The complete mitochondrial genome of *Sesarmops sinensis* reveals gene rearrangements and phylogenetic relationships in Brachyura

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Abstract

Mitochondrial genome (mitogenome) is very important to understand molecular evolution and phylogenetics. Herein, in this study, the complete mitogenome of *Sesarmops sinensis* was reported. The mitogenome was 15,905 bp in size, and contained 13 protein-coding genes (PCGs), two ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and a control region (CR). The AT skew and the GC skew are both negative in the mitogenomes of *S. sinensis*. The nucleotide composition of the *S. sinensis* mitogenome was also biased toward A + T nucleotides (75.7%). All tRNA genes displayed a typical mitochondrial tRNA cloverleaf structure, except for the *trnS1* gene, which lacked a dihydroxyuridine arm.

*S. sinensis* exhibits a novel rearrangement compared with the Pancrustacean ground pattern and other Brachyura species. Based on the 13 PCGs, the phylogenetic analysis showed that *S. sinensis* and *Sesarma neglectum* were clustered on one branch with high nodal support values, indicating that *S. sinensis* and *S. neglectum* have a sister group relationship. The group (*S. sinensis* + *S. neglectum*) was sister to (*Parasesarmops tripectinis* + *Metopaulias depressus*), suggesting that *S. sinensis* belongs to Grapsoidea, Sesarmidae. Phylogenetic trees based on amino acid sequences and nucleotide sequences of mitochondrial 13 PCGs using BI and ML respectively indicate that section Eubrachyura consists of four groups clearly. The resulting phylogeny supports the establishment of a separate subsection Potamoida. These four groups correspond to four subsections of Raninoida, Heterotremata, Potamoida, and Thoracotremata.
**Introduction**

The Brachyura mostly live in littoral regions of tropical shallow seas and about 7000 species have been described and is the most species rich infraorder within Decapoda [1]. Phylogenetic relationships within the Brachyura are complicated because of the extreme morphological and ecological diversity within the group [2]. Four groups of Brachyura (Dromiacea, Raninoida, Heterotremata and Thoracotremata) were recognized [3–5]. Raninoida, Heterotremata and Thoracotremata should be attributed to Eubrachyura, which is a sister group to Dromiacea [6,7].

Animal mitochondrial DNA (mtDNA), a double-stranded circular molecule, ranging from 14 to 19 kilobases (kb) in size, containing 37 genes, including 13 PCGs, ATPase subunits 6 and 8 of the ATPase (atp6 and atp8), cytochrome c oxidase subunits 1–3 (cox1–cox3), cytochrome B (cob), NADH dehydrogenase subunits 1–6 and 4 L (nad1–6 and nad4L), 22 tRNA genes, two rRNA genes and CR [8]. The mtDNA can provide important information on rearrangement trends and phylogeny because of its rapid evolutionary rate and lack of genetic recombination [8]. Using complete mitogenomes is becoming increasingly common for phylogenetic reconstruction [9–11]. The AT-content, secondary structures of tRNAs, and gene rearrangements can be inferred from animal mitogenomes at the genome level [12]. Partial DNA sequences are often too short to contain sufficient phylogenetic information [13]. Further, the addition of rRNA makes alignment ambiguous [2]. It is becoming increasingly common to use complete animal mitogenomes for phylogenetic reconstruction [9–11].

To date, there has been no reports of the complete mitogenome of *S. sinensis*. Thus, in this paper, the complete mitogenome of *S. sinensis* was sequenced and compared with other Brachyura mitogenomes. The available complete mitogenomes were used to provide insight into the phylogenetic relationship of *S. sinensis* and related species. These results will help us to understand features of *S. sinensis* mitogenome and the evolutionary relationships within Brachyura.

**Materials and methods**

**Ethics statement**

There are no specific permits for crabs collection in the selected locations. The sampling locations are not privately-owned or natural protected areas. Crabs used for the experiments are not considered endangered or protected species, and its collection is legal in China.

**Sampling and DNA extraction**

Adult specimens of *S. sinensis* were collected from Fujian province in China in June 2014. The total genomic DNA of *S. sinensis* was isolated from single specimens using the Aidlab Genomic DNA Extraction Kit (Aidlab, China) according to the manufacturer’s instructions. DNA from an individual *S. sinensis* crab was used to amplify the complete mitogenome.

