Comparative Mitochondrial Genome Analyses of Sesarmid and Other Brachyuran Crabs Reveal Gene Rearrangements and Phylogeny

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Mitochondrial genomes (mitogenomes) are important for understanding molecular evolution and phylogenetic relationships. The complete mitogenome of Perisesarma bidens was determined, which is 15,641 bp in length. The A + T content of P. bidens mitogenome was 74.81%. The AT skew was slightly negative (−0.021). The 22 tRNAs ranged from 65 to 73 bp and were highly A + T biased. All tRNA genes had typical cloverleaf structures, except for the trnS1 gene, which lacked a dihydrouridine (DHU) arm. The gene order within the P. bidens mitogenome was identical to the pancrustacean ground pattern, except for the translocation of the trnH. Additionally, the gene order of trnI-trnQ-trnM in pancrustacean ground pattern became trnQ-trnI-trnM in P. bidens. Phylogenetic analyses supported the inclusion of P. bidens in Sesarmidae and the promotion of Sesarminae to Sesarmidae. The results will help us to better understand the status and evolutionary history of Grapsoidea crabs.

Keywords: mitochondrial genomes, phylogeny, gene order, crustacean, Perisesarma bidens

INTRODUCTION

Decapoda is the most diverse, species-rich group of crustaceans, containing many well-known animal species, such as crayfish, lobsters, shrimps, hermit crabs, and “true” crabs (Shen et al., 2013; Basso et al., 2017). The true crabs belong to Brachyura, which is a diverse, economically important group, with about 7200 described species (De Grave et al., 2009; Ahyong et al., 2011). Brachyura is highly adaptable and can live on land and in both marine and fresh water. Therefore, crabs have become

Abbreviations: A, adenine; Atp6 and Atp8, genes for the ATPase subunits 6 and 8; BI, Bayesian inference; BP, Base pair; C, cytosine; Cox1-cox3, genes for cytochrome C oxidase subunits I–III; G, guanine; l-rRNA (large), rRNA subunit; Mitogenomes, Mitochondrial genomes; ML, maximum likelihood; mtDNA, mitochondrial DNA; Nad1–nad6 and nad4L, genes for NADH dehydrogenase subunits 1–6 and 4L; PCGs, protein-coding genes; rRNA, ribosomal RNA genes subunit; s-rRNA, (small); T, thymine; tRNAX, transfer RNA, where X is replaced by three letters amino acid code of the corresponding amino acid.
important groups for the study of evolution (Castro et al., 2015). Some Brachyura are edible and medicinal and have economic importance (Carpenter and Niem, 1998).

Most Brachyura are grouped into the Podotremata, Heterotremata, and Thoracotremata, with the latter two referred to as the Eubrachyura. However, the phylogenetic relationships within Eubrachyura remain controversial, particularly the relationships of the Sesarmidae and Varunidae, and between these two and Grapsidae (Schubart et al., 2000, 2002; Kitaura et al., 2002). The traditional classification of Grapsidae contains four subfamilies: Grapsinae, Plagusiinae, Sesarminae, and Varuninae (Schubart et al., 2000). Traditional methods place the following in the Sesarminae: Perisesarma bidens, Sesarmops sinensis, Clistocoeloma sinensis, Helice tientsinsensis, Helice latimera, Helice wuana, and Metaplax longipes. Of these, P. bidens and S. sinensis should be Sesarmops crabs; C. sinensis should be a Clistocoeloma crab; H. tientsinsensis, H. latimera, and H. wuana should be Helice crabs and M. longipes should be a Metaplax crab (Schubart et al., 2000). However, some scholars have suggested that Grapsidae should be promoted and should be a Sesarmops, i.e., Grapsidae, Varunidae, Sesarmidae and Plagusiidae. Other scholars have suggested that Grapsidae should be promoted and should be a Sesarmops, i.e., Grapsidae, Varunidae, Sesarmidae and Plagusiidae. Other scholars have suggested that Grapsidae should be promoted and should be a Sesarmops, i.e., Grapsidae, Varunidae, Sesarmidae and Plagusiidae. Other scholars have suggested that Grapsidae should be promoted and should be a Sesarmops, i.e., Grapsidae, Varunidae, Sesarmidae and Plagusiidae.

The classification of these taxa remains unresolved. Sesarmid crabs are common in mangroves and areas of the controversial taxa, Sesarmidae and Grapsidae.

