrysoidea supported the placement of this superfamily belonging to the order Littorinimorpha, just as in a previously published study.
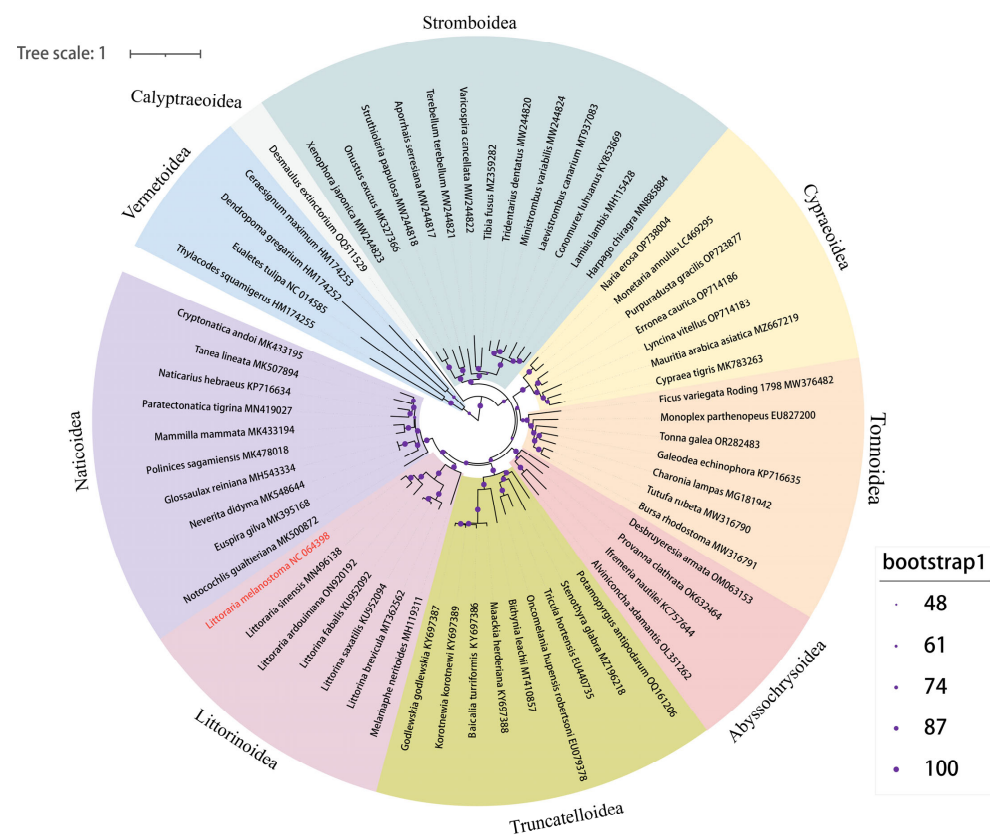
Figure 4. Maximum likelihood phylogenetic tree inferred from the 13 PCGs of Littorinimorpha. Different background colors mark the 9 superfamilies. Bootstraps values were indicated by different sizes of the purple symbol, as shown in the legend. The species which sequenced the mt genome in this study was colored in red.

4. Conclusions

In this study, the mitogenomes of *L. melanostoma* were sequenced, and 37 genes (13 PCGs, 22 tRNA genes, and 2 rRNA genes) and 1 control region were located in positions considered typical of a Littorinoidea mitogenome. The ML phylogenetic relationships based on the 13 PCGs of the order Littorinimorpha were analyzed, indicating that the basis for the relationship based on a molecular analysis is consistent with that of the traditional morphological method.

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