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Complete mitochondrial genome of *Clistocoeloma sinensis* (Brachyura: Grapsoidea): Gene rearrangements and higher-level phylogeny of the Brachyura

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Deciphering the animal mitochondrial genome (mitogenome) is very important to understand their molecular evolution and phylogenetic relationships. In this study, the complete mitogenome of *Clistocoeloma sinensis* was determined. The mitogenome of *C. sinensis* was 15,706 bp long, and its A+T content was 75.7%. The A+T skew of the mitogenome of *C. sinensis* was slightly negative (−0.020). All the transfer RNA genes had the typical cloverleaf structure, except for the *trnS1* gene, which lacked a dihydroxyuridine arm. The two ribosomal RNA genes had 80.2% A+T content. The A+T-rich region spanned 684 bp. The gene order within the complete mitogenome of *C. sinensis* was identical to the pancrustacean ground pattern except for the translocation of *trnH*. Additionally, the gene order of *trnI-trnQ-trnM* in the pancrustacean ground pattern becomes *trnQ-trnI-trnM* in *C. sinensis*. Our phylogenetic analysis showed that *C. sinensis* and *Sesarmops sinensis* cluster together with high nodal support values, indicating that *C. sinensis* and *S. sinensis* have a sister group relationship. The results support that *C. sinensis* belongs to Grapsoidea, Sesarmidae. Our findings also indicate that Varunidae and Sesarmidae species share close relationships. Thus, mitogenomes are likely to be valuable tools for systematics in other groups of Crustacea.

Mitochondrial DNA (mtDNA) is a typically closed circular molecule approximately ranging in size from 14 to 18 kb. It contains 13 protein-coding genes (PCGs), 2 ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and control region (CR)[1,2]. mtDNA is characterized by maternal inheritance, simple structure, a small genome size, conserved gene content and organization, high mutation rate, and accelerated rate of nucleotide substitution[3–7]. The mitogenomes of animal mtDNA can provide important information on rearrangement laws and phylogenetic analysis because of their rapid evolutionary rate and lack of genetic recombination[1]. It is becoming increasingly common to use complete animal mitogenomes for phylogenetic reconstruction[8–10]. Partial DNA sequences are often too short to contain sufficient phylogenetic information[11], and combination of mitochondrial and nuclear genomes makes model selection difficult[12]. Further, the addition of rRNA makes alignment ambiguous[13].

The infraorder Brachyura contains about 7000 described species in 98 families[14]. *C. sinensis* is one of the most important Brachyura species, and is used as a good indicators of environmental changes and water pollutions in China[15]. Although *C. sinensis* was described over 80 years ago[16], it is still very poorly understood. Earlier studies classified *C. sinensis* into Grapsidae, Sesarminae[17]. In recent years, some researchers have classified *C. sinensis* into Grapsoidea, Sesarmidae[18]. Gene rearrangements in mitogenomes are useful in reconstruction of Brachyuran phylogeny[19]. In the present study, we sequenced the complete mitogenome of *C. sinensis* with the aim of elucidating

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Primer	Sequence (5′-3′)	annealing temperature	Location
F1	GGTCAACAAATCATAAAGATATTGG	55 °C	*cox1*
R1	TAAACTTCAGGGTGACCAAAAAATCA		*cox1*
F2	TAGTWATHAANGGHCTACGVTGRGG	50 °C	*cox3*
R2	AAGTCCRTGRAAYCCDGTDGCHAC		*cox3*
F3	TATGTGGDWTWCCTTTTWTAGCDGG	48 °C	*nad5*
R3	ATHTCAAGMTAARCHAGCHCCHCC		*nad5*
F4	GTGCCAGCCGCCGCGGTTA	52 °C	*rrnS*
R4	ATGCACTTTCCAGTACATCTA		*rrnS*
F5	CCCACGCAGGAGCTTCAGTAG	56 °C	*cox1-cox3*
R5	AGTCTTTGGATTGCTTGGTTGTG		*cox1-cox3*
F6	TTCCCCTTTTAAATACAACTA	56 °C	*cox3-nad5*
R6	GCTAATGCAGGGATACTAAC		*cox3-nad5*
F7	GCAGGTATCAAGCAGAAAAAG	56 °C	*nad5-rrnS*
R7	TTTAAAAATTTGGCGGTGAT		*nad5-rrnS*
F8	ATCAAATCCTCCTTCATAATA	56 °C	*rrnS-cox1*
R8	GCAGCAGCTAGAGGAGGATAAA		*rrnS-cox1*

Table 1. Primers used in this study.

