The mitochondrial genome of *Grapsus albolineatus* (Decapoda: Brachyura: Grapsidae) and phylogenetic associations in Brachyura

Jiayin Lü1,3, Liping Xia1,3, Xiaojuan Liu2, Yanwen Ma1, Jiji Li1,3*, Yingying Ye1,2* & Baoying Guo1

Complete mitochondrial genomes (mitogenomes) can provide useful information for phylogenetic relationships, gene rearrangement, and evolutionary traits. In this study, we determined the complete mitochondrial DNA sequence of the herbivorous crab *Grapsus albolineatus*. It is a typical metazoan mitochondrial genome. The total size is 15,583 bp, contains the entire set of 37 genes, and has an AT-rich region. Then, 23 of the 37 genes were encoded by the heavy (+) strand while 14 are encoded by the light (−) strand. Compared with the pan-crustacean ground pattern, two tRNA genes (*tRNA-His* and *tRNA-Gln*) were rearranged and the tandem duplication/random loss model was used to explain the observed gene rearrangements. The phylogenetic results showed that all Grapsidae crabs clustered together as a group. Furthermore, the monophyly of each family was well supported, with the exception of Menippidae. In general, the results obtained in this study will contribute to the better understanding of gene rearrangements in Grapsidae crab mitogenomes and provide new insights into the phylogeny of Brachyura.

Brachyura crab is the largest clade in the Decapod crustacean group, with more than 7250 known species, including 98 families of marine, freshwater, and terrestrial habitats, most of which are economically important1. However, the phylogenetic relationships among members of Brachyura and their evolutionary origin continue to be controversial due to the high morphological similarity and ecological diversity2–4. Initially, Brachyura was divided into Podotremata, Heterotremata, and Thoracotremata5. Subsequently, it was segmented into Dromiacea and Eubrachyura (including Thoracotremata, Raninoida, and Heterotremata)6. However, the latest classification scheme divides Brachyura into Cyclodorippoida, Eubrachyura, Dromicea, and Raninoida7,8. Although the phylogenetic relationship within Brachyura is still uncertain, the current classification system has been recognized by most scholars.

According to WoRMS (http://www.marinespecies.org/), the family Grapsidae has 8 genera and 49 species in total. However, only five species sequences of Grapsidae have been published4,9–12. The herbivorous crab (*Grapsus albolineatus*) is one of the marine crustaceans that live on rocky shores which belongs to the phylum Arthropod, subphylum Crustacea, order Decapoda, infraorder Brachyura, clade Thoracotremata, family Grapsidae, genus *Grapsus*. They are mainly distributed in Japan, Hawaii, Australia and China's Guangdong, Hainan Island, Xisha Islands, Taiwan. So far, most studies of this species have focused on the morphology and growth13,14. Although there are few studies on the molecular level, most of them were based on partial mitochondrial and nuclear ribosomal RNA gene sequences15.

The mitochondrial genome (mitogenome) of metazoans is usually 14–20 kb in size and encoded with a set of 37 genes, including 13 protein coding genes (*cox1-3, cob, nad1-6, nad4L, atp6, and atp8*), 2 ribosomal RNA genes (*rrnL and rrns*), 22 transport RNA genes (tRNAs), and an AT-rich region (also called control region, CR) which contains some initiation sites for transcription and replication of the genome16. Mitochondrial DNA forms a separate unit of genetic information that evolved independently from the nuclear genome. Due to its haploid
properties, matrilineal inheritance, limited recombination, and rapid rate of evolution\textsuperscript{17}, the mitogenome is increasingly being used in evolutionary and phylogenetic studies. With the rapid development of sequencing technology, next-generation sequencing has become a fast and low-cost method to provide complete mitotic genomes\textsuperscript{23}. Gene rearrangements in the mitogenomes of crabs are relatively common\textsuperscript{1,19,20}. So far, several hypotheses have been suggested to help explain gene rearrangements in animal mitogenomes. Recombination model and tandem duplication/random loss (TDLR) model are more commonly accepted. Recombination models are involved in the breaking and reconnecting of DNA strands\textsuperscript{21}. The TDLR model assumes that the rearranged gene order occurs via tandem duplications followed by random deletion of certain duplications\textsuperscript{22}. This model has been widely used to explain the translocation of genes encoded on the same strand\textsuperscript{23}. Model tRNA mis-priming model and the tandem duplication/non-random loss model (TNDL) are less commonly used.

In this study, we successfully sequenced the complete mitogenome of \textit{G. albolineatus} and used existing complete mitogenomes to compare it with other Brachyura species. In addition, a phylogenetic analysis of 70 brachyuran species was conducted based on the nucleotide sequences of 13 PCGs (Protein-coding gene). These results will help us to understand features of the \textit{G. albolineatus} mitogenome and the evolutionary relationships within Brachyura.

**Results and discussion**

**Genome structure and composition.** The complete mitogenome sequence of \textit{G. albolineatus} is a typical closed-circular molecule of 15,583 bp in size (GenBank accession number MZ262276), which is similar in length to the published Grapsidae mitogenomes\textsuperscript{45-11}, a size range from 15,406 to 15,920 bp (Table 1). The mitogenome contents of \textit{G. albolineatus} is the same as most other published Brachyura which includes 37 genes, 13 PCGs, 22 tRNAs, and 2 rRNA (\textit{rrnL} and \textit{rrnS}), as well as a brief non-coding region, all the genes were identified (Fig. 1, Table 2). Most of the 37 genes are located on the heavy (H-) strand, except 4 PCGs (ND5, ND4, ND4L, ND1), 8 tRNAs (\textit{tRNA-Gys}, Tyr, Gln, Val, Leu, Pro, Phe, and His), and 2 rRNA which are located on the light (L-) strand (Fig. 1, Table 2). There are 13 regions with overlap in the total \textit{G. albolineatus} mitogenome, with 3 of them more than 10 bp (\textit{tRNA-Phe} (41 bp), \textit{trnL} (25 bp), and \textit{cox2/trnS} (20 bp)) and the other 10 shorter than 10 bp (\textit{nad1} (7 bp), \textit{atp8} (4 bp), \textit{cox3/atp6/mnk/nad6/trnW} (1 bp), \textit{trnG} (3 bp), and \textit{nad3/nad2} (2 bp)) (Table 2). The \textit{G. albolineatus} mitogenome also contains 328 bp of intergenic spacers located in 17 regions, ranging from 1 to 122 bp (Table 2) and indicating the occurrence of tandem duplications and the deletions of redundant genes. GC-skew of the complete mitogenomes of 6 Grapsidae species were calculated and compared (Tables 3, 4). The nucleotide composition of the \textit{G. albolineatus} mitogenome is A (33.4%), T (34.04%), G (12.02%), and C (20.54%), with a high A–T bias. The A + T (%) content of the mitogenomes was 66.74%. The AT-skew and GC-skew value are calculated for the chosen complete mitogenomes (Table 3). Both AT-skew and GC-skew of the \textit{G. albolineatus} mitogenome are slightly negative, −0.009 and −0.262, informing T's and C's are more abundant than A's and G's. Similar results were observed for the other selected Grapsidae mitogenomes. In general, the AT-skew and GC-skew of the overall mitogenomes, nucleotide composition, and gene lengths of the \textit{G. albolineatus} were the same as those of the other Grapsidae species\textsuperscript{15-17}.