**Materials and Methods**

**Ethics Statement**

We have taken a close look at the website1. We found that the species *Perisesarma bidens* is not considered endangered or protected species, the IUCN status for this species is “Not evaluated.” Similarly, the species *Perisesarma bidens* is also Not endangered or protected species in China. No special permit is required to collect crabs at selected sites in China. The sampling locations are Not privately-owned or natural protected areas, the collection of this species is legal in China. So we can use this species for experiments and subsequent analysis.

**Sample Collection**

Specimens of *P. bidens* were collected from the seaside of Zhangzhou City, Fujian Province, China, identified using the morphological methods of Dai (1999) and molecular identification with COI marker, and preserved in 95% ethanol at –20°C until DNA extraction. Voucher specimens of *P. bidens* were deposited in the Jiangsu Provincial Key Laboratory of Coastal Wetland Biodiversity and Environmental Protection, School of Ocean and Biological Engineering, Yancheng Teachers University, Yancheng, China.

**DNA Extraction, PCR, and Genome Sequencing**

Total genomic DNA was extracted from muscle using a genomic DNA extraction kit (Sangon, China), following the manufacturer’s instructions, and was visualized on 1.0% agarose gels. The complete mitogenome was obtained using a combination of conventional PCR and long PCR to amplify overlapping fragments spanning the entire mitogenome. Initially, conserved sequences, such as *cox1*, *cox3*, *nad5*, *nad4*, and *rrnS*, were amplified by conventional PCR using universal primers synthesized by Beijing Sunbiotech (Tang et al., 2003, 2017, 2018; Liu et al., 2015; Xin et al., 2017a,b).

We designed species-specific primers to amplify large overlapping regions of the mitogenome based on conserved sequences using Primer Premier 5 (Supplementary Table S1). All amplifications were performed on a Mastercycler (Eppendorf) and Mastercycler gradient. The reactions were 50 µL and contained 34.65 µL ddH2O, 5 µL 10 × LA PCR buffer II (Mg2+ Plus, AIDLAB), 4 µL dNTPs (10 mM), 2 µL each primer (10 µM), 0.35 µL red *Taq* DNA Polymerase (5 U/µL, AIDLAB), and 2 µL DNA template (~30 ng).

The PCR conditions for conserved sequences followed a standard three-step protocol, with an initial denaturing at 96°C

1https://www.gbif.org/en/species/4382775
### TABLE 1 | List of brachyuran species with their GenBank accession numbers.