Species	Family	Size (bp)	Accession No.
Clistocoeloma sinensis	**Sesarmidae**	**15,706**	**KU589292**
Sesarmops sinensis	Sesarmidae	15,905	KR336554
Helice latimera	Varunidae	16,246	KU589291
Pachygrapsus crassipes	Grapsidae	15,652	KC878511
Eriocheir japonica sinensis	Varunidae	16,378	KM516908
Eriocheir japonica hepuensis	Varunidae	16,335	FJ455506
Eriocheir japonica japonica	Varunidae	16,352	FJ455505
Xenograpsus testudinatus	Xenograpsidae	15,798	EU727203
Homologenus malayensis	Homolidae	15,793	KJ612407
Pseudocarcinus gigas	Menippidae	15,515	AY562127
Damithrax spinosissimus	Mithracidae	15,817	KM405516
Geothelphusa dehaani	Potamidae	18,197	AB187570
Portunus pelagicus	Portunidae	16,157	KM977882
Callinectes sapidus	Portunidae	16,263	AY363392
Portunus trituberculatus	Portunidae	16,026	AB093006
Portunus sanguinolentus	Portunidae	16,024	KT438509
Charybdis japonica	Portunidae	15,738	FJ460517
Scylla paramamosain	Portunidae	15,824	JX457150
Scylla olivacea	Portunidae	15,723	FJ827760
Scylla tranquebarica	Portunidae	15,833	FJ827759
Scylla serrata	Portunidae	15,775	FJ827758
Charybdis feriata	Portunidae	15,660	KF386147
Umalia orientalis	Raninidae	15,466	KM365084
Lyreidus brevifrons	Raninidae	16,112	KM983394
Gandalfus yunohana	Bythograeidae	15,567	EU647222
Gandalfus puia	Bythograeidae	15,548	KR002727
Austinograea alayseae	Bythograeidae	15,620	JQ035660
Austinograea rodriguezensis	Bythograeidae	15,611	JQ035658
Ilyoplax deschampsi	Dotillidae	15,460	JF909979

Table 2. List of Brachyura species analysed in this study with their GenBank accession numbers.

its evolutionary status and rearrangement information by comparing it with complete Brachyuran mitogenomes available to date[20,21]. This information may provide insights into phylogenetic rearrangement and enable phylogenetic analysis.

Gene	Direction	Location	Size	Intergenic nucleotides	Anticodon	Start codon	Stop codon
cox1	F	1–1535	1535	0		ATG	TA
trnL2	F	1536–1601	66	6	TAA		
cox2	F	1608–2295	688	0		ATG	T
trnK	F	2296–2365	70	0	TTT		
trnD	F	2366–2433	68	0	GTC		
atp8	F	2434–2592	159	−7		ATG	TAA
atp6	F	2586–3259	674	0		ATT	TA
cox3	F	3260–4050	791	0		ATG	TA
trnG	F	4051–4115	65	0	TCC		
nad3	F	4116–4466	351	2		ATT	TAA
trnA	F	4469–4532	64	5	TGC		
trnR	F	4538–4601	64	2	TCG		
trnN	F	4604–4674	71	1	GTT		
trnS1	F	4676–4743	68	−1	TCT		
trnE	F	4743–4810	68	9	TTC		
trnH	R	4820–4886	67	0	GTG		
trnF	R	4887–4951	65	4	GAA		
nad5	R	4956–6686	1731	0		ATG	TAA
nad4	R	6687–8065	1379	0		ATG	TA
nad4L	R	8066–8361	296	7		ATG	A
trnT	F	8369–8434	66	0	TGT		
trnP	R	8435–8502	68	2	TGG		
nad6	F	8505–9008	504	0		ATT	TAA
cob	F	9009–10,143	1135	0		ATT	A
trnS2	F	10,144–10,212	69	18	TGA		
nad1	R	10,231–11,169	939	39		ATA	TAA
trnL1	R	11,209–11,276	68	0	TAG		
rrnL	R	11,277–12,612	1336	0			
trnV	R	12,613–12,685	73	0	TAC		
rrnS	R	12,686–13,517	832	0			
CR	—	13,518–14,201	684	0			
trnQ	R	14,202–14,269	68	70	TTG		
trnI	F	14,340–14,405	66	12	GAT		
trnM	F	14,418–14,487	70	0	CAT		
nad2	F	14,488–15,493	1006	0		ATG	T
trnW	F	15,494–15,562	69	11	TCA		
trnC	R	15,574–15,637	64	0	GCA		
trnY	R	15,638–15,706	69	—	GTA		

Table 3. Summary of *Clistocoeloma sinensis* mitogenome.

Methods

Sample and DNA Extraction. Adult specimens of *C. sinensis* were captured from Yancheng, Jiangsu province, China. Total genomic DNA was isolated from individual specimens using the Aidlab Genomic DNA Extraction Kit (Beijing, China). All procedures were completed following the manufacturer's instructions. The complete mitogenome was amplified from the DNA from one *C. sinensis* crab.