**PCGs and codon usage.** The initial and terminal codons of all PCGs of \textit{G. albolineatus} are listed in Table 2. \textit{G. albolineatus} has 13 PCGs in the typical order found in Brachyuran species, containing 7 NADH dehydrogenase (\textit{nad1-nad6}, \textit{nad4L}), 3 cytochrome c-oxidases (\textit{cox1–cox3}), two ATPases (\textit{atp6}, \textit{atp8}), and cytochrome b (\textit{cob}). The total length of the 13 PCGs is 11,323 bp. The length of the 13 PCGs range from 303 to 1371 bp (Tables 2, 3).

The average A + T content is 65.26%, ranging from 39.63% (ND5) to 74.21% (ATP8) (Table 3). The AT-skew and GC-skew are −0.159 and −0.034, respectively (Table 3). All of the PCGs are initiated by the start codon (Tables 2, 3).

**Transfer RNAs and ribosomal RNAs.** Like most Grapsidae species, \textit{G. albolineatus} mitogenome contains 22 tRNA genes\textsuperscript{20,25,26}. Fourteen of them are encoded by the heavy strain (H-) and the rest are encoded by the light strain (L-). In the whole mitogenome, the size of tRNAs range from 50 to 73 bp and have a total length of 1402 bp, with an obvious AT bias (71.54%) (Table 2). The AT-skew and GC-skew are −0.009 and 0.158, respectively, showing a slight bias toward the use of Ts and an apparent bias toward Cs (Table 3). The most frequently used amion acid in \textit{G. albolineatus} is Leu, and the least common anion acid is Trp (Fig. 2). The relative synonymous codon usage (RSCU) values for \textit{G. albolineatus} of the 13 PCGs are shown in Table 5 and Fig. 24. The three most frequently detected codons are GCC (Ala), UCU (Ser2), and GUA (Val), whereas GCC (Ala) is the least common codon. Based on CDspT and RSCU, comparative analyses showed that the codon usage pattern of \textit{G. albolineatus} is conserved. The codon usage patterns of 13 PCGs are similar to those of other Grapsidae species.
| Superfamily | Family       | Species               | Size (bp) | Accession.no |
|------------|-------------|-----------------------|-----------|--------------|
| Grapsioidea | Grapsidae   | Grapsus marmoratus    | 15,406    | MF457403.1   |
|            |             | Grapsus albolineatus  | 15,583    | MZ262276     |
|            |             | Metopograpsus frontalis | 15,587  | NC_042152.1  |
|            |             | Metopograpsus quadridentatus | 15,520 | MH310445    |
|            |             | Grapsus tenuicrustatus  | 15,858    | NC_029724    |
|            |             | Pachygrapsus crassipes  | 15,652    | NC_021754    |
|            | Sesarmidae  | Pachygrapsus pictum   | 15,611    | NC_038066    |
|            |             | Pachygrapsus tricarinatus | 15,612  | NC_030046    |
|            |             | Perciesarma bidens     | 15,641    | NC_051868    |
|            |             | Pachygrapsus affine    | 15,638    | NC_039990    |
|            |             | Chiromantes haenatocheir | 15,899  | NC_042142.1  |
|            |             | Sesarma neglectum      | 15,920    | NC_031851.1  |
|            | Varunidae   | Pseudohelice subquadrata | 16,898  | MH718959     |
|            |             | Hemigrapsus penicillatus | 16,486  | MG71772.1    |
|            |             | Varuna yui             | 15,915    | NC_035715    |
|            |             | Varuna littorata       | 16,378    | MF198,252.1  |
|            |             | Cyclograpsus intermedia | 16,154  | MZ421398.1   |
|            |             | Cyclograpsus granulosus | 16,300  | NC_025571    |
|            |             | Metaplax longipes      | 16,424    | MF198,248    |
|            |             | Eriocheir sinensis     | 16,378    | KM516908     |
|            |             | Chasmagnathus convexus  | 15,107    | NC_051834.1  |
|            |             | Gecarcoidea lalandii    | 15,575    | NC_054751.1  |
|            |             | Gecarcoidea natatil     | 15,545    | NC_039811.2  |
|            | Xenograpsida | Xenograpsus ngatiama    | 15,798    | EU722703     |
|            |             | Xenograpsus testudinatus | 15,798  | NC_013480.1  |
| Ocypodoidea | Dotillidae  | Ilyoplax deschampsi    | 15,460    | NC_020040    |
|            | Macrophthalmidae | Macrothelasma pacificus | 17,226   | NC_046039    |
|            |             | Macrothelasma lateralei | 15,747   | MW423579     |
|            |             | Macrothelasma abbreviatus | 16,322  | MN393095     |
|            | Mictyridae   | Mictyris longicarpus    | 15,548    | LN611670     |
|            |             | Mictyris thailandensis  | 15,557    | MW697086     |
| Ocypodeida | Ocypodidae  | Ocypode ceratophthalmus | 15,564   | NC_025324    |
|            |             | Ocypode stimpsoni      | 15,575    | NC_046797    |
|            |             | Austruca lactea        | 15,659    | NC_042401    |
|            |             | Cranuca inversa        | 15,677    | MF457405     |
| Bythograeidea | Bythograeidae | Gandalfus pusa       | 15,548    | NC_027414    |
|            |             | Austringnarea alystraeae | 15,611  | KC851803     |
| Calappoidea | Calappidae  | Calappa bilineata     | 15,606    | NC_047195    |
|            | Matutidae   | Asthoret lunaris      | 15,807    | NC_024435    |
|            |             | Matutia planipes      | 15,751    | MK281334     |
|            | Carpiliidae  | Carpillus convexus    | 15,766    | MT780873     |
| Eriphioidea | Menippidae  | Myomemippe fornaxinii  | 15,658    | NC_024437    |
|            |             | Pseudocarcinus gigas   | 15,515    | AY562127     |
|            | Oziidae     | Epixanthus frontalis  | 15,993    | MF457404     |

