

1 **The complete mitogenome of *Lysmata vittata* (Crustacea:**
2 **Decapoda: Hippolytidae) and its phylogenetic position in**
3 **Decapoda**

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11

12 **Abstract**

13 In this study, the complete mitogenome of *Lysmata vittata* (Crustacea: Decapoda:
14 Hippolytidae) has been determined. The genome sequence was 22003 base pairs (bp)
15 and it included thirteen protein-coding genes (PCGs), twenty-two transfer RNA genes
16 (tRNAs), two ribosomal RNA genes (rRNAs) and three putative control regions
17 (CRs). The nucleotide composition of AT was 71.50%, with a slightly negative AT
18 skewness (-0.04). Usually the standard start codon of the PCGs was ATN, while *coxI*,
19 *nad4L* and *cox3* began with TTG, TTG and GTG. The canonical termination codon
20 was TAA, while *nad5* and *nad4* ended with incomplete stop codon T, and *coxI* ended
21 with TAG. We compared the order of genes of Decapoda ancestor and found that the
22 positions of the two tRNAs genes (*trnA* and *trnR*) of the *L. vittata* were translocated.
23 The phylogenetic tree showed that *L. vittata* was an independent clade, namely
24 Hippolytidae.

25 **Introduction**

26 *Lysmata vittata* (Crustacea: Decapoda: Hippolytidae) belongs to a small marine
27 ornamental shrimp, commonly known as peppermint shrimp, which is popular in the
28 marine aquarium trade. The species has a special sexual system, ie, protandric
29 simultaneous hermaphrodite (PSH) [1]. It is a member of the clean shrimp family, a
30 common marine ornamental species that originated in the Indian Ocean-Pacific region,
31 including coastal areas such as China, Japan, Philippines and Australia [2-4]. *L.*
32 *vittata* prefers to move in the range of 2~50 m below the sea surface, usually hiding in

33 the reef during the day and activating at night [5]. In view of the research needs of *L.*
34 *vittata*, we sequenced its mitogenome sequence.

35 The mitogenome is a significant tool for studying identification and phylogenetic
36 relationships in the different species [6]. In shrimps, the mitochondria is maternally
37 inherited, usually is circular and approximately 15 to 20 kb in length, including
38 thirteen PCGs, two rRNAs, twenty-two tRNAs and one CR. The mitogenome is a
39 complete system, which not only contains abundant information, but also the
40 phylogenetic tree based on the genome has the advantages of stable and reliable
41 structure.

42 Decapoda includes the largest number of species in crustaceans (8000 ~ 10000
43 species), with the greatest economic value and the most widely known invertebrates
44 [7]. It includes many aquatic products with important economic value, such as
45 lobsters, prawns and crabs. Therefore, the phylogeny and classification of decapod
46 crustaceans have been the focus of research for many years. The classification of
47 Hippolytidae was the most controversial family in Decapoda, especially the
48 monophyly of Hippolytidae and the position of the genus *Lysmata* [1, 8]. The
49 Hippolytidae is an important group of marine benthic organisms and a common group
50 in shallow sea biomes. Most species of the Hippolytidae are small shrimps living in
51 shallow water, which are distributed worldwide. It occupies an important position in
52 the animal classification system. However, we are the first to publish the
53 mitochondrial genome sequence of the Hippolytidae species in the GenBank database,
54 which is of great significance for us to expand the database of Hippolytidae.

55 In this study, the mitogenome of *L. vittata* has been successfully determined, which
56 helps us to understand the characteristics of mtDNA of *L. vittata*. Furthermore,
57 phylogenetic analysis using the nucleotide and amino acid sequences of thirteen PCGs
58 helps us to reconstruct the phylogenetic relationship between *L. vittata* and related
59 species. The addition of newly determined mitogenome complements the record of
60 the mitochondrial gene library of Hippolytidae from scratch.