PCR Amplification and Sequencing. The complete mitogenome was obtained using a combination of conventional PCR and long PCR to amplify overlapping fragments spanning the whole mitogenome. Universal and specific primers were designed based on the conserved nucleotide sequences of known mitochondrial sequences in Brachyura (Table 1) and synthesized by Beijing Sunbiotech[22–26]. The fragments were amplified using Aidlab Red Taq (Beijing, China) according to the manufacturer's instructions. All amplifications were performed on an Eppendorf Mastercycler and Mastercycler gradient in 50 µl reaction volumes with 5 µl 10 × Taq Buffer (Mg^{2+}) (Aidlab), 4 µl of dNTPs (2.5 mM, Aidlab), 2 µl of each primer (10 µM), 2 µl of DNA temple (~30 ng), 34.5 µl ddH_2O, and 0.5 µl Red Taq DNA polymerase (5U, Aidlab). PCR was performed using the following procedure: 94 °C for 3 min; followed by 40 cycles of 30 s at 94 °C, annealing for 35 s at 48–56 °C (depending on primer combination), and elongation at 72 °C for 30 s to 4 min (depending on the fragment length); and final extension at 72 °C for 10 min. The PCR products were separated by agarose gel electrophoresis (1% w/v) and purified using a DNA

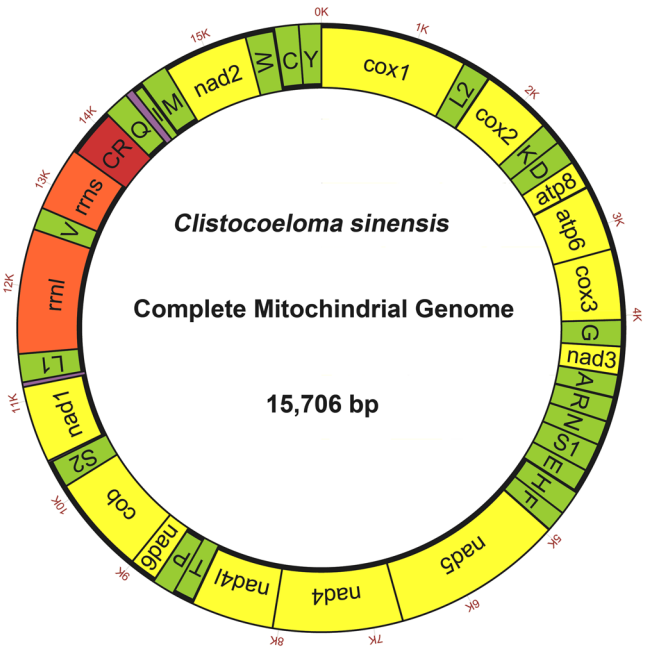

Figure 1. Graphical map of the mitogenome of *Clistocoeloma sinensis*. Protein-coding and ribosomal RNA genes are shown using standard abbreviations. Genes for transfer RNAs are abbreviated using a single letter. S1 = AGN, S2 = UCN, L1 = CUN, L2 = UUR. CR = control region. The 13 protein-coding genes are yellow, tRNAs are green, rRNAs are red, and CRs are dark red.

species	Size (bp)	A %	G %	T %	C %	A+T %	A+T skew	G+C skew
C. sinensis	**15,706**	**37.1**	**9.4**	**38.6**	**14.9**	**75.7**	**−0.020**	**−0.228**
S. sinensis	15,905	37.4	9.4	38.3	14.9	75.7	−0.012	−0.228
H. latimera	16,246	34.0	11.0	35.1	19.9	69.1	−0.017	−0.290
G. puia	15,548	35.1	10.3	34.8	19.8	69.9	0.006	−0.313
P. sanguinolentus	16,024	31.6	12.9	34.0	21.5	65.6	−0.037	−0.243
E. j. sinensis	16,378	35.2	10.8	36.4	17.6	71.6	−0.016	−0.243
E. j. hepuensis	16,335	35.1	10.8	36.4	17.7	71.5	−0.018	−0.245
E. j. japonica	16,352	35.2	10.7	36.5	17.7	71.7	−0.018	−0.245
X. testudinatus	15,798	36.7	9.3	37.2	16.8	73.9	−0.007	−0.286
P. gigas	15,515	35.0	10.8	35.5	18.7	70.5	−0.006	−0.268
G. dehaani	18,197	36.9	8.3	38.0	16.8	74.9	−0.014	−0.341
L. brevifrons	16,112	34.2	11.3	36.4	18.1	70.6	−0.031	−0.231
C. sapidus	16,263	34.2	11.1	34.9	19.8	69.1	−0.011	−0.279
P. trituberculatus	16,026	33.3	11.3	36.9	18.5	70.2	−0.051	−0.241
H. malayensis	15,793	37.3	10.0	34.4	18.3	71.7	0.040	−0.292
C. japonica	15,738	33.8	11.9	35.4	18.9	69.2	−0.024	−0.228
S. paramamosain	15,824	34.9	10.1	38.2	16.8	73.1	−0.045	−0.247
U. orientalis	15,466	33.1	11.8	34.9	20.2	68.0	−0.027	−0.262
S. olivacea	15,723	33.5	11.2	35.9	19.4	69.4	−0.035	−0.267
S. tranquebarica	15,833	35.0	9.8	38.7	16.5	73.7	−0.050	−0.258
S. serrata	15,775	34.5	10.4	38.0	17.1	72.5	−0.047	−0.242
D. spinosissimus	15,817	33.3	10.5	36.8	19.4	70.1	−0.050	−0.294
C. feriata	15,660	34.1	11.2	36.1	18.6	70.2	−0.028	−0.246
G. yunohana	15,567	34.3	10.8	35.6	19.3	69.9	−0.019	−0.281
P. pelagicus	16,157	33.7	12.2	35.0	19.1	68.8	−0.019	−0.219
A. alayseae	15,620	34.4	11.4	32.4	21.8	66.8	0.029	−0.316
A. rodriguezensis	15,611	35.3	10.3	33.5	20.9	68.8	0.025	−0.341
P. crassipes	15,652	30.5	12.7	35.8	21.0	66.3	−0.080	−0.245
I. deschampsi	15,460	34.1	10.7	35.5	19.7	69.6	−0.019	−0.294