Continued
| Superfamily | Family | Species          | Size (bp) | Accession.no |
|------------|--------|------------------|----------|--------------|
| Xanthoidea | Xanthidae | *Etisus anaglyptus* | 16,435 | NC_042208   |
|            |        | *Etisus dentatus*  | 15,884  | NC_054248    |
|            |        | *Atergatis integerrimus* | 15,924 | NC_037172    |
|            |        | *Atergatis floridus* | 16,180  | NC_037201    |
| Majoidea   | Oregoniidae | *Chinoecetes japonicus* | 15,341 | AB735678     |
|            | Majidae | *Maja crispata*   | 16,592  | NC_035424.1  |
|            |        | *Maja squinado*    | 16,598  | NC_035425.1  |
| Portunoidea | Geryonidae | *Chaceon granulatus* | 16,135 | NC_023476.1  |
|            |        | *Chaceon sp.*      | 16,126  | KU507298     |
|            | Portunidae | *Thalamita crenata* | 15,787 | NC_024438    |
|            |        | *Thalamita sima*   | 15,831  | NC_039640    |
|            |        | *Portunus trituberculatus* | 16,026 | AB093006    |
|            |        | *Portunus gracilimanus* | 15,990 | NC_040124    |
|            |        | *Charybdis natator* | 15,664 | MF285241     |
|            |        | *Charybdis japonica* | 15,738 | FJ460517     |
|            |        | *Charybdis feriata* | 15,660 | KF386147     |
| Outgroup   |        | *Pagurus nigrofascia* | 15,423 | NC_042412    |
|            |        | *Pagurus gracilipes* | 16,051 | LC222534     |

Table 1. List of Brachyuran species with their GenBank accession numbers.

Figure 1. Circular mitogenome map of *Grapsus albolineatus*. Protein coding, ribosomal, and tRNA genes are shown with standard abbreviations. Arrows indicate the orientation of gene transcription. The inner circles show the G–C content and GC-skew, which are plotted as the deviation from the average value of the entire sequence.
However, the gene order in 4 families (Sesarmidae, Varunidae, Macrophthalmidae, and Xenograpsidae) displace the usual location between trnH and nad5. Additionally, 45 species from 14 families (Grapsidae, Mictyridae, Ocypodidae, Bythograeidae, Calappidae, Dotillidae, Matutidae, Menippidae, Oziidae, Xanthidae, Oregoniidae, Geryonidae, Portunidae, and Oregoniidae) have shown that gene rearrangements in metazoan mitochondrial genomes are conserved and the occurrence of gene rearrangements is relatively random and rare. However, it can be used as direct evidence of evolutionary relationships between species, as it can be used as a tool for studying mitochondrial evolution.

### Table 2. Nucleotide composition and skewness of *Grapsus albolineatus* mitochondrial genome.

|            | A% | T% | G% | C% | (A+T)% | AT-skew | GC-skew | Length (bp) |
|------------|----|----|----|----|--------|---------|---------|-------------|
| Mitogenome | 33.4 | 34.04 | 12.02 | 20.54 | 67.44 | −0.009 | −0.262 | 15,583 |
| PCGs       | 27.44 | 37.82 | 16.78 | 17.96 | 65.26 | −0.159 | −0.034 | 11,323 |
| cox1       | 26.90 | 34.50 | 16.31 | 22.29 | 61.40 | −0.124 | −0.155 | 1539 |
| cox2       | 30.79 | 32.77 | 14.69 | 21.75 | 63.56 | −0.031 | −0.194 | 708 |
| atp8       | 28.93 | 7.55 | 45.28 | 18.24 | 74.21 | 0.586 | 0.426 | 159 |
| atp6       | 37.05 | 28.27 | 12.20 | 22.47 | 65.33 | 0.134 | −0.296 | 672 |
| nad3       | 28.41 | 33.71 | 15.78 | 22.10 | 62.12 | −0.085 | −0.167 | 792 |
| nad4L      | 26.84 | 38.70 | 22.03 | 22.03 | 65.54 | −0.181 | 0.000 | 354 |
| nad5       | 29.29 | 38.30 | 20.68 | 11.73 | 70.30 | −0.133 | 0.276 | 1751 |
| nad6       | 27.80 | 39.61 | 22.65 | 9.94 | 39.61 | −0.175 | 0.390 | 1338 |
| nad4       | 27.80 | 39.61 | 22.65 | 9.94 | 67.41 | −0.175 | 0.390 | 1338 |
| nad4L      | 28.71 | 41.58 | 21.45 | 8.25 | 70.30 | −0.183 | 0.444 | 303 |
| cox1       | 23.49 | 43.37 | 10.64 | 22.49 | 66.87 | −0.297 | −0.358 | 498 |
| cob        | 26.52 | 35.51 | 14.19 | 23.79 | 62.03 | −0.145 | −0.253 | 1135 |
| nad1       | 23.95 | 41.77 | 22.57 | 11.71 | 65.72 | −0.271 | 0.317 | 948 |
| nad2       | 25.62 | 39.86 | 10.88 | 23.64 | 65.48 | −0.217 | −0.370 | 1011 |
| tRNAs      | 35.45 | 36.09 | 16.48 | 11.98 | 71.54 | −0.009 | 0.158 | 1402 |
| tRNAs      | 36.24 | 36.33 | 17.61 | 9.82 | 72.57 | −0.001 | 0.284 | 2158 |
| AT-rich    | 45.54 | 32.09 | 8.91 | 13.45 | 77.63 | 0.173 | −0.203 | 617 |