61 **Materials and methods**

62 **Mitochondria DNA sequencing and genome assembly**

63 Specimens of *L. vittata* were collected in Xiamen, Fujian province, China. The
64 morphological characteristics of the species follow the previous description of
65 Abdelsalam [9]. Approximately 5g of fresh leaves was harvested for mtDNA isolation
66 using an improved extraction method [10]. After DNA isolation, the isolated DNA

67 was purified according to manufacturer's instructions (Illumina), and then 1 µg was
68 taken to create short-insert libraries, whose insertion size was 430 bp, followed by
69 sequencing on the Illumina Hiseq 4000 [11] (Shanghai BIOZERON Co., Ltd). The
70 high molecular weight DNA was purified and used for PacBio library prep,
71 BluePippin size selection, then sequenced on the Sequel Sequencer.
72 The raw data obtained by sequencing was processed and then the duplicated
73 sequences were assembled. The mitogenome was reconstructed using a combination
74 of the PacBio Sequel and the Illumina Hiseq data. Assemble the genome framework
75 by the both Illumina and PacBio using SOAPdenovo2.04 [12]. Verifying the
76 assembly and completing the circle or linear characteristic of the mitogenome, filling
77 gaps if there were. Finally, the clean data were mapped to the assembled draft
78 mitogenome to correct the wrong bases, and the most of the gaps were filled through
79 local assembly.

80 **Validation of mitogenome data**

81 In order to ensure the accuracy of the *L. vittata* mitogenome data, we resequenced the
82 samples on the Illumina HiSeq X10 platform (Nanjing GenePioneer Biotechnologies
83 Co. Ltd).

84 **Genome annotation and sequence analysis**

85 Mitogenome sequences were annotated using homology-based prediction and de novo
86 prediction, and the EVidenceModeler v1.1 [13] was used to integrate the complete
87 genetic structure. Twenty-two tRNAs and two rRNAs were predicted by
88 tRNAscan-SE [14] and rRNAmmer 1.2 [15]. The circular of the complete *L. vittata*
89 mitogenome graphical map was drawn using OrganellarGenomeDRAW v1.2 [16].
90 The RSCU of thirteen PCGs (remove incomplete codons) was calculated using
91 MEGA 5.0 [17]. The composition skewness of each component of the genome was
92 calculated according to the following formulas: AT-skew = $(A-T) / (A+T)$; GC-skew
93 = $(G-C) / (G+C)$ [18]. The secondary cloverleaf structure of tRNAs was examined
94 with MITOS WebServer (<http://mitos2.bioinf.uni-leipzig.de/index.py>) [19].

95 **Phylogenetic analysis**

96 To reconstruct the phylogenetic relationship among shrimp, the PCGs sequences of
97 the 51 Decapoda species were downloaded from GenBank database (S1 Table). The
98 PCGs sequences of *Euphausia superba* (NC_040987.1) were used as outgroup. The
99 nucleotide and amino acid sequences of 13 PCGs were aligned using MEGA 5.0 [17].

100 Gblocks was used to identify and selected the conserved regions [20]. Subsequently,
101 Bayesian inference (BI) and Maximum likelihood (ML) analysis were utilized for
102 reconstructing phylogenetic tree by MrBayes v3.2.6 [21] and PhyML 3.1 [22].
103 According to the Akaike Information Criterion (AIC) [23], GTR + I + G model was
104 considered as the best-fit model for analysis with nucleotide alignments using
105 jModeltest [24], and MtArt + I + G + F model was the optimal model for the amino
106 acid sequence dataset using ProtTest 3.4.2 [25]. In BI analysis, two simultaneous runs
107 of 10000000 generations were conducted for the matrix. Sampling trees every 1000
108 generations, and diagnostics were calculated every 5000 generations, with three
109 heated and one cold chains to encourage swapping among the Markov-chain Monte
110 Carlo (MCMC) chains. Additionally, the standard deviation of split frequencies was
111 below 0.01 after 10000000 generations, and the potential scale reduction factor (PSRF)
112 was close to 1.0 for all parameters. Posterior probabilities over 0.9 or bootstrap
113 percentage over 75%, the results were regarded as credible [26, 27]. The resulting
114 phylogenetic trees were visualized in Fig Tree v1.4.0.