Table 4. Composition and skewness of mitogenome in 29 Brachyura species.

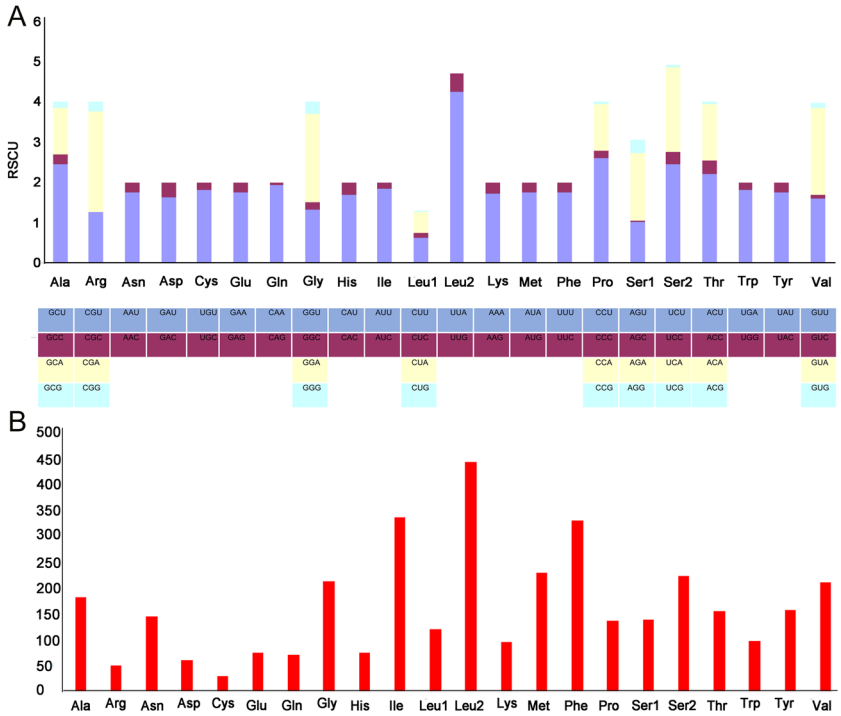

Figure 2. Relative synonymous codon usage in *Clistocoeloma sinensis* mtDNAs. Codon families are provided on the x axis (**A**). (**B**) Nucleotide composition conditions.

nt %	PCGs	tRNAs	rRNAs	CR
A%	36.1	37.7	40.4	43.4
T%	38.1	38.5	39.8	39.5
C%	15.9	12.8	13.0	10.5
G%	9.9	11.0	6.8	6.6
A+T%	74.2	76.2	80.2	82.9
C+G%	25.8	23.8	19.8	17.1
AT-Skew	−0.026	−0.010	0.007	0.047
GC-skew	−0.233	−0.075	−0.313	−0.228

Table 5. Composition and skewness of *Clistocoeloma sinensis* mitogenome. CR = control region.

gel extraction kit (Transgen, Beijing, China). The purified products were then ligated into the T-vector (Sangon, Shanghai, China) and sequenced.

Complete Mitogenome Analysis. The graphical map of the complete mitogenome was drawn using the online mitochondrial visualization tool mtviz[27]. The secondary cloverleaf structure and anticodon of transfer RNAs were identified using the tRNA-scan SE webserver[28]. Codon usage and the nucleotide composition of the mitogenome were determined using MEGA6. The sequences of 29 Brachyura species and *Alpheus distinguendus* were aligned using MAFFT[29].

Phylogenetic Analysis. Twenty-eight complete Brachyura mitogenomes were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). In addition, the mitogenome of *A. distinguendus* was downloaded from GenBank and used as an outgroup taxon. GenBank sequence information is shown in Table 2.

The sequences were aligned with the mitochondrial sequences of closely related species. In order to remove the gaps in sequences, poorly aligned positions and divergent regions were removed using Gblocks[25]. Then, fasta sequences were converted to nex format sequences and phylip format sequences for Bayesian inference (BI) and Maximum likelihood (ML) analyses using online software (http://sequenceconversion.bugaco.com/converter/biology/sequences/fasta_to_phylip.php). We used DAMBE to detect the saturation status of the sequences[30].