**Gene rearrangement.** Mitochondrial gene rearrangement is an important molecular marker and is considered to be an effective tool for studying mitochondrial evolution. A large number of studies and results have shown that gene rearrangements in metazoan mitochondrial genomes are conserved and the occurrence of gene rearrangements is relatively random and rare. However, it can be used as direct evidence of evolutionary relationships between species. Mapping the gene layout based on the complete mitochondrial sequences of 70 species. Through comparison and analysis with the ancestor of Decapoda (Fig. 3A), we found that *G. albolineatus* and another 5 species from Grapsidae have a trnH translocation, which the trnH shifted into trnE instead of the usual location between nad5 and nad4 (Fig. 3C). It is widely believed that the tandem duplication/random loss model (TDRL) can explain the movement of trnH, which occurs from tandem duplication in the region between trnE and nad4, followed by deletion of redundant genes producing trnH-trnF-nad5. Additionally, 45 species from 14 families (Grapsidae, Mictyridae, Ocypodidae, Bythograeidae, Calappidae, Dotillidae, Matutidae, Menippidae, Oziidae, Xanthidae, Oregoniidae, Geryonidae, Portunidae, and Carpiliidae) have shown that gene rearrangements, which are consistent with the ancestral of Brachyura (Fig. 3B). However, the gene order in 4 families (Sesarmidae, Varunidae, Macrobrachidae, and Xenograpasidae) displayed 4 patterns of gene rearrangements. The family Sesarmidae observed trnQ and trnl inverted, which has been described in previous studies (Fig. 3D). The gene order of the Varunidae (Grapsoidae) and Macrobrachidae (Ocypodoidea) have the same high level rearrangements (Fig. 3E). It is worth noting that the two families come from different superfamilies, but they form a sister clade in phylogenetic trees. The gene order of the Xenograpasidae have a more complex rearrangement and such within-genus rearrangements were infrequent (Fig. 3F, G), which seems to be related to their particular habitat. Xenograpasidae have been found thus far only in shallow-water, volcanically active, and sulphur-rich hydrothermal vents.

**Phylogenetic relationships.** In the present study, the phylogenetic relationships were analyzed based on the sequences of the 13 PCGs to clarify the relationships in Brachyura. *G. albolineatus* and 68 other known brachyuran specie were analyzed, with *P. nigrofascia* and *P. gracilipes* as outgroups. The two phylogenetic trees (Maximum Likelihood (ML) tree and Bayesian Inference (BI) tree) resulted in identical topological structuring with different supporting value. Then, only one topology (ML) with both support values was presented displayed (Fig. 4). Both trees showed that all the species of Grapsidae clustered together as a solid monophyletic group and consist of three sister clades ((Grapsus + Pachygrapsus) + Metapograpsus). It is obvious that *G. albolineatus* had the closest relationship with *G. tenuicrustatus*, and that these two species form a sister clade with high support values (BI posterior probabilities PP = 1, ML bootstrap BP = 100), constituting a Grapsus group. However, recent molecular studies, including our dataset, have not reached an agreement about closest relatives in Grapsidae. Our phylogenetic tree showed that Grapsidae and Dotillidae form a sister clade, which was in concordance with Wang et al. While Wang et al. and Ng, N. K. et al. found that Grapsidae do not have any close relatives, Li et al. found that Grapsidae and Ocypodida form a sister clade.

Among the 21 families included in our phylogenetic tree, except Menippidae, each family in the tree forms a monophyletic clade with high nodal support values. At a higher level of classification, most Brachyura superfamilies were found to be monophyletic, except Ocypodoidea, Grapsidea and Eriphioidea, which is in line with previous studies. It showed that Grapsidea was divided into three clades.
(((Seasamidae + Gecarcinidae + Xengrapsidae) + Grapsidae) + Varunidae), Ocypodoidea was divided in three clades ((Ocypodidae + Dotillidae) + Macrophthalmidae + Mictyridae) and Eriphioidea was divided into two clades (Oziidae + Menippidae). Within Thoracotremata, the superfamilies Ocypodoidea and Grapsoidea supported paraphletic and 9 families showed the following relationship: ((((Seasamidae + Gecarcinidae) + Xengrapsidae) + Ocypodidae) + (Grapsidae + Dotillidae) + (Varunidae + Macrophthalmidae) + Mictyridae) (Fig. 4).

The main phylogenetic structure of our tree is consistent with previous results, but some controversial findings were observed. Here, the families Macrophthalmidae and Varunidae were grouped into one clade, and Mictyridae as basal group which supports the previous findings revealed in Wang et al. and Zhang et al.9,33. However, previous researchers revealed that Macrophthalmidae and Varunidae were grouped into one clade, then into another clade with Varunidae (((Macrophthalmidae + Varunidae) + Mictyridae)38,39, which was conflict with our results. The classification of Grapsoidea and Ocypodoidea has long been controversial. Previous studies based on morphological characteristics considered them to be monophyletic branches. However, an increasing number of molecular studies, including ours, challenge the inconsistent views on the traditional classification system that are put forward. Although the polyphyly of Grapsidae, Ocypodoidea, and Eriphioidea is well supported, the phylogenetic relationships of these superfamilies need to be further analyzed by integrating additional molecular data32–36. Previous studies on mitochondrial phylogeny have confirmed the importance of mitochondrial