115 **Results and discussion**

116 **Genome structure, organization and composition**

117 The mitogenome of *L. vittata* was a typical circular molecule of 22003 bp in size. It
118 contained 37 mitochondrial genes (thirteen PCGs, twenty-two tRNAs, two rRNAs and
119 three CRs) (Fig 1 and S2 Table). Among the 37 genes, the coding direction of the
120 twenty-three genes was clockwise (F-strand), and the coding direction of the
121 remaining fourteen genes was counterclockwise (R-strand) (Fig 1 and S2 Table). The
122 nucleotide composition of the mitogenome was biased toward A and T (T=37.15%,
123 A=34.35%, C=16.69%, G=11.80%) (Table 1). The relatively AT contents of the
124 complete mitogenome were calculated [mitogenome (71.50%), PCGs (69.79%),
125 tRNAs (69.58%) and rRNAs (69.29%)] (Table 1). The AT-skew values (-0.04) and
126 GC-skew values (-0.17) for the entire mitogenome were negative, showing that there
127 were higher Ts than As and Cs than Gs (Table 1). All original sequence data in this
128 study were submitted to the NCBI database under accession number MT478132.

129

130 **Fig 1. Mitogenome map of *Lyasmata vittata*.** The genes outside the map were coded on the F
131 strand, whereas the genes on the inside of the map are coded on the R strand. The middle black
132 circle displays the GC content and the inside purple and green circle displays the GC skew.

133

134 **Table 1. Composition and skewness of *Lysmata vittata* mitogenome.**

<i>Lysmata vittata</i>	Size(bp)	T (%)	C (%)	A (%)	G (%)	A+T (%)	AT-skew	GC-skew
Mitogenome	22003	37.15	16.69	34.35	11.80	71.50	-0.04	-0.17
PCGs	11144	41.09	15.25	28.70	14.96	69.79	-0.18	-0.01
<i>atp6</i>	675	40.15	19.41	28.30	12.15	68.44	-0.17	-0.23
<i>atp8</i>	165	43.64	15.76	35.15	5.45	78.79	-0.11	-0.49
<i>cob</i>	1137	39.40	20.14	27.88	12.58	67.28	-0.17	-0.23
<i>cox1</i>	1614	37.73	17.91	27.76	16.60	65.49	-0.15	-0.04
<i>cox2</i>	693	37.95	19.77	28.43	13.85	66.38	-0.14	-0.18
<i>cox3</i>	756	39.29	18.25	27.91	14.55	67.20	-0.17	-0.11
<i>nad1</i>	927	44.01	10.79	27.29	17.91	71.31	-0.23	0.25
<i>nad2</i>	1005	43.28	18.01	29.05	9.65	72.34	-0.20	-0.30
<i>nad3</i>	354	42.66	18.93	26.27	12.15	68.93	-0.24	-0.22
<i>nad4</i>	1336	43.11	9.51	28.59	18.79	71.70	-0.20	0.33
<i>nad4l</i>	246	45.12	7.72	26.02	21.14	71.14	-0.27	0.46
<i>nad5</i>	1732	41.17	9.82	31.64	17.38	72.81	-0.13	0.26
<i>nad6</i>	504	44.64	17.06	28.57	9.72	73.21	-0.22	-0.27
tRNAs	1512	33.27	14.02	36.31	16.40	69.58	0.04	0.08
rRNAs	2315	32.40	11.88	36.89	18.83	69.29	0.06	0.23
CR1	650	42.15	9.85	38.31	9.69	80.46	-0.05	-0.01
CR2	3821	38.50	14.39	33.73	13.37	72.23	-0.07	-0.04
CR3	888	42.34	13.51	34.91	9.23	77.25	-0.10	-0.19

135

136 **PCGs and codon usage**

137 The PCGs region was 11144 bp long, and accounted 50.6% of the *L. vittata*
 138 mitogenome. Nine of thirteen PCGs (*atp6*, *atp8*, *cob*, *cox1-3*, *nad2-3* and *nad6*) were
 139 encoded on the light (F) strand, while the other four genes (*nad1*, *nad4L* and *nad4-5*)
 140 were encoded on the heavy (R) strand (Table 1). Each PCG was initiated by a