**PCR amplification and sequencing**

To amplify the entire mitogenome of *S. sinensis*, specific primers were designed based on the conserved nucleotide sequences of known mitochondrial sequences in the Brachyura [14–18]. The complete mitogenome of *S. sinensis* was obtained using a combination of conventional PCR and long PCR to amplify overlapping fragments spanning the whole mitogenome. First, six conserved genes (cox1, cox3, 12S, 16S, nad4, cob) were sequenced using the universal primer of crabs. Then the specific primers were designed by conservative sequences. Finally the complete mitogenome was generated by overlapping PCR with specific primers. The fragments...
were amplified using Aidlab Red Taq (Aidlab) according to the manufacturer’s instructions. PCR reactions for the fragments were performed in a 50μL volume with 5μL of 10×Taq plus Buffer (Mg²⁺), 4μL of dNTPs, 2μL each of the primers, 2μL of DNA, 34.5μL of ddH₂O, and 0.5μL Red Taq DNA polymerase. The PCR reactions were performed using the following procedures: 94°C for 3 min; followed by 40 cycles of 30s at 94°C, annealing for 35s at 48–56°C (depending on primer combination), elongation for 1–3 min (depending on length of the fragments) at 72°C; and a final extension step of 72°C for 10 min. The PCR products were separated by agarose gel electrophoresis (1% w/v) and purified using the DNA gel extraction kit (Aidlab, China). The purified PCR products were ligated into the T-vector (SangonBiotech, China) and sequenced at least three times.

**Sequence alignment and gene annotation**

Thirty-nine complete Brachyura mitogenomes were downloaded from GenBank. In addition, the mitogenomes of *Cherax destructor* and *Cambaroides similis* were downloaded from GenBank and used as outgroup taxa. Detailed information is shown in Table 1.

Sequence annotation was performed using NCBI BLAST and the DNAStar package (DNASTar Inc. Madison, WI, USA). Alignments of sequences for each of the available Brachyura mitogenomes were performed using default settings in MAFFT, and then concatenated [19]. The sequences were aligned with those of closely related species. To remove the gaps in sequences and align each gene, poorly aligned positions and divergent regions were removed using Gblocks in our study [20]. The fasta sequences were converted to the nex format and phylip format for BI and ML, respectively. We then used DAMBE to detect the saturated conditions of the sequences [21]; the result of the DAMBE analysis was that ISS was less than ISS.c and p value was extremely significant (0.0000), suggesting that sequences was unsaturated and suit to construct phylogenetic tree. Cloverleaf secondary structure and anticodons of transfer RNAs were identified using the web-server of tRNA-scan SE [22].

**Phylogenetic analysis**

We estimated the taxonomic status of *S. sinensis* within the Decapoda by constructing the phylogenetic tree. Two concatenated datasets: amino acid alignments (AA dataset) and nucleotide alignments (NT dataset) from 42 mitogenomes PCGs were combined. Each dataset was processed using two inference methods: Bayesian inference (BI) and Maximum likelihood (ML). BI was performed using MrBayes v 3.2.1 [23]. ML was performed using raxmlGUI [24]. Nucleotide substitution models were selected using Akaike information criterion implemented in Mrmodeltest v 2.3 [25]. The GTR+I+G model was chosen as the best model of nucleotide phylogenetic analysis and molecular evolution. MtArt + I+ G +F was chosen as the best model for the AA dataset, according to the results of Prottest version 1.4 [26]. There was no MtArt + I+ G +F model in MrBayes; therefore, MtREV + I+ G +F was selected as the second best model. ML analyses were performed on 1000 bootstrapped replicates [27]. The Bayesian analysis ran as 4 simultaneous MCMC chains for 10,000,000 generations, sampled every 100 generations, and a burn-in of 2,500,000 generations was used. Convergence was deduced for the Bayesian analysis on the following basis: the average standard deviation of split frequencies was less than 0.01. Additionally, we observe sufficient parameter sampling by using software Tracer v1.6. The value of ESS is more than 200. The above two points show that our data is convergent [28]. BI was performed under the GTRCAT model with the NT dataset. ML was performed with ML+rapid bootstrap under the GTRCAT model with the NT dataset. BI and ML were performed under the MtREV + I+ G +F model with the AA dataset.
Results and discussion