We determined the taxonomic status of *C. sinensis* within Brachyura by reconstructing the phylogenetic tree. Nucleotide sequences from 30 mitogenome PCGs were combined. The dataset was run using two inference methods: BI and ML analyses. The former was performed using Mrbayes v3.2.1[31], while ML analysis was performed using raxmlGUI[32]. The nucleotide substitution model was selected using Akaike information criterion implemented in Mrmodeltest v2.3[33,34]. The GTR+I+G model was the best model to examine nucleotide phylogenetic

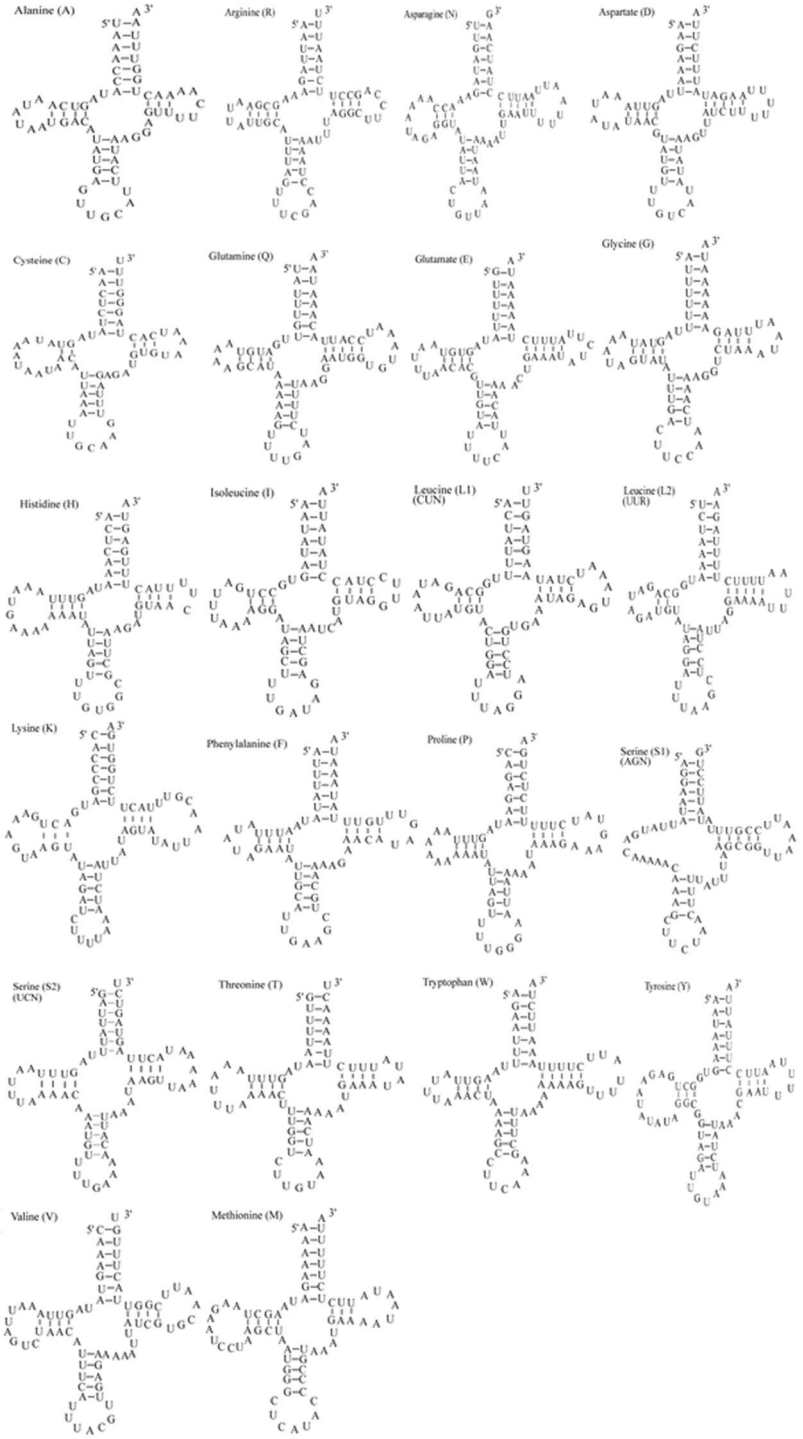

Figure 3. Secondary structures of the 22 transfer RNA genes of *Clistocoeloma sinensis*. The tRNAs are labelled with the abbreviations of their corresponding amino acids. Dashes (−) indicate Watson-Crick pairing.

analysis and molecular evolution. BI and ML analyses were performed under the GTRCAT model with nucleotide alignment (NT dataset) of the 13 mitochondrial PCGs. ML analyses were performed on 1000 bootstrapped datasets. The BI analysis ran as 4 simultaneous MCMC chains for 10,000,000 generations, sampled every 100 generations, and a burn-in of 5000 generations was used. The average standard deviation of split frequencies was less than 0.01, and the effective sample size determined using tracer v1.6 exceeded 200. These two findings indicate that our data was convergent. The resulting phylogenetic trees were visualized using FigTree v1.4.2.

Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	291	1.75	UCU(S)	111	2.44	UAU(Y)	140	1.76	UGU(C)	27	1.8
UUC(F)	42	0.25	UCC(S)	14	0.31	UAC(Y)	19	0.24	UGC(C)	3	0.2
UUA(L)	401	4.24	UCA(S)	96	2.11	UAA(*)	8	2	UGA(W)	89	1.82
UUG(L)	45	0.48	UCG(S)	3	0.07	UAG(*)	0	0	UGG(W)	9	0.18
CUU(L)	60	0.63	CCU(P)	90	2.61	CAU(H)	64	1.68	CGU(R)	16	1.25
CUC(L)	10	0.11	CCC(P)	6	0.17	CAC(H)	12	0.32	CGC(R)	0	0
CUA(L)	49	0.52	CCA(P)	40	1.16	CAA(Q)	70	1.94	CGA(R)	32	2.51
CUG(L)	2	0.02	CCG(P)	2	0.06	CAG(Q)	2	0.06	CGG(R)	3	0.24
AUU(I)	312	1.85	ACU(T)	86	2.21	AAU(N)	128	1.74	AGU(S)	47	1.03
AUC(I)	26	0.15	ACC(T)	13	0.33	AAC(N)	19	0.26	AGC(S)	1	0.02
AUA(M)	203	1.76	ACA(T)	55	1.41	AAA(K)	82	1.71	AGA(S)	77	1.69
AUG(M)	28	0.24	ACG(T)	2	0.05	AAG(K)	14	0.29	AGG(S)	15	0.33
GUU(V)	85	1.6	GCU(A)	113	2.46	GAU(D)	50	1.64	GGU(G)	72	1.34
GUC(V)	5	0.09	GCC(A)	11	0.24	GAC(D)	11	0.36	GGC(G)	9	0.17
GUA(V)	115	2.17	GCA(A)	54	1.17	GAA(E)	66	1.74	GGA(G)	117	2.18
GUG(V)	7	0.13	GCG(A)	6	0.13	GAG(E)	10	0.26	GGG(G)	17	0.32

Table 6. The codon number and relative synonymous codon usage in *Clistocoeloma sinensis* mitochondrial protein coding genes.

Results and Discussion

Genome Structure and Organization. The mitogenome of *C. sinensis* is 15,706 bp long, and its gene content is same as that most known Brachyura: 13 PCGs, 2 rRNA genes, and 22 tRNA genes plus CR (Table 3 and Fig. 1). Twenty-three genes are coded on the J strand and the remaining 14 genes are transcribed on the N strand. It has been deposited in GenBank under accession number KU589292. The genome composition (A: 37.1%, T: 38.6%, C: 14.9%, G: 9.4%) shows a strong A+T bias, which account for 75.7% of the bases, and exhibits a negative AT skew ([A − T]/[A+T] = −0.020) and GC skew ([G − C]/[G+C] = −0.228). The A+T skew of other previously sequenced Brachyura mitogenomes ranged from −0.080 (*Pachygrapsus crassipes*) to 0.040 (*Homologenus malayensis*), while the G+C skew ranged from −0.341 (*Austinograea rodriguezensis*, *Geothelphusa dehaani*) to −0.219 (*Portunus pelagicus*) (Table 4). However, different regions have different A+T contents. The CR had the highest A+T content (82.9%), whereas the PCG region had the lowest A+T content (74.2%) (Table 5).

Protein-Coding Genes. Among the 13 PCGs, 9 (*nad2*, *cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad6*, and *cob*) were coded on the J strand, while the rest (*nad5*, *nad4*, *nad4L*, and *nad1*) were on the N strand. The 13 PCGs ranged in size from 159 to 1731 bp (Table 3). Their A+T content was 74.2% and AT skew was −0.026 (Table 5). The relative synonymous codon usage for *C. sinensis* at the third position is shown in Fig. 2. The usage of both two- and four-fold degenerate codons was biased toward the use of codons abundant in A or T (Table 6), which is consistent with other Brachyura species[35–37].

Transfer RNAs, Ribosomal RNAs, and A+T-Rich Region. Like most Brachyura mtDNA, the *C. sinensis* mitogenome contains a set of 22 tRNAs genes (Fig. 3), although this feature is not very well conserved in animal mtDNA. The tRNAs ranged in size from 64 to 73 bp and showed a strong A+T bias, as these bases accounted for 76.2% of the DNA. Further, they exhibited a negative AT skew (−0.010) (Table 5). Fourteen tRNA genes were present on the J strand and eight were on the N strand. All the tRNA genes had the typical cloverleaf structure, except for the *trnS1* gene, whose dihydroxyuridine arm was instead just a simple loop (Fig. 3). These features are common in most Brachyura mitogenomes[35–37]. The secondary cloverleaf structure of 18 tRNAs was examined using tRNA-scan SE; 4 tRNAs not detected by tRNAscan-SE were found in the unannotated regions by sequence similarity to the tRNAs of other crabs. The 2 rRNA genes with 80.2% total A+T content and positive AT skew (0.007) (Table 5) were located between *trnL1* and *trnV* and between *trnV* and CR. *rrnL* is 1336 bp while *rrnS* is 832 bp. The CR located between *rrnS* and *trnQ*, spans 684 bp. This region contains 82.9% AT nucleotides, with a positive AT skew (0.047) and negative GC skew (−0.228) (Table 5).