141 canonical ATN codon (ATG for *atp6*, *atp8*, *nad2-5* and *cob*; ATT for *cox2* and *nad1*;
 142 ATC for *nad6*), except for *cox1* (TTG), *nad4L* (TTG) and *cox3* (GTG) (S2 Table).
 143 Two of the thirteen PCGs (*nad5* and *nad4*) terminated with incomplete stop codon T,
 144 one PCG (*cox1*) terminated with stop codon TAG, and the other ten PCGs terminated
 145 with the canonical termination codon TAA (S2 Table).
 146 The RSCU values of *L. vittata* mitogenome were analyzed and the results were shown
 147 in Table 2. The total number of codons in thirteen PCGs was 3714 except eleven
 148 canonical stop codons and two incomplete stop codons and the most common amino
 149 acids were Ile (AUR) (499), Phe (UUR) (357) and Leu2 (UUR) (315), whereas
 150 codons encoding Cys (UGR) (41) and Met (AUR) (24) were rare (Fig 2). The overall
 151 A + T content of thirteen PCGs was 69.79%, the AT-skews and GC-skews were
 152 negative which implied a higher occurrence of Ts and Cs than As and Gs (Table 1).

153

154 **Table 2. The codon number and relative synonymous codon usage (RSCU) in *L. vittata***
 155 **mitochondrial protein coding genes.**

Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	300	1.68	UCU(S)	129	2.46	UAU(Y)	101	1.57	UGU(C)	32	1.56
UUC(F)	57	0.32	UCC(S)	29	0.55	UAC(Y)	28	0.43	UGC(C)	9	0.44
UUA(L)	283	3.13	UCA(S)	92	1.76	UAA(*)	10	0.29	UGA(W)	92	2.68
UUG(L)	32	0.35	UCG(S)	12	0.23	UAG(*)	1	0.03	UGG(W)	15	1
CUU(L)	131	1.45	CCU(P)	101	2.71	CAU(H)	53	1.47	CGU(R)	12	0.4
CUC(L)	33	0.36	CCC(P)	14	0.38	CAC(H)	19	0.53	CGC(R)	2	0.07
CUA(L)	59	0.65	CCA(P)	28	0.75	CAA(Q)	55	1.62	CGA(R)	38	1.26
CUG(L)	5	0.06	CCG(P)	6	0.16	CAG(Q)	13	0.38	CGG(R)	11	0.36
AUU(I)	266	1.6	ACU(T)	85	1.95	AAU(N)	108	1.65	AGU(S)	45	0.86
AUC(I)	42	0.25	ACC(T)	23	0.53	AAC(N)	23	0.35	AGC(S)	7	0.13
AUA(I)	191	1.15	ACA(T)	61	1.40	AAA(K)	83	1.77	AGA(S)	93	3.08
AUG(M)	24	1	ACG(T)	5	0.11	AAG(K)	11	0.23	AGG(S)	25	0.83
GUU(V)	95	1.82	GCU(A)	93	2.14	GAU(D)	56	1.51	GGU(G)	61	1.06

GUC(V)	8	0.15	GCC(A)	25	0.57	GAC(D)	18	0.49	GGC(G)	20	0.35
GUA(V)	87	1.67	GCA(A)	50	1.15	GAA(E)	64	1.35	GGA(G)	106	1.84
GUG(V)	19	0.36	GCG(A)	6	0.14	GAG(E)	31	0.65	GGG(G)	43	0.75

156

157 **Fig 2. RSCU and Codon distribution in the mitogenome of *L. vittata*.** The left ordinate
158 represents RSCU, and the right ordinate represents the number of the Codon distribution.