| Gene  | Position From | To   | Length | Amino acid | Start/stop codon | Anticodon | Intergenic region | Strand |
|-------|---------------|------|--------|------------|-----------------|-----------|------------------|--------|
| cox1  | 1             | 1539 | 1539   | 513        | ATG/TAG         | 0         | H                |        |
| trnlL2| 1535          | 1602 | 68     | TAA        | 10              | H         |                  |        |
| cox2  | 1613          | 2320 | 708    | 236        | ATG/TAA         | −20       | H                |        |
| trnK  | 2301          | 2370 | 70     | TTT        | −1              | H         |                  |        |
| trnD  | 2370          | 2433 | 64     | GTC        | 0               | H         |                  |        |
| atp8  | 2434          | 2592 | 159    | 53         | GTG/TAA         | −4        | H                |        |
| atp6  | 2589          | 3260 | 672    | 224        | ATA/TAA         | −1        | H                |        |
| cox3  | 3260          | 4051 | 792    | 264        | ATG/TAA         | −1        | H                |        |
| trnG  | 4051          | 4113 | 63     | TCC        | −3              | H         |                  |        |
| nad3  | 4111          | 4464 | 354    | 118        | ATA/TAA         | −2        | H                |        |
| trnA  | 4463          | 4526 | 64     | TGC        | 6               | H         |                  |        |
| trnR  | 4533          | 4596 | 64     | TCG        | 1               | H         |                  |        |
| trnN  | 4598          | 4662 | 65     | GTT        | 4               | H         |                  |        |
| trnS1 | 4667          | 4733 | 67     | TCT        | 2               | H         |                  |        |
| trnE  | 4736          | 4803 | 68     | TTC        | 3               | H         |                  |        |
| trnH  | 4807          | 4871 | 65     | GTG        | 4               | L         |                  |        |
| trnF  | 4876          | 4940 | 65     | GAA        | 52              | L         |                  |        |
| nad5  | 4993          | 6723 | 1731   | 577        | ATT/TAA         | 44        | L                |        |
| nad4  | 6768          | 8105 | 1338   | 446        | ATG/TAG         | −7        | L                |        |
| nad4L | 8099          | 8401 | 303    | 101        | ATG/TAA         | 5         | L                |        |
| trnF  | 8416          | 8481 | 50     | TGT        | −41             | H         |                  |        |
| trnP  | 8482          | 8550 | 69     | TGG        | 8               | L         |                  |        |
| nad6  | 8559          | 9056 | 498    | 166        | ATT/TAA         | −1        | H                |        |
| cob   | 9056          | 10,190| 1134   | 378        | ATG/TAA         | 0         | H                |        |
| trnS2 | 10,191        | 10,258| 927    | 309        | TCT             | 0         | H                |        |
| nad1  | 10,286        | 11,233| 948    | 316        | ATT/TAA         | 23        | L                |        |
| trnL1 | 11,257        | 11,323| 67     | TAG        | −25             | L         |                  |        |
| trnL  | 11,299        | 12,629| 1331   | 21         | L                |        |                  |        |
| trnV  | 12,651        | 12,723| 73     | TAC        | 0               | L         |                  |        |
| trnS  | 12,724        | 13,550| 827    | 122        | L                |        |                  |        |
| CR    | 13,551        | 14,167| 617    | 0          | H                |        |                  |        |
| trnI  | 14,168        | 14,234| 155    | GAT        | 70              | H         |                  |        |
| trnQ  | 14,232        | 14,300| 69     | TTG        | 7               | L         |                  |        |
| trnM  | 14,308        | 14,378| 71     | CAT        | 0               | H         |                  |        |
| nad2  | 14,379        | 15,389| 1011   | 367        | ATT/TAG         | −2        | H                |        |
| trnW  | 15,388        | 15,456| 69     | TCA        | −1              | H         |                  |        |
| trnC  | 15,456        | 15,519| 64     | GCA        | 0               | L         |                  |        |
| trnY  | 15,520        | 15,583| 64     | GTA        | 0               | L         |                  |        |

Table 3. Organization of the *Grapsus albolineatus* mitochondrial genome.
genomic data in elucidating the Grapsidae phylogeny. On the contrary, many families contained only one representative, which may produce unstable phylogenetic relationships. Therefore, it is necessary to perform further mitogenome sequence studies to obtain a more comprehensive taxon sampling and understand the phylogeny and evolution of Grapsidae.

**Materials and methods**

**Sampling and DNA extraction.** A specimen of *G. albolineatus* was collected from Yangjiang, Guangdong Province, China (21°28’45” N, 111°16’35” E). The specimen was immediately preserved in absolute ethanol after collection and then stored at −20 °C. This specimen was identified by morphology and fresh tissues were dissected from the operculum and preserved in absolute ethanol before DNA extraction. The total genomic DNA was extracted using the salt-extraction procedure with a slight modification and stored at −20 °C.

### Table 4. Nucleotide composition in regions of the mitogenomes of six Grapsidae species.

| Species              | Total size | Complete mitogenome | A   | T   | G   | C   | A+T% | AT-skew | GC-skew |
|----------------------|------------|---------------------|-----|-----|-----|-----|------|---------|---------|
| *Pachygrapsus crassipes* | 15,652     | 36.61 38.2 10.06 15.13 | 74.81 | −0.021 | −0.201 |
| *Pachygrapsus marmoratus* | 15,406     | 31.4 36.99 12.13 19.49 | 68.38 | −0.082 | −0.233 |
| *Grapsus albolineatus*  | 15,583     | 33.4 34.04 12.02 20.54 | 67.44 | −0.009 | −0.262 |
| *Grapsus tenuicrustatus* | 15,858     | 31.92 33.11 12.13 22.85 | 65.03 | −0.018 | −0.306 |
| *Metopograpsus frontalis* | 15,587     | 32.77 36.95 11.01 19.27 | 69.72 | −0.060 | −0.273 |
| *Metopograpsus quadridentatus* | 15,520    | 34.25 26.01 10.21 19.53 | 70.26 | 0.137 | −0.313 |

**Figure 2.** Codon usage patterns in the mitogenome of *Grapsus albolineatus* CDspT, codons per thousand codons. Codon families are provided on the x-axis (A), and the relative synonymous codon usage (RSCU) (B).
In this study, the mitogenome of *G. albolineatus* was sequenced by next-generation sequencing, thereby generating new mitochondrial data for Grapsidae and confirming its ancestral gene order. The mitogenome is a typical closed-circular molecule including 13 PCGs, 22 tRNA genes, two rRNA genes, and a control region (CR). The AT-skew and GC-skew are both negative in the mitogenome of *G. albolineatus*, showing an obvious strand asymmetry. A full description of the mitogenome is provided in the table below.