Genome structure and organization

The *S. sinensis* mitogenome is a closed circular molecule of 15,905 bp in length. It has been deposited in GenBank under the accession number KR336554 and contains typical animal mitochondrial genes, including 13 PCGs, 22 tRNA genes, a large ribosomal RNA (rRNA)
gene and a small ribosomal RNA (srRNA) genes, and CR (Table 2 and Fig 1). Twenty-three genes are coded on the majority strand and the remaining fourteen genes are transcribed on the minority strand. The S. sinensis nucleotide composition is (A) 37.4%, (T) 38.3%, (G) 9.4%, and (C) 14.9%. It shows a high A+T bias: the A+T nucleotide content is 75.7%. In addition, the A+T skew value ([A–T]/[A+T]) is –0.012, and the G+C skew value ([G–C]/[G+C]) is –0.228 [29]. The AT skew and GC skew were calculated for the selected complete mitogenomes (Table 3). The A+T skew value is in the range from –0.080 (Pachygrapsus crassipes) to 0.040 (Homologenus malayensis). The GC skew values were negative in all sequenced Brachyura

Table 2. Summary of mitogenome of S. sinensis.

| Gene   | Direction | Location | Size | Anticodon | Start codon | Stop codon | Intergenic nucleotides |
|--------|-----------|----------|------|-----------|-------------|------------|------------------------|
| cox1   | F         | 1–1559   | 1559 | —         | ATG         | TAA        | -24                    |
| tmL2   | F         | 1536–1601| 66   | TAA       | —           | —          | 6                      |
| cox2   | F         | 1608–2315| 708  | —         | ATG         | TAA        | -20                    |
| tmK    | F         | 2296–2365| 70   | TTT       | —           | —          | -1                     |
| tmD    | F         | 2365–2428| 64   | GTC       | —           | —          | 0                      |
| atp8   | F         | 2429–2587| 159  | —         | ATG         | TAA        | -7                     |
| atp6   | F         | 2581–3254| 674  | —         | ATG         | TA         | 0                      |
| cox3   | F         | 3255–4045| 791  | —         | ATG         | TA         | 0                      |
| tmG    | F         | 4046–4110| 65   | TCC       | —           | —          | 0                      |
| nad3   | F         | 4111–4461| 351  | —         | ATT         | TAA        | 4                      |
| tmA    | F         | 4466–4529| 64   | TGC       | —           | —          | 10                     |
| tmR    | F         | 4540–4605| 66   | TCG       | —           | —          | 2                      |
| tmN    | F         | 4608–4674| 67   | GTT       | —           | —          | 3                      |
| tmS1   | F         | 4678–4744| 67   | TCT       | —           | —          | 0                      |
| tmE    | F         | 4745–4810| 66   | TTC       | —           | —          | 4                      |
| tmH    | R         | 4815–4878| 64   | TAC       | —           | —          | 2                      |
| tmF    | R         | 4881–4945| 65   | GAA       | —           | —          | 7                      |
| nad5   | R         | 4953–6680| 1728 | —         | ATG         | TAA        | 19                     |
| nad4   | R         | 6700–8061| 1392 | —         | ATG         | TAA        | -7                     |
| nad4L  | R         | 8055–8357| 303  | —         | ATG         | TAA        | 9                      |
| tmT    | F         | 8367–8432| 66   | TGT       | —           | —          | 0                      |
| tmP    | R         | 8433–8499| 67   | TGG       | —           | —          | 2                      |
| nad6   | F         | 8502–9004| 503  | —         | ATT         | TA         | 0                      |
| cob    | F         | 9005–10,159| 1155 | —         | ATG         | TAA        | -20                    |
| tmS2   | F         | 10,140–10206| 67   | TGA       | —           | —          | 19                     |
| nad1   | R         | 10,226–11164| 939 | —         | ATA         | TAA        | 40                     |
| tmL1   | R         | 11,205–11,268| 64   | TAG       | —           | —          | 0                      |
| rrL    | R         | 11,269–12,267| 999 | —         | —           | —          | 340                    |
| tmV    | R         | 12,608–12680| 73   | TAC       | —           | —          | 0                      |
| rrS    | R         | 12,681–13,502| 822 | —         | —           | —          | 0                      |
| CR     | —         | 13,503–14,253| 751 | —         | —           | —          | 0                      |
| tmQ    | R         | 14,254–14,322| 69   | TTG       | —           | —          | 192                    |
| tmI    | F         | 14,515–14,581| 67   | GAT       | —           | —          | 47                     |
| tmM    | F         | 14,629–14,698| 70   | CAT       | —           | —          | 0                      |
| nad2   | F         | 14,699–15,706| 1008 | —         | ATG         | TAG        | 2                      |
| tmW    | F         | 15,709–15,778| 70   | TCA       | —           | —          | -3                     |
| tmC    | R         | 15,776–15,839| 64   | GCA       | —           | —          | 0                      |
| tmY    | R         | 15,840–15,905| 66   | GTA       | —           | —          | —                      |
mitogenomes, ranging from \(-0.349\) (Macrophthalmus japonicus) to \(-0.215\) (P. tripectinis).