159

160 **Transfer RNAs and Ribosomal RNAs**

161 The mitogenome of *L. vittata* contained twenty-two tRNAs and these genes ranged
162 from 60 (*trnA*) to 77 bp (*trnN*) (S2 Table). The tRNAs showed a strong A +T bias
163 (69.58%), while they also exhibited positive AT-skew (0.04) and GC-skew (0.08)
164 (Table 1). Eight tRNAs [*trnQ* (CAA), *trnC* (UGC), *trnY* (UAC), *trnF* (UUC), *trnH*
165 (CAC), *trnP* (CCA), *trnL1* (CUA) and *trnV* (GUA)] were present on the R strand and
166 the remaining fourteen were present on the F strand (S2 Table). The examined
167 secondary structure of twenty-two tRNAs was shown in S1 Fig. The other twenty-one
168 tRNAs had typical cloverleaf secondary structure except that *trnS1* (AGA) lacked the
169 dihydropyridine (DHU) arm [18, 19, 27, 28] (S1 Fig). In the secondary structure of
170 the tRNAs, the most common non-Watson–Crick base pair was G–U (e.g. *trnC*, *trnE*),
171 followed by U–U (e.g. *trnA*, *trnC*) [19]. In addition, several mismatches were
172 common in tRNAs, such as A–C (e.g. *trnA*), C–U (e.g. *trnA*, *trnG*) and A–A (e.g.
173 *trnM*, *trnS1*) (S1 Fig).

174 Two rRNA genes were found on the R strand. The *rrnL* was 1494 bp and *rrnS* was
175 821 bp, one located between *trnL1* and *trnV* and another located between *trnV* and
176 CR1 (S2 Table and Fig 1). The total A+T content of the two rRNAs was 69.29%, with
177 a positive AT-skew (0.06) (Table 1).

178 **Overlapping and intergenic regions**

179 The mitogenome of *L. vittata* contained four overlapping regions, these four pairs of
180 genes were presented: *atp8* / *atp6*, *trnE* / *trnF*, *nad4* / *nad4L* and *trnL1* / *rrnL*, with
181 the longest 23 bp overlap located between *trnL1* and *rrnL* (S2 Table). The 27
182 intergenic regions were found with a length varying from 2 ~ 3821 bp (S2 Table).
183 Three putative CRs had been identified in *L. vittata* mitogenome. The CR1 was
184 located between *rrnS* and *trnI*, with a length of 650 bp, and the A+T content was
185 80.46%. The CR2 was located between *cox1* and *trnL2*, with a length of 3821 bp, and

186 the A+T content was 72.23%. The CR3 was located between *trnL2* and *cox2*, with a
187 length of 888 bp, and the A+T content was 77.25% (Table 1 and S2 Table).

188 To our knowledge, this study is the first reported mitogenome from the genus
189 *Lysmata*. How multiple CRs were generated and evolved in the mitogenome of
190 *Lysmata* is a novel problem that has not yet been solved, and more mitogenomes of
191 *Lysmata* are still needed to clarify the mechanism forming this phenomenon.

192 **Gene rearrangement**

193 Compared with the gene order of a Decapoda ancestor [20, 29], two tRNA gene (*trnA*
194 and *trnR*) positions of *L. vittata* had translocated, which indicates that the *L. vittata*
195 was quite unconserved in its evolution (Fig 3). In fact, gene rearrangement was a very
196 common phenomenon in the mitogenome and the rearrangement mainly occurred in
197 tRNA genes. Gene arrangement was stable, and it could be used as an important
198 phylogenetic marker in the analysis of evolutionary perspective on shrimp. At present,
199 no other species in the Hippolytidae have been tested for mitogenome, and the
200 common characteristics of gene order were not easy to determine.