Gene Arrangement. Gene order within the complete mitogenome of *C. sinensis* is similar to the pancrustacean ground pattern[38–40] (Fig. 4A), except for the translocation of *trnH*. Typically, the *trnH* gene is located between the *nad4* and *nad5* genes in the pancrustacean ground pattern, but in *C. sinensis*, it is between the *trnE* and *trnF* genes (Fig. 4B). This translocation was also observed in the mitogenomes of Brachyura crabs available in GenBank that were compared with the *C. sinensis* mitogenome. In addition, in the pancrustacean ground pattern, the tRNA gene order between the CR and *nad2* is *trnI-trnQ-trnM*. However, in *C. sinensis*, it is *trnQ-trnI-trnM* (Fig. 4B). The tRNA rearrangements are generally considered to be a consequence of tandem duplication of part of the mitogenome[41]. Similar non-coding sequences are present at the position of *trnI* originally occupied by the transposed *trnQ* in *C. sinensis*. Because these intergenic sequences have similar lengths to those of typical tRNA genes, they were presumed to be remnants of the *trnQ* gene and its boundary sequences[42]. The gene order

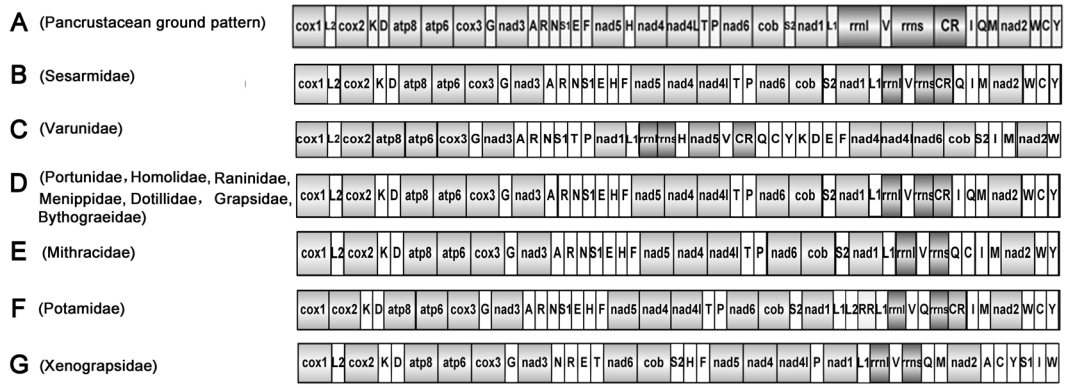

Figure 4. Linear representation of gene rearrangements of Brachyura mitogenomes. All genes are transcribed from left to right. tRNA genes are represented by the corresponding single-letter amino acid code. S1 = AGN, S2 = UCN, L1 = CUN, L2 = UUR. CR = control region. *rrnL* and *rrnS* are the large and small ribosomal RNA subunits.

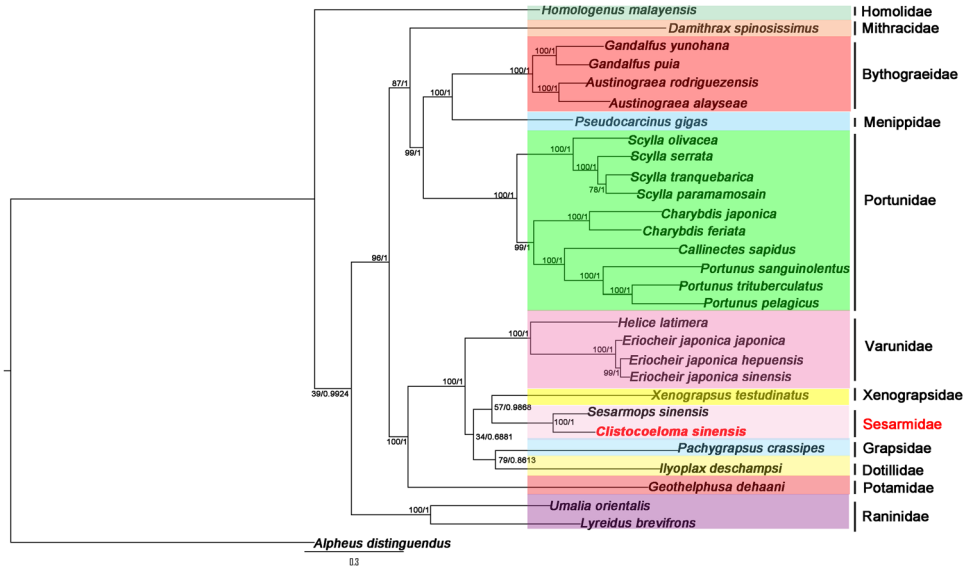

Figure 5. Inferred phylogenetic relationships among Brachyura based on nucleotide sequence of 13 mitochondrial PCGs using maximum likelihood (ML) and Bayesian inference (BI). *Alpheus distinguendus* was used as the outgroup. The bootstrap value (BP) and Bayesian posterior probability (BPP) of each node are shown as BP based on the NT dataset/BPP based on the NT dataset, 100/1.00.