Although the AT skew and GC skew of S. sinensis are all negative, GC skew is far lower than that of AT indicates an obvious bias toward the use of As and Cs.

### Protein-coding genes

The mitogenome of S. sinensis contains 13 PCGs, starting with the typical ATN codons (Table 2). One (nad1) starts with ATA, two (nad3, nad6) with ATT, and ten (cox1, cox2, atp8, atp6, cox3, cob, nad2, nad5, nad4, and nad4l) with ATG. Nine PCGs (cox1, cox2, atp8, nad3, nad5, nad4, nad4l, cob, and nad1) have a complete TAA stop codon, while the remaining four terminate with either TA (atp6, cox3, and nad6) or TAG (nad2). Nine PCGs (cox1, cox2, atp8, atp6, cox3, nad3, nad6, cob, and nad2) are encoded on the majority strand, while the rest are encoded on the minority strand. The A+T content was 74.0% and AT skew was \(-0.163\)
The RSCU (relative synonymous codon usage) values for *S. sinensis* for the third positions is shown in Fig 2A and Table 4. The codon usage is biased: there is a high frequency of AT compared with GC in the third codon position, which is consistent with other crabs. The most common amino acids in mitochondrial proteins are Leu (UUR), Ile and Phe (Fig 2B).

**Table 3.** The A+T skew value and the G+C skew value of 40 Brachyura species.

| Species            | Size (bp) | A %  | G %  | T %  | C %  | A+T % | A+T skew | G+C skew |
|--------------------|-----------|------|------|------|------|-------|----------|----------|
| *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   | -0.297   |
| *P. gigas*         | 15,515    | 35.0 | 10.8 | 35.5 | 18.7 | 71.5  | -0.006   | -0.268   |
| *G. dehaani*       | 15,620    | 34.4 | 11.4 | 32.4 | 21.8 | 66.8  | 0.029    | -0.316   |
| *A. alayseae*      | 15,611    | 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. xerita*        | 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   |
| *O. cordimus*      | 15,604    | 31.8 | 11.9 | 34.5 | 21.8 | 66.3  | -0.043   | -0.293   |
| *P. tripectinis*   | 15,612    | 36.2 | 10.1 | 38.0 | 15.7 | 74.2  | -0.023   | -0.215   |
| *M. japonicus*     | 16,170    | 33.6 | 10.9 | 32.8 | 22.7 | 66.4  | 0.014    | -0.349   |
| *S. neglectum*     | 15,920    | 37.4 | 9.5  | 38.2 | 14.9 | 75.6  | -0.010   | -0.219   |
| *M. depressus*     | 15,765    | 37.9 | 8.7  | 39.4 | 14.0 | 77.3  | -0.0038  | -0.231   |
| *O. ceratophthalmus*| 15,564   | 33.7 | 11.1 | 35.8 | 19.4 | 69.5  | -0.029   | -0.269   |
| *D. pilumnoides*   | 16,475    | 37.5 | 9.5  | 34.7 | 18.3 | 72.2  | 0.037    | -0.316   |
| *M. majora*        | 15,903    | 38.4 | 9.8  | 35.5 | 16.3 | 73.9  | 0.039    | -0.248   |
| *H. lichuanense*   | 15,380    | 35.8 | 9.3  | 37.4 | 17.5 | 73.2  | -0.023   | -0.305   |
| *M. fornasiini*    | 15,658    | 35.5 | 9.9  | 36.1 | 18.5 | 71.6  | -0.0087  | -0.303   |
| *C. granulosus*    | 16,300    | 33.2 | 11.2 | 36.1 | 19.5 | 69.3  | -0.043   | -0.272   |
| *M. longicarpus*   | 15,548    | 32.4 | 11.8 | 36.6 | 19.2 | 69.0  | -0.060   | -0.236   |