| Species                        | Family               | Superfamily | Size (bp) | Accession No. |
|--------------------------------|----------------------|-------------|-----------|---------------|
| Sesarmops sinensis             | Sesarmidae           | Grapsoidea  | 15,905    | KR336554      |
| Clitocoleoma sinensis          | Sesarmidae           | Grapsoidea  | 15,706    | KUS89292      |
| Perisesarma bidens             | Sesarmidae           | Grapsoidea  | 15,641    | KY808394      |
| Metaplax longipes              | Varunidae            |             | 16,424    | MF198248      |
| Helice latimera                | Varunidae            |             | 16,246    | KUS89291      |
| Helice bientinensis            | Varunidae            |             | 16,212    | KR336555      |
| Helice wuana                   | Varunidae            |             | 16,359    | KO344898      |
| Sesarma neglectum             | Sesarmidae           | Grapsoidea  | 15,920    | KX156964      |
| Metopaullia depressus          | Sesarmidae           | Grapsoidea  | 15,765    | KX118277      |
| Parasesarmops trpectinis       | Sesarmidae           | Grapsoidea  | 15,612    | KU343209      |
| Eriocheir japonica japonica   | Varunidae            |             | 16,352    | FJ455505      |
| Eriocheir japonica sinensis    | Varunidae            |             | 16,378    | KMM16908      |
| Eriocheir japonica hepuensis   | Varunidae            |             | 16,335    | FJ455506      |
| Cyclograpsus granulosus        | Varunidae            |             | 16,300    | LN624373      |
| Pachygrapsus crassipes         | Grapsidae            |             | 15,652    | KCB878511     |
| Grapsus tenuicrastus           | Grapsidae            |             | 15,858    | KT787821      |
| Xenograpsus testudinitus       | Xenograpsidae        |             | 15,798    | EU727203      |
| Xenograpsus ngatama            | Xenograpsidae        |             | 16,106    | KYY85236      |
| Portunus pelagicus             | Portunidae           | Portunoidea  | 16,157    | KMM77882      |
| Callinctesapidus               | Portunidae           |             | 16,263    | AY863392      |
| Portunus triturcularatus       | 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      |
| Charybdis natator              | Portunidae           |             | 15,664    | MF285241      |
| Thalamita crenata              | Portunidae           |             | 15,787    | LK391945      |
| Chaceon granulatus             | Geryonidae           |             | 16,135    | AB769383      |
| Chaceon sp.                     | Geryonidae           |             | 16,126    | KUS507298     |
| Gandatus yunohana              | Bythograeidae        | Bythograeoida | 15,567    | EU647222      |
| Gandatus puia                   | Bythograeidae        | Bythograeoida | 15,548    | KRP02727      |
| Austrograea alayseae           | Bythograeidae        |             | 15,620    | JQ305660      |
| Austrograea Rodriguesensis     | Bythograeidae        |             | 15,611    | JQ305658      |
| Sagonzacija mesatlantica       | Bythograeidae        |             | 15,521    | KYS41839      |
| Homologenus malayensis         | Homolidae            | Homoloidae   | 15,793    | KJ612407      |
| Moloha majora                  | Homolidae            | Homoloidae   | 15,903    | KT182069      |
| Geothelphusa dehaani           | Potamidae            | Potamoidea   | 18,197    | AB187570      |
| Longpotamon xiaoshuense         | Potamidae            |             | 18,460    | KU024041      |
| Huananpotamon lichuanense      | Potamidae            |             | 15,380    | KX639824      |
| Somannialtheplpha boyangensis  | Parathelphusidae     |             | 17,032    | KU024042      |
| Pseudocarcinus gigas           | Eriphidae            | Xanthoidea   | 15,515    | AY962127      |
| Leptodius sanguineus           | Xanthidae            |             | 15,480    | KT986744      |
| Myomenippe fornasinii          | Menippidae           | Erphoidea    | 15,658    | LK391943      |
| Ocypode cordimanus             | Ocypodidae           | Ocypodoidea  | 15,604    | KT896743      |
| Ocypode ceratophthalmus        | Ocypodidae           |             | 15,584    | LN611669      |
| Ilyoplax deschampsi            | Doliidae             |             | 15,460    | JF909979      |
| Mictyris longipenis            | Mictyridae           |             | 15,548    | LN611670      |
| Macrophthalmus japonicus       | Macrophthalmidae     |             | 16,170    | KU343211      |
| Umalia orientalis              | Raninidae            | Raninoidea   | 15,466    | KMM35084      |
| Lyneidus brevisfons            | Raninidae            |             | 16,112    | KMM38394      |
| Ranina ranina                  | Raninidae            |             | 15,563    | KMM189817     |

(Continued)
for 3 min, then 40 cycles of 94°C for 30 s, annealing at the recommended temperature for each primer for 30 s, and elongation at 72°C for 45 s, with a final 5 min extension at 72°C.

The PCR conditions for large overlapping regions followed a standard two-step protocol with 3 min at 94°C, followed by 35 cycles of 35 s at 94°C, 3–6 min at 50–56°C, and 10 min at 72°C. All PCR products were sent to General Biosystems, Anhui for Sanger sequencing.

Annotation and Alignment

The sequence was annotated using DNASTAR (DNASTAR, Madison, WI, United States). The locations of the PCGs, rRNA genes, tRNA genes, and CR were initially identified using the MITOS Web Server. The PCG coding regions were further identified using the NCBI ORF Finder. Two rRNA genes were identified by alignment with published brachyuran sequences. Codon usage and the nucleotide composition of the mitogenomes were determined using MEGA6 (Tamura et al., 2013). The nucleotide sequence of the complete P. bidens mitogenome was deposited in the NCBI database under accession no. KY808394. Gene orders in the complete mitogenome were also inferred through the MITOS Web Server.

Phylogenetic Analyses

We used nucleotide (NT) sequences for phylogenetic analyses. The sequences were aligned using MAFFT using the default settings (Katoh et al., 2002). Gaps in the sequences were removed using Gblocks (Castresana, 2000), and the saturation of the sequences was examined using DAMBE (Xia and Xie, 2001), which indicated that the sequences were not saturated and were suitable for phylogenetic analyses. Complete mitogenomes of 65 decapods (60 crabs plus 5 outgroups) were downloaded from NCBI (Table 1). The five outgroups were Cherax destructor, Cambaroides similis, Neopetrolisthes maculatus, Paralithodes camtschaticus, and Pagurus longicarpus.