of *C. sinensis* is identical to that of *S. sinensis* (Fig. 4B), which indicates that *C. sinensis* may belong to the group Sesarmidae of the superfamily Grapsoidea and that *C. sinensis* and *S. sinensis* probably belong to sister groups.

The gene sequences of Varunidae species (*Eriocheir japonica sinensis*, *E. j. hepuensis*, *E. j. japonica*, and *Helice latimera*) are identical (Fig. 4C). As shown in Fig. 4D, the order and orientation of genes in 7 families are uniform. The order of genes in *C. sinensis* sequences is different from that in the sequences of the mitogenomes of these 7 families because of the rearrangement of two tRNA genes between CR and *trnM*: the placement of genes between CR and *trnM* in *C. sinensis* is CR-*trnQ*-*trnI*-*trnM*, while that in the 7 families is CR-*trnI*-*trnQ*-*trnM*. In this case, tandem duplication of gene regions may be the most likely mechanism for mitochondrial gene rearrangement, which includes *trnI* and *trnQ*, followed by loss of supernumerary genes[43,44]. Slipped-strand mispairing occurred first, followed by gene deletion[45]. Partial PCGs, tRNAs, and rRNAs of *Damithrax spinosissimus*, *G. dehaani*, and *Xenograpsus testudinatus* appear to be rearranged compared to *C. sinensis* (Fig. 4E–G).

Phylogenetic analysis. Our analyses were based on the NT dataset in mitogenomes derived from 29 Brachyura species belonging to 12 families (Varunidae, Xenograpsidae, Homolidae, Menippidae, Mithracidae, Potamidae, Portunidae, Raninidae, Bythograeidae, Sesarmidae, Grapsidae, and Dotillidae). The data matrix (15,706 bp in all) was analysed using the model-based evolutionary methods of BI and ML analyses (Fig. 5). The ML and BI analyses of the dataset gave the same tree topology. It is obvious that *C. sinensis* and *S. sinensis* clustered in one branch in the phylogenetic tree with high nodal support values (Fig. 5), indicating that *C. sinensis*

and *S. sinensis* have a sister group relationship. This result supported that *C. sinensis* belongs to Grapsoidea, Sesarmidae. From the phylogenetic tree, we found that *X. testudinatus* and two Sesarmidae species formed a group and showed close relationships. *X. testudinatus*, which was originally placed in Varunidae, has been transferred to its own family (Xenograpsidae)[21,46]. Analysis of the nucleotide sequences of the 13 mitochondrial PCGs using BI and ML showed that *E. j. sinensis*, *E. j. hepuensis*, *E. j. japonica*, and *H. latimera* clustered together with high statistical support, showing that these species have a sister group relationship and belong to Grapsoidea, Varunidae. Our phylogenetic analysis indicated that Sesarmidae species, Xenograpsidae species and Varunidae species have close relationships[47]. In addition, *P. crassipes* belongs to Grapsoidea, Grapsidae[48].

The phylogenetic position of *Ilyoplax deschampsi* is always within Grapsoidea[21,47,49,50]. *I. deschampsi* belongs to the family Dotillidae, Ocypodoidea. The real phylogenetic position of *I. deschampsi* should be closer to the Grapsoidea species that shown in Fig. 5. Recent studies on the genus *Ucides* have also shown similar classification[51,52]. *G. dehaani* belongs to Potamidae, Potamoidea[53]. However, the phylogenetic tree showed that Potamidae are associated closely with Varunidae, Grapsidae, Sesarmidae, Dotillidae, and Xenograpsidae. This result is in agreement to that inferred from 23 Brachyuran crabs, in which the author use the two mitogenomes[21]. Phylogenetic relationships between *I. deschampsi*, *G. dehaani* and Grapsoidea species need to be reconsidered by integrating more mitogenomic data. More mitogenomic data will also lead to a better overall understanding the phylogenetic relationships among Brachyuran crabs.

Availability of data and materials. The data set supporting the results of this article is available at NCBI (KU589292).

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Author Contributions

Q.N.L. and B.P.T. conceived and designed the experiments. Q.N.L., Z.Z.X., and X.Y.C. performed the experiments. Q.N.L., Z.F.W., Y.L., H.B.Z. and Z.Z.X. analyzed the data. D.Z.Z., C.L.Z. and B.P.T. contributed reagents and materials. Q.N.L. and Z.Z.X. wrote the paper. Z.Z.X., and Q.N.L. revised the paper.

Additional Information

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