https://doi.org/10.1371/journal.pone.0179800.t003
Transfer RNAs, ribosomal RNAs, and control region

The *S. sinensis* mitogenome contains 22 tRNA genes, as do most Brachyura mtDNAs. The tRNA genes range from 64 to 73 bp. Fourteen tRNA genes are encoded on the majority strand and eight are encoded on the minority strand (Table 2). All the tRNA genes have a typical cloverleaf structure, except for the *trnS*1 gene, whose dihydroxyuridine (DHU) arm had been simplified down to a loop (Fig 3). The loss of the DHU arm is common in animal mitogenomes and has been considered a typical feature of metazoan mitogenomes [30–34]. The cloverleaf secondary structures of 19 transfer RNAs were identified using the web-server of tRNA-scan SE. The three tRNAs not detected by tRNA-scan-SE were determined in the unannotated regions by sequence similarity to tRNAs of other crabs. The average AT content of the tRNA genes is 74.6%; their AT skew and GC skew are all negative (S1 Table), showing an obvious bias toward the use of Ts and Cs. Two rRNA genes were identified on the minority strand in
the *S. sinensis*, with the IrRNA gene located between *trnL* (CUN) and *trnV*, and the srRNA gene located between *trnV* and CR, respectively. The IrRNA gene is 999 bp and the srRNA gene is 822 bp long. The AT-skew of rRNAs (0.001), the GC-skew of rRNAs (–0.296) shows clearly that more As and more Cs than Ts and Gs in rRNAs (S1 Table). CR is located between *rrns* and *trnQ*. The average AT content of the CR is 83.2%. The overall AT-skew and GC-skew in the CR of *S. sinensis* are 0.107 and –0.111, respectively (S1 Table), indicating an obvious bias toward the use of As and Cs.

**Gene rearrangement**

In Pancrustaceans, the tRNA gene order between CR and *trnM* is *trnI-trnQ* [35,36] (Fig 4A). The arrangement of the tRNA genes between CR and *trnM* is *trnQ-trnI* in *S. sinensis* (Fig 4G). The tRNA rearrangements are generally considered to be a consequence of tandem duplication of part of the mitogenome [37,38]. The arrangement of the tRNA gene between *trnE* and *trnF* is *trnH* in *S. sinensis*, which is different from the tRNA genes arrangement of the Pancrustacean ground pattern. In most arthropods, *trnH* is between *nad4* and *nad5* [39], whereas it was found between *trnE* and *trnF* in *S. sinensis*. The phenomenon of gene rearrangements in the mitochondrial genome is a relatively common event in crustacean species [40]. The gene order of *S. sinensis* is identical to that of *S. neglectum* [41], *M. depressus* and *P. tricentinis* (Fig 4G), which supports the view that *S. sinensis* belongs to the Grapsoidea, Sesarmidae. The above results suggested that *S. sinensis*, *S. neglectum*, *M. depressus* and *P. tricentinis* are sister groups.