**Table 5.** Codon number and relative synonymous codon usage in the mitochondrial genome of *Grapsus albolineatus*.

| Codon | Count | RSCU | Codon | Count | RSCU | Codon | Count | RSCU | Codon | Count | RSCU |
|-------|-------|------|-------|-------|------|-------|-------|------|-------|-------|------|
| UUU(F) | 253 | 1.43 | UCU(S) | 127 | 1.6 | UAU(Y) | 219 | 1.31 | UGU(C) | 57 | 1.07 |
| UCG(F) | 102 | 0.57 | UCC(S) | 73 | 0.92 | UAC(Y) | 115 | 0.69 | UGC(C) | 50 | 0.93 |
| UUA(L) | 179 | 1.55 | UCA(S) | 103 | 1.3 | UAA(*) | 233 | 1.51 | UGA(W) | 59 | 1.22 |
| UUG(L) | 62 | 0.54 | UCG(S) | 33 | 0.42 | UAG(*) | 76 | 0.49 | UGG(W) | 38 | 0.78 |
| CUA(L) | 163 | 1.41 | CUC(P) | 93 | 1.43 | CAU(H) | 88 | 1.18 | CGU(R) | 19 | 0.93 |
| CUC(L) | 80 | 0.69 | CCC(P) | 63 | 0.97 | CAC(H) | 61 | 0.82 | CGC(C) | 19 | 0.93 |
| CUA(L) | 156 | 1.35 | CCA(P) | 88 | 1.35 | CAA(Q) | 99 | 1.4 | CGA(R) | 31 | 1.51 |
| CUG(L) | 54 | 0.47 | CGG(P) | 17 | 0.26 | CAG(Q) | 42 | 0.6 | CGG(R) | 13 | 0.63 |
| AUC(U) | 194 | 1.32 | ACC(T) | 118 | 1.57 | AUA(M) | 189 | 1.12 | AGU(S) | 86 | 1.08 |
| AUC(U) | 99 | 0.68 | ACC(T) | 72 | 0.96 | AAC(N) | 148 | 0.88 | AGC(S) | 71 | 0.89 |
| AUA(M) | 172 | 1.48 | ACA(T) | 87 | 1.16 | AAA(K) | 221 | 1.51 | AGA(S) | 87 | 1.1 |
| AUG(M) | 60 | 0.52 | ACG(T) | 24 | 0.32 | AAG(K) | 72 | 0.49 | AGG(S) | 55 | 0.69 |
| GUU(V) | 53 | 1.45 | GCU(A) | 57 | 1.64 | GAU(D) | 58 | 1.27 | GGU(G) | 32 | 1.17 |
| GUC(V) | 23 | 0.63 | GCC(A) | 43 | 1.24 | GAC(D) | 33 | 0.73 | GGC(G) | 25 | 0.92 |
| GUA(L) | 59 | 1.62 | GCA(A) | 30 | 0.86 | GAA(E) | 65 | 1.46 | GGA(G) | 37 | 1.36 |
| GUG(V) | 11 | 0.3 | GCG(A) | 9 | 0.26 | GAG(E) | 24 | 0.54 | GGG(G) | 15 | 0.55 |

**Phylogenetic analysis.** The phylogenetic relationships within Brachyura were reconstructed using the sequences of the 13 PCGs of a total of 57 complete mitogenome sequences downloaded from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) and adding two species of Paguridae to serve as the outgroup (Table 1). The phylogenetic relationships were analyzed with Maximum Likelihood (ML) by using IQ-TREE 1.6.2 and Bayesian Inference (BI) methods in MrBayes 3.2 version program. The ML analysis was inferred with 1000 ultrafast likelihood bootstrap replicates by using IQ-TREE 1.6.2. The best-fit model for each partition was selected by Akaike Information Criterion (AIC) in MrModelTest 2.3. The Bayesian phylogenetic analyses were performed using the parameters estimated with the commands in MrModelTest or ModelTest (nst = 6, rates = invgamma). With three hot chains and one cold chain, they were run simultaneously twice by Markov Chain Monte Carlo (MCMC) sampling, and the posterior distribution was estimated. The MCMC chains were set for 2,000,000 generations and sampled every 1000 steps, with a relative burn-in of 25%. The convergence of the independent runs was evaluated by mean standard deviation of the split frequencies (< 0.01). The phylogenetic trees were visualized and edited using Figure Tree v1.4.3 software.
bias towards the use of T's and C's, consistent with published findings in most Brachyura crabs. *G. albolineatus* exhibits a novel gene rearrangement, which is similar to *G. tenuicrustatus*, *P. crassipes*, *P. marmoratus*, *M. frontalis*, and *M. quadridentatus*. Compared with the pan-crustacean ground pattern, the trnH of *G. albolineatus* shifted into trnE and trnF instead of the usual location between nad5 and nad4. By adding 62 Brachyura mitochondrial genomes, rearrangement and the phylogeny of Brachyura was reanalyzed. The phylogenetic analyses indicated
Figure 4. The phylogenetic tree was inferred from the nucleotide sequences of 13 mitogenome PCGs using BI and ML methods. Numbers on branches indicate posterior probability (BI) and bootstrap support (ML). The node marked with a solid circle indicates 100 ML bootstrap support (BS) and 100% BI posterior probability (PP).
that *G. albolineatus* has close relationships with *G. tenuicrustatus*, *P. crassipes*, *P. marmoratu*, *M. frontalis*, and *M. quadridentatus*, belonging to Grapsoida, part of the Grapsidae family.

### Data availability

The complete mitogenome of *Grapsus albolineatus* has been submitted to GenBank under the accession number of MZ262276. The data that support the findings of this study are openly available in Microsoft OneDrive at https://1drv.ms/u/s!Apz_mHDHDIq1UHXhxoLoLR0_NEHF?e=ug7Ne8W.