Phylogenetic analyses were performed using Bayesian inference (BI) and maximum likelihood (ML) methods.

**TABLE 1** Continued

| Species                  | Family          | Superfamily  | Size (bp) | Accession No. |
|--------------------------|-----------------|--------------|-----------|---------------|
| Dynomene pilumnoides     | Dynomenidae     | Dromioidea   | 16,475    | KT1820200     |
| Ashtorei lunaris         | Matutidae       | Calapoidea   | 15,807    | LK931941      |
| Maja squinado            | Majidae         | Majoidea     | 16,598    | KY650652      |
| Maja crispata            | Majidae         |              | 16,592    | KY650651      |
| Chionoecetes japonicus   | Majidae         |              | 15,341    | AB735678      |
| Damithrax spinosissimus  | Mithracidae     |              | 15,817    | KM405516      |
| Cherax destructor        | Parastacidae    | Paguroidea   | 15,713    | HG799087      |
| Cambaroides similis      | Cambaridae      | Astacoidea   | 16,220    | NC016925      |
| Neopetrolisthes maculatus| Porcellanidae  | Galatheoidea | 15,324    | KC107816      |
| Paralithodes camtschaticus| Lithodiidae  |              | 16,720    | NC020029      |
| Pagurus longicarpus      | Paguridae       |              | 15,630    | AF150765      |

**TABLE 2** Summary of the P. bidens mitogenome.

| Gene | Direction | Location | Size (bp) | Intergenic nucleotides |
|------|-----------|----------|-----------|------------------------|
| cox1 | F         | 1–1560   | 1560      | −25                    |
| trnL2 | F         | 1536–1604| 69        | 5                      |
| cox2 | F         | 1610–2317| 708       | −20                    |
| trnK | F         | 2298–2366| 69        | 0                      |
| trnD | F         | 2367–2434| 68        | 0                      |
| atp8 | F         | 2435–2593| 159       | −7                     |
| atp6 | F         | 2587–3261| 675       | −1                     |
| cox3 | F         | 3261–4052| 792       | −1                     |
| trnG | F         | 4052–4116| 65        | 0                      |
| nad3 | F         | 4117–4467| 351       | 2                      |
| trnA | F         | 4470–4536| 67        | 10                     |
| trnR | F         | 4547–4612| 66        | 2                      |
| trnN | F         | 4615–4681| 67        | 0                      |
| trnS1| F         | 4682–4748| 67        | 1                      |
| trnE | F         | 4750–4815| 66        | 4                      |
| trnH | R         | 4820–4884| 65        | 0                      |
| trnF | R         | 4885–4950| 66        | 1                      |
| nad5 | R         | 4962–6682| 1731      | 41                     |
| nad4 | R         | 6742–8073| 1250      | −7                     |
| nad4L| R         | 8067–8369| 303       | 8                      |
| trnT | F         | 8378–8442| 66        | 0                      |
| trnP | R         | 8444–8509| 66        | 2                      |
| nad6 | F         | 8512–9015| 504       | −1                     |
| cob  | F         | 9015–10,149| 1135   | 0                      |
| trnS2| F         | 10,150–10,217| 68   | 15                     |
| nad1 | R         | 10,233–11,171| 939  | 34                     |
| trnL1| R         | 11,206–11,271| 66  | 0                      |
| mL   | R         | 11,272–12,612| 1341| 0                      |
| trnV | R         | 12,613–12,685| 73   | 0                      |
| ms   | R         | 12,686–13,515| 830  | 0                      |
| CR   | —         |          | 13,516–14,193| 678| 0                      |
| trnQ | R         | 14,194–14,263| 70  | 23                     |
| trnL | F         | 14,287–14,354| 68  | 8                      |
| trnW | F         | 14,363–14,431| 69  | 0                      |
| nad2 | F         | 14,432–15,439| 1008| 2                      |
| trnW | F         | 15,442–15,511| 70  | −3                     |
| trnC | R         | 15,509–15,573| 65  | 0                      |
| trnY | R         | 15,574–15,641| 68  | −                      |
using MrBayes v 3.2.2 (Ronquist et al., 2012) and IQ-Tree (Nguyen et al., 2014; Kalyaanamoorthy et al., 2017; Hoang et al., 2018), respectively. The GTR model was selected by MrModeltest 2.3 (Nylander, 2004). The BI analyses ran four independent chains for 10,000,000 generations, sampled every 100 generations, with a burn-in of 25,000 generations. The average standard deviation of split frequencies was < 0.01. Convergence was assessed using Tracer v1.6 and the effective sampling size for all parameters was > 200. The ML analyses were performed on 1000 bootstrap replications. The resulting phylogenetic trees were visualized using FigTree v1.4.2.