201

202 **Fig 3. Comparison of the order of mitochondrial genes of *Lysmata vittata* and the ancestor of**
203 **Decapoda.**

204

205 **Phylogenetic analysis**

206 Using ML and BI analysis methods, phylogenetic analysis was performed based on
207 the nucleotide and amino acid sequences of thirteen PCGs of the species in S1 Table,
208 and the analysis results were presented (Fig 4 and Fig 5). The phylogenetic tree based
209 on the nucleotide sequence of thirteen PCGs showed that the monophyly of each
210 family was basically well supported, especially the clade of the Hippolytidae was
211 strongly supported (ML BP = 100%; BI PP = 1). A basal split separates two clades,
212 with insignificant support (Fig 4). The first clade revealed the two phylogenetic
213 relationships: (Hippolytidae + (Atyidae + (Alpheidae + Palaemonidae))) and
214 (Palinuridae + (Astacidae + (Nephropsidae + Enoplometopidae))). The second clade
215 revealed the one phylogenetic relationship: (Sergestidae + (Solenoceridae +
216 Penaeidae)) (Fig 4). The phylogenetic tree based on the amino acid sequence of 13
217 PCGs revealed that the phylogenetic relationship between Hippolytidae and Atyidae
218 has changed as follows: (Atyidae + (Hippolytidae + (Alpheidae + Palaemonidae))).
219 However, the clade of the Hippolytidae was very weak support (ML BP = 52%; BI PP

220 = 0) (Fig 5). We could still reach a conclusion that the Hippolytidae was an older
221 family than Atyidae, and the Atyidae formed a sister group to Alpheidae –
222 Palaemonidae. The Caridea were dominated by Palaemonidae, followed by Alpheidae,
223 Atyidae and Hippolytidae [30]. At present, the phylogenetic study of the Hippolytidae
224 was limited to the partial fragments of mitochondrial genes *16S* or *12S* of individual
225 species in several genera (such as *Lysmata*, *Exhippolysmata*, *Ligur*, *Mimocaris* and
226 *Lysmatella*) [31-34]. The successful determination of the mitogenome of *L. vittata*
227 could provide a deeper understanding of the phylogenetic status of the Hippolytidae.

228

229 **Fig 4. Phylogenetic tree inferred from nucleotide sequences of 13 PCGs of the mitogenome**
230 **using ML and BI methods (BP / PP).**

231

232 **Fig 5. Phylogenetic tree inferred from amino acid sequences of 13 PCGs of the mitogenome**
233 **using ML and BI methods (BP / PP).**

234

235 Conclusion

236 In this study, we successfully obtained the mitogenome sequence of the *L. vittata*,
237 which was also the first species of the Hippolytidae to publish the mitogenome
238 sequence in the GenBank database. The genome sequence was 22003 base pairs (bp)
239 and it included 37 genes and three CRs. Each PCGs was initiated by a canonical ATN
240 codon, except for *cox1*, *nad4L* and *cox3*, which were initiated by a TTG, TTG and
241 GTG. Two of the thirteen PCGs (*nad5* and *nad4*) terminated with incomplete stop
242 codon T, and one (*cox1*) terminated with stop codon TAG. The AT-skew (-0.04) and
243 the GC-skew (-0.17) were both negative in the mitogenomes of *L. vittata*. Compared
244 with the gene order of a Decapoda ancestor, the gene arrangement order of the *L.*
245 *vittata* has changed. Furthermore, phylogenetic analyses showed that *L. vittata* was not
246 in the clades of other families, but was an independent clade, namely the
247 Hippolytidae.

248

249 Supporting information

250 **S1 Table. List of species used to construct the phylogenetic tree.**

251 (DOC)

252 **S2 Table. Summary of *Lysmata vittata* mitogenome.**

253 (DOC)

254 **S1 Fig. Predicted secondary structure for the tRNAs of *Lysmata vittata***
255 **mitogenome.**

256 (TIF)

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259 **Author contributions**

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266 **Data Availability Statement**