As shown in Fig 4B, the gene orders of these species are identical. The order of the genes in the mitogenome of *S. sinensis* is different from that in these Brachyura mitogenomes sequences because of the rearrangement of two tRNA genes between CR and *trnM*. The arrangement of the tRNA genes is *trnQ-trnI* between CR and *trnM* in *S. sinensis*, which is different from the *trnL-trnQ* of the these Brachyura species. In this case, the tandem duplication of the gene regions that include *trnL*, *trnQ*, and *trnM*, followed by losses of the supernumerary genes might represent the most ideal mechanism for mitochondrial gene rearrangement [42–44]. It is believed that slipped-strand mispairing takes place first, followed by gene deletion [45]. The gene orders of *Eriocheir japonica sinensis* [46], *E. j. hepuensis*, *E. j. japonica*, *Helice latimera*,

**Table 4. RSCU (relative synonymous codon usage) of *S. sinensis***

| Codon | Count | RSCU | Codon | Count | RSCU | Codon | Count | RSCU | Codon | Count | RSCU |
|-------|-------|------|-------|-------|------|-------|-------|------|-------|-------|------|
| UUU(F) | 314 | 1.76 | UCU(S) | 106 | 2.41 | UAU(Y) | 132 | 1.69 | UGU(C) | 32 | 1.83 |
| UUC(F) | 42 | 0.24 | UCC(S) | 14 | 0.32 | UAC(Y) | 24 | 0.31 | UGC(C) | 3 | 0.17 |
| UUA(L) | 408 | 4.2 | UCA(S) | 102 | 2.32 | UAA(*) | 11 | 1.83 | UGA(W) | 98 | 1.9 |
| UUG(L) | 35 | 0.36 | UGG(S) | 3 | 0.07 | UAG(*) | 1 | 0.17 | UGG(W) | 5 | 0.1 |
| CUU(L) | 70 | 0.72 | CUC(P) | 77 | 2.15 | CAU(H) | 59 | 1.55 | CGU(R) | 19 | 1.41 |
| CUG(L) | 9 | 0.09 | CCC(P) | 14 | 0.39 | CAC(H) | 17 | 0.45 | CGC(R) | 0 | 0 |
| CUA(L) | 55 | 0.57 | CCA(P) | 50 | 1.4 | CAA(Q) | 68 | 1.81 | CGG(R) | 5 | 0.37 |
| CUG(L) | 6 | 0.06 | CGC(P) | 2 | 0.06 | CAG(Q) | 7 | 0.19 | CCG(R) | 0 | 0 |
| AUU(I) | 325 | 1.83 | ACU(T) | 88 | 2.11 | AAU(N) | 140 | 1.74 | AGU(S) | 40 | 0.91 |
| AUC(I) | 31 | 0.17 | ACC(T) | 10 | 0.24 | AAC(N) | 21 | 0.26 | AGC(S) | 1 | 0.02 |
| AUA(M) | 209 | 1.81 | ACA(T) | 67 | 1.6 | AAA(K) | 86 | 1.74 | AGA(S) | 74 | 1.68 |
| AUG(M) | 22 | 0.19 | ACG(T) | 2 | 0.05 | AAG(K) | 13 | 0.26 | AGG(S) | 12 | 0.27 |
| GUU(V) | 92 | 1.69 | GCU(A) | 109 | 2.26 | GAU(D) | 63 | 1.77 | GGU(G) | 82 | 1.48 |
| GUC(V) | 7 | 0.13 | GCC(A) | 19 | 0.39 | GAC(D) | 8 | 0.23 | GGC(G) | 6 | 0.11 |
| GAU(V) | 99 | 1.82 | GCA(A) | 60 | 1.24 | GAA(E) | 70 | 1.82 | GGA(G) | 119 | 2.15 |
| GUG(V) | 20 | 0.37 | GCC(A) | 5 | 0.1 | GAG(E) | 7 | 0.18 | GGG(G) | 14 | 0.25 |

https://doi.org/10.1371/journal.pone.0179800.t004
Fig 3. Secondary structures of 22 transfer tRNA genes of *S. sinensis.*

https://doi.org/10.1371/journal.pone.0179800.g003
Phylogenetic analysis

Phylogenetic analyses were based on two datasets: the AA datasets and the NT datasets using two methods (BI and ML) and alignment method of MAFFT, the topologies of phylogenetic analysis in BI and ML were roughly the same, with some slight differences. *S. sinensis* and *S. neglectum* were clustered in one branch in the phylogenetic tree with high nodal support values in BI and ML trees. (*S. sinensis + S. neglectum*) clade is well supported to be the sister group to the (*P. tripectinis + M. depressus*) clade. This supported the view that *S. sinensis* belongs to the Grapsoidea, Sesarmidae, which is consistent with the results of the gene rearrangement analysis. *H. latimera, C. granulosus, E. j. sinensis, E. j. hepuensis, and E. j. japonica* clustered together with high statistical support (Figs 5 and 6), showing that these species have sister group relationships and belong to Grapsoidea, Varunidae [48].