Received: 28 September 2021; Accepted: 11 January 2022
Published online: 08 February 2022

### References

1. Basso, A. et al. The highly rearranged mitochondrial genomes of the crabs *Maja crispata* and *Maja squinado* (Majidae) and gene order evolution in Brachyura. *Sci. Rep.* 7(1), 4096 (2017).
2. Liu, H. et al. Novel insights into mitochondrial gene rearrangement in thrips (Insecta: Thysanoptera) from the grass thrips, *Anaphothrips obscursus*. *Sci. Rep.* 7(1), 4284 (2017).
3. Li, Q., Xu, C., Wan, C. & Liu, G. The complete mitochondrial genome of red-clawed crab *Chirontomaes haematochir* (Sesarmidae: Grapsidae). *Mitochondr. DNA B Resour.* 4(1), 53–54 (2019).
4. Guan, M. et al. The whole mitochondrial genome of the mangrove crab, *Metopograpsus frontalis* (Miers, 1880) (Decapoda, Grapsidae) and its phylogenetic relationship. *Mitochondr. DNA B Resour.* 3(1), 368–369 (2018).
5. Gregersen, H. M., Oram, P. & Spears, J. Priorities for forestry and agroforestry policy research. *Class. Rev.* 54(1), 138–139 (1992).
6. Martin, J. W. & Davis, G. W. An updated classification of the recent Crustacea. *Natural history museum of Los Angeles county.*
7. Ming, T. L.
8. Ahyong, S. T.
9. Wang, Z.
12. Yu, Y. Q., Ma, W. M., Yang, W. J. & Yang, J. S. The complete mitogenome of the lined shore crab *Maja midae* and phylogenetic analysis of the Brachyura: Sesarmidae: *Maja* species. *Sci. Rep.* 12(2), 2123 (2016).
10. Wang, Q. et al. Comparative mitochondrial genomic analysis of *Macrothelphusa pacifica* and insights into the phylogeny of the Octopodoidea and Grapsoida. *Genomics* 112(1), 82–91 (2020).
11. Sato, M. & Sato, K. Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA.
12. Chionocephalus major
13. Pachygrapsus crassipes
14. Li, M. H. Fluctuating asymmetry and intersexuality in the shore crab *Grapsus grapsus* and comparison with other Brachyuran crabs. *Genomics* 112(1), 10–19 (2020).
15. Guan, M. et al. The whole mitochondrial genome of the mangrove crab, *Metopograpsus frontalis* (Miers, 1880) (Decapoda, Grapsidae) and its phylogenetic relationship. *Mitochondr. DNA B Resour.* 4(1), 53–54 (2019).
16. Guan, M. et al. The whole mitochondrial genome of the mangrove crab, *Metopograpsus frontalis* (Miers, 1880) (Decapoda, Grapsidae) and its phylogenetic relationship. *Mitochondr. DNA B Resour.* 3(1), 368–369 (2018).
17. Sato, M. & Sato, K. Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA.
18. Chionocephalus major
19. Zhang, Y. et al. Characterization of the complete mitochondrial genome of *Uca lacteus* and comparison with other Brachyuran crabs. *Sci. Rep.* 12(2), 2123 (2016).
20. Maynard, S. J. & Smith, N. H. Recombination in animal mitochondrial DNA.
21. Arndt, A. & Smith, M. J. Mitochondrial gene rearrangement in the sea cucumber genus *Cucumaria*.
22. Schubart, C. D., Cannicci, S., Vannini, M. & Fratini, S. Molecular phylogeny of grapsoid crabs (Decapoda, Brachyura) and allies based on two mitochondrial genes and a proposal for reframing from current superfamily classification. *J. Zool. Syst. Evol. Res.* 44(3), 193–199 (2006).
23. Boore, J. L. Animal mitochondrial genomes. *Nucl. Acids Res.* 27(8), 1767–1780 (1999).
24. Sato, M. & Sato, K. Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. *BBA Biomemb.* 1833(8), 1979–1984 (2013).
25. Mun, H. T. et al. Comparative mitogenomes of the Decapoda reveals evolutionary heterogeneity in architecture and composition. *Sci. Res.* 9(2), 221–229 (2019).
26. Zhang, Y. et al. Gene rearrangements in the mitochondrial genome of *Chirontomaes eurumene* (Brachyura: Sesarmidae) and phylogenetic implications for Brachyura. *Int. J. Biol. Macromol.* 162, 704–714 (2020).
27. Wang, Z. et al. Complete mitochondrial genome of *Parasesarma affine* (Brachyura: Sesarmidae): Gene rearrangements in Sesarmidae and phylogenetic analysis of the Brachyura. *Int. J. Mol. Biol.* 118, 31–40 (2018).
28. Maynard, S. J. & Smith, N. H. Recombination in animal mitochondrial DNA. *Mol. Biol. Evol.* 12, 23–33 (2002).
29. Moritz, C., Dowling, T. E. & Brown, W. M. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annu. Rev. Ecol. Syst.* 18(1), 269–292 (1987).
30. Arndt, A. & Smith, M. J. Mitochondrial gene rearrangement in the sea cucumber genus *Cucumaria*. *Mol. Biol. Evol.* 15(8), 1009–1016 (1998).
31. Postaire, B., Bruggemann, J. H., Magalon, H. & Faure, B. Evolutionary dynamics in the southwest Indian ocean marine biodiversity hotspot: a perspective from the rocky shore gastropod genus *Nerita*. *PLoS ONE* 9(4), e95040 (2014).
32. Grantham, R., Gautier, C. & Gouy, M. Codon catalog usage and the genome hypothesis. *Nucl. Acids Res.* 8(1), 49–62 (1980).
33. Tan, M. H., Gan, H. M., Lee, Y. P., Linton, S. & Austin, C. M. ORDER within the chaos: insights into phylogenetic relationships within the Anomura (Crustacea: Decapoda) from mitochondrial sequences and gene order rearrangements. *Mol. Phylogenet. Evol.* 127, 320 (2018).
34. Zhang, K. Z. et al. Novel gene rearrangement in the mitochondrial genome of *Muraenosax cinereus* and the phylogenetic relationship of Anguilliformes. *Sci. Rep.* 11(1), 2411 (2021).
35. Gong, L. et al. Novel gene rearrangement in the mitochondrial genome of *Coenobita brevispinus* (Anomura: Coenobitidae) and phylogenetic implications for Anomura. *Genomics* 112(2), 1804–1812 (2020).