267 Data are available from the NCBI database (accession number MT478132).

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275 **Competing Interests**

276 The authors declare there are no competing interests.

277

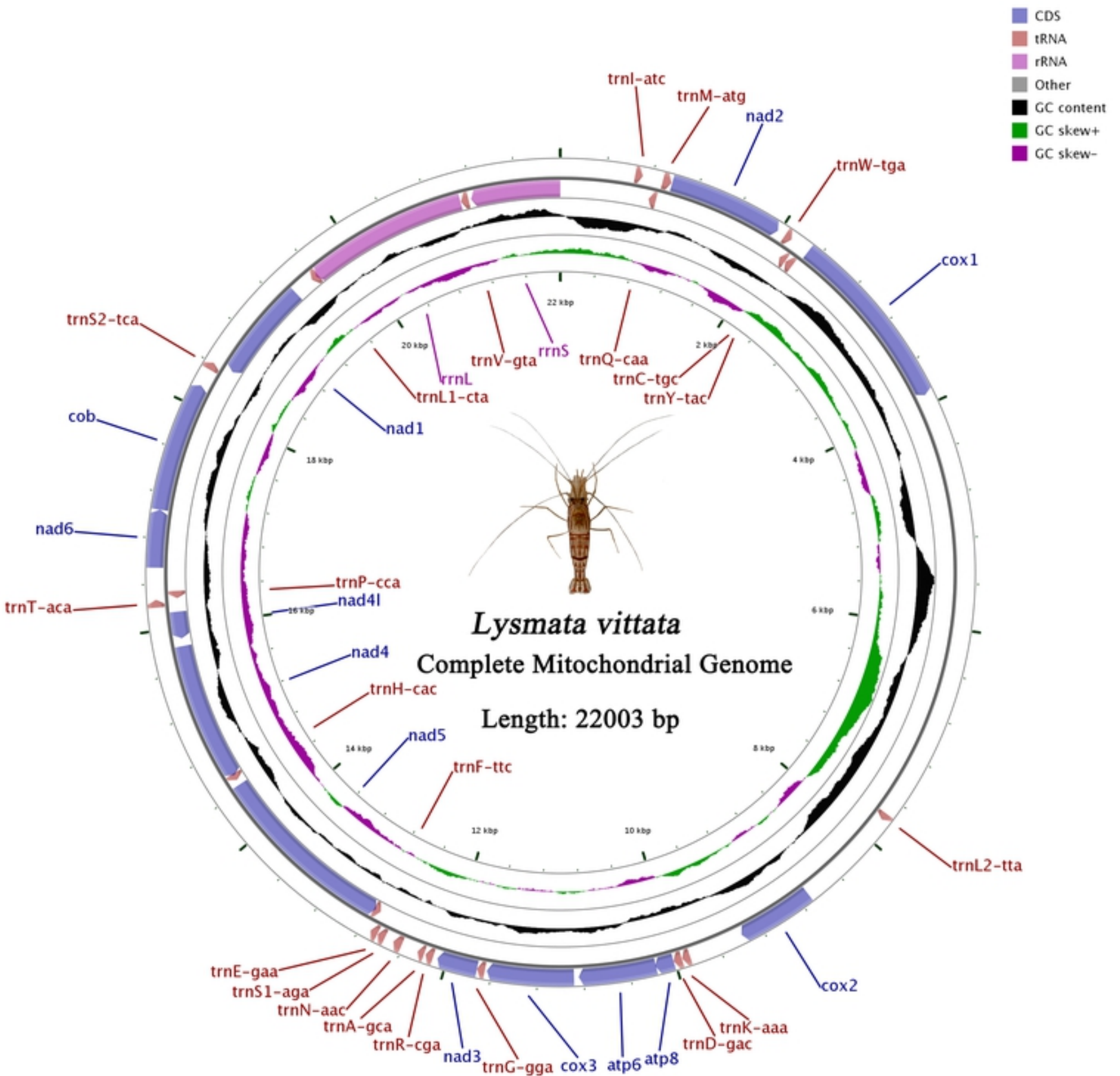
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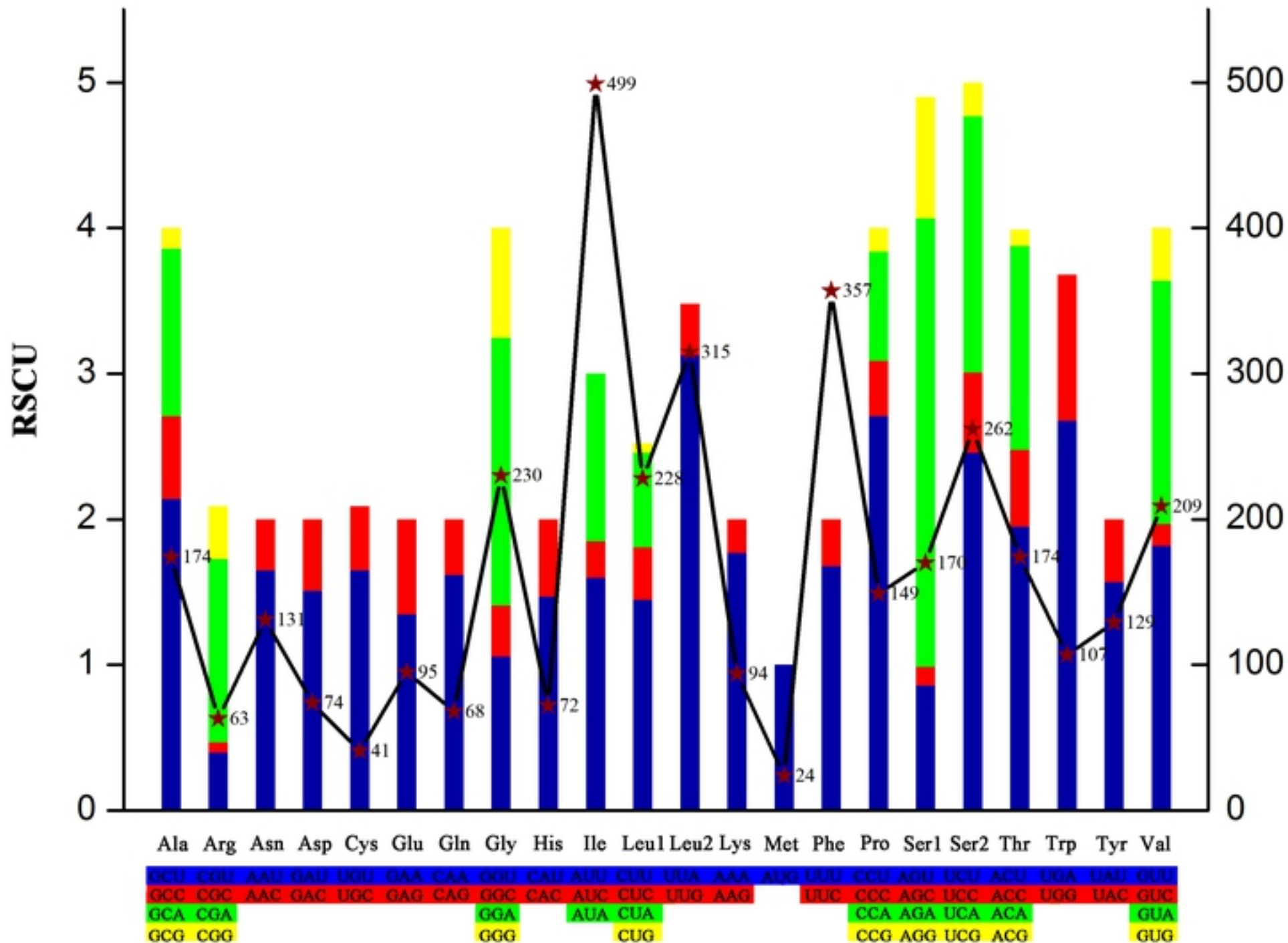
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Ancestor of Decapoda

cox1 L2 cox2 K D atp8 atp6 cox3 G nd3 A R N S1 E F nd5 H nd4 nd4L T P nd6 cob S2 nd1 L1 rrmL V rrmS CR I Q M nd2 W C Y

Lysmata vittata

cox1 CR2 L2 CR3 cox2 K D atp8 atp6 cox3 G nd3 R A N S1 E F nd5 H nd4 nd4L T P nd6 cob S2 nd1 L1 rrmL V rrmS CR1 I Q M nd2 W C Y