Cyclograpsus granulosus and *M. japonicus* are same (Fig 4C). *H. latimera, C. granulosus, E. j. sinensis, E. j. hepuensis*, and *E. j. japonica* belong to the Grapoidea, Varunidae [47].

Fig 4. Linearized representation of gene rearrangements of Brachyura circle mitogenomes. All genes are transcribed from left to right. tRNA genes are exhibited by the corresponding single-letter amino acid code with S1 = AGN, S2 = UCN, L1 = CUN, and L2 = UUR. CR represents control region. rml, rns, large and small subunit ribosomal RNA.

https://doi.org/10.1371/journal.pone.0179800.g004
topologies. The section Eybrachyura crabs consist of four groups, one comprising families of Heterotremata, the second Thoracotremata families, the third Potamoidea crabs, and the fourth Raninoida species. All the bootstrap values for the branches separating these groups are high. The resulting phylogeny supports the establishment of a separate subsection Potamoida corresponding to Group 3. The present molecular study gives additional evidence for the Potamoida status in these taxa. In all trees Potamoida does closely cluster with subsection Thoracotremata. The relationship between Potamoida and Thoracotremata is much closer than it between the former and Heterotremata, which was proposed by Bowman and Abele [4]. These four subsections (groups) constitute a monophyletic sister group to Section Dromiacea (Group 5) in all phylogenetic trees.

Conclusion

This study presents one mitogenome of *S. sinensis*. The mitogenome contains 13 PCGs, 22 tRNA genes, two tRNA genes and CR. The AT skew and the GC skew are both negative in the mitogenomes of *S. sinensis*, indicating an obvious bias toward the use of Ts and Cs, which is consistent with most sequenced brachyuran crabs. The gene arrangement of *S. sinensis* is identical to that of *S. neglectum*, *P. tripectinis* and *M. depressus*. In comparison to Pancrustacean ground pattern and common arrangement for brachyuran crabs, *S. sinensis* exhibits a novel rearrangement. Tandem duplication followed by random deletion is widely considered to explain generation of gene rearrangement of mitogenome in *S. sinensis*. The phylogenetic analyses indicate that *S. sinensis* and *S. neglectum* have sister group relationships, and the clade (*S. sinensis* + *S. neglectum*) is well supported to be the sister group to (*P. tripectinis* + *M. depressus*),
suggesting that *S. sinensis* should belong to Grapoidea, Sesarmidae. The topology of BI and ML trees of Brachyura species (Fig 5) inferred from nucleotide sequences of mitochondrial 13 PCGs sequences are similar to those of Fig 6 constructed from amino acid sequences of whole mitogenomes. The resulting phylogeny strongly supports the establishment of a separate subsection Potamoida, so section Eubrachyura consists of four subsections which are Raninoida, Heterotremata, Potamoida, and Thoracotremata. However, the four subsections and two sections are monophyletic, respectively, whereas the relationships within families of each subsection were not resolved absolutely in the present study.

**Supporting information**

S1 Table. Composition and skewness in the *S. sinensis* mitogenome.

(DOCX)

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (31672267 and 31640074), the Natural Science Foundation of Jiangsu Province (BK20160444), the Natural Science Research General Program of Jiangsu Provincial Higher Education Institutions (15KJB240002, 12KJA18009 and 16KJA18008), Jiangsu Provincial Key Laboratory of Coastal Wetland Bioresources and Environmental Protection (JLCBE14006), the Special Guide Fund Project of Agricultural Science and Technology Innovation of Yancheng city.
(YKN2014022), the Jiangsu Provincial Key Laboratory for Bioresources of Saline Soils (JKLBS2014013 and JKLBS2015004).

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