36. Gong, L., Liu, Z. M., Guo, B. Y., Ye, Y. Y. & Liu, L. Q. Characterization of the complete mitochondrial genome of the *tidewater goby*, *Eucyclogobius newberryi* (Gobiiformes, Gobiidae, Gobionellinae) and its phylogenetic implications. *Conserv. Genet. Resour.* 10(1), 93–97 (2018).
37. Ng, N. K., Suzuki, H., Shih, H. T. & Dewa, S. I. The hydrothermal crab, *xenograpsus testudinatus* ng, hung & ho, 2000 (crustacea: decapoda: brachyura: Grapsidae) in southern japan. *Proc. Biol. Soc. Wash.* 127(2), 391–399 (2014).
38. Tan, M. H., Gan, H. M., Lee, Y. P. & Austin, C. M. The complete mitogenome of the ghost crab *ocypode ceratophthalmus* (pallas, 1772) (crustacea: decapoda: ocypodidae). *Mitochondr. DNA.* 2123 (2016).
39. Kim, S. J., Kim, H. S. & Ju, S. J. Mitochondrial genome of the hydrothermal vent crab *austinograea alyssae* (crustacea: bythograeidae): genetic differences between individuals from tofua arc and manus basin. *Mitochondr. DNA.* 25(4), 251–252 (2014).
40. Zhang, Y., Gong, L., Lu, X., Jiang, L. & Zhang, X. Gene rearrangements in the mitochondrial genome of *Chirontomaes eurumene* (Brachyura: Sesarmidae) and phylogenetic implications for Brachyura. *Int. J. Biol. Macromol.* 162, 704–714 (2021).
34. Wang, Q. et al. Insights into the evolution of Brachyura (Crustacea: Decapoda) from mitochondrial sequences and gene order rearrangements. Int. J. Biol. Macromol. 170, 2 (2021).
35. Ng, N. K. et al. Xenograpsidae, a new family of grapsoid crabs (Crustacea: Brachyura) associated with shallow water hydrothermal vents. Raffles Bull. Zool. 16, 233–256 (2007).
36. Li, Y. et al. Comparative mitochondrial genome analyses of sesarmid and other brachyuran crabs reveal gene rearrangements and phylogeny. J. Front. Genet. (2020).
37. Xinting, L. et al. The complete mitochondrial genome of Calappa bilineata: the first representative from the family Calappidae and its phylogenetic position within Brachyura. J. Genom. 112, 3 (2020).
38. Xu, X. et al. The entire mitochondrial genome of Macropodatus Abbreviatus reveals insights into the phylogeny and gene rearrangements of Brachyura. Biochem. Genet. 59(3), 211–219 (2021).
39. Tan, M. H., Gan, H. M., Schultz, M. B. & Austin, C. M. MitoPhAST, a new automated mtogenomic phylogeny tool in the post-genomic era with a case study of 89 decapod mitogenomes including eight new freshwater crayfish mitogenomes. Mol. Phylogenet. Evol. 85, 180–188 (2015).
40. Aljanabi, S. M. & Martinez, I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 22, 4692–4693 (1997).
41. Dierckxsens, N., Mardulyn, P. & Smits, G. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res. 45(4), e18 (2017).
42. Bernt, M. et al. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol. Phylogenet. Evol. 69(2), 313–319 (2013).
43. Alschul, S. F. et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl. Acids Res. 25(17), 3389–3402 (1997).
44. Grant, J. R. & Stothard, P. The CGView server: a comparative genomics tool for circular genomes. Nucl. Acids Res. 36, 181–184 (2008).
45. Xia, X. DAMBE5: comprehensive software package for data analysis in molecular biology and evolution. Mol. Biol. Evol. 2013(30), 1720–1728 (2013).
46. Kumar, S., Stecher, G. & Tamura, K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33(7), 1870–1874 (2016).
47. Perna, N. T. & Kocher, T. D. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. J. Mol. Evol. 41(3), 353–358 (1995).
48. Nguyen, L. T., Schmidt, H. A., Haeseler, A. & Minh, B. Q. IQTREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32(1), 268–274 (2015).
49. Ronquist, F. et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61(3), 539–542 (2012).
50. Ma, X. M. Study on complete mitochondrial genome of Cypridopsis vidua and molecular phylogeny of ostracoda. PhD thesis, Shanghai, China: East China Normal University. (2016).
51. Huelsenbeck, J. P. & Ronquist, F. MRBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17(8), 754–755 (2001).
52. Nylander, J. A., Ronquist, F., Huelsenbeck, J. P. & Nieves, J. L. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53(1), 47–67 (2004).
53. Posada, D. & Crandall, K. A. Modeltest: testing the model of DNA substitution. Bioinformatics 14(9), 817–818 (1998).
54. Rambaut, A. Fig Tree, version 1.4.3, http://tree.bio.ed.ac.uk/software/fgtree/accessed 1 July. (2016).

Author contributions
Conceptualization, J.L. and Y.Y., methodology, J.L. and L.X., software, J.L. and L.X., formal analysis, Y.M. and X.L., writing—original draft preparation, J.L. and L.X., writing—review and editing, J.L. and Y.Y., supervision, B.G., funding acquisition, J.L. and Y.Y. All authors have read and agreed to the published version of the manuscript.

Funding
This work was financially supported by the Fundamental Research Funds for Zhejiang Provincial Universities and Research Institutes (No. 2021C21026 and No. 2021C21017). The Foundation of Guangdong Provincial Key Laboratory of Marine Environmental Engineering (No. 2020C21026 and No. 2021C21017).

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to J.L. or Y.Y.

Publisher’s note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access
This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022