RESULTS AND DISCUSSION

Genome Structure, Organization, and Composition

The complete mitogenome of *P. bidens* was a circular of 15,641 bp (GenBank accession no. KY808394). Its size was within the

![Diagram of P. bidens mitogenome](image)

**Perisesarma bidens**

Complete mitochondrial genome

15,641 bp

**FIGURE 1** | Map of the *P. bidens* mitogenome. Protein-coding and ribosomal genes are shown with standard abbreviations. Genes for tRNAs are abbreviated by single letters, with S1 = AGN, S2 = UCN, L1 = CUN, and L2 = UUR. CR, control region.
range observed in completely sequenced brachyuran species. The mitogenome composition (A: 36.61%, T: 38.20%, C: 15.13%, G: 10.06%) was strongly A + T biased which accounts for 74.81%, and exhibited with negative AT-skew (−0.021). The AT-skew of the mitogenomes of most crabs were negative, for example, *H. wuana* (Tang et al., 2018), *S. sinensis* (Tang et al., 2017), *H. tientsinensis* (Xin et al., 2017b), *C. sinensis* (Xin et al., 2017a), the AT-skew value of mitogenomes in other crabs had also been calculated and counted in related studies (Xin et al., 2017a,b). The genes were typical of animal mitogenomes, with 22 tRNA genes, 13 PCGs, 2 rRNA genes, and a CR (*Table 2*). Overall, 4 of the 13 PCGs (*nad5*, *nad4*, *nad4L*, and *nad1*), 8 tRNAs [*trnQ*, *trnC*, *trnY*, *trnF*, *trnP*, *trnL* (CUN), and *trnV*], and 2 rRNAs (*rrnL* and *rrnS*) were encoded by the minority strand, while the other 23 genes were encoded by the majority strand (*Table 2* and *Figure 1*). The 13 PCGs ranged from 159 to 1731 bp. Of 22 tRNA genes, 8 were encoded by the L-strand and the remaining 14 by the H-strand. All tRNAs had the typical clover-leaf secondary structures observed in mitochondrial tRNA genes, except for *trnS1* (AGN), which lacked a stable dihydrouridine (DHU) arm; this has been observed in several animals, including insect and brachyuran mitogenomes (Liu et al., 2015; Xin et al., 2017a,b). *Figure 2* shows the relative synonymous codon usage (RSCU) of *P. bidens*. The codon usage was biased with a high frequency of AT compared to GC in the third codon position. The codon usage analysis revealed that the leucine 2 (*Leu2*), isoleucine (*Ile*), phenylalanine (*Phe*) codon families were most frequently utilized, while cysteine (*Cys*) family was the least used (*Figure 3*).

**Gene Order in Sesarmidae**

The gene order of *P. bidens* was identical to other Sesarmidae species in our study. In contrast to the inferred ancestral gene sequences of Pancrustaceans, where *trnH* was located between *nad5* and *nad4*, here it was found between *trnE* and *trnF*. In Pancrustaceans, the tRNA gene sequences between CR and *trnM* was *trnI-trnQ*, but here was *trnQ-trnI* (*Figure 4A*).

The duplication/random loss model was used to explain the rearrangements seen in Sesarmidae (Moritz and Brown, 1987; Macey et al., 1997; Boore and Brown, 1998). The movement of *trnH* can be explained as follows. First, gene duplication occurred in *trnF*, *nad5*, and *trnH*, changing the arrangement of *trnF-nad5-trnH* to *trnF-nad5-trnH-trnF-nad5-trnH*. Then, the redundant *trnF*, *nad5*, and *trnH* genes were lost at random. Finally, the new gene order of *trnH-trnF-nad5* was formed (*Figure 4B*). The order principles of *trnQ* moving from the junction between *trnI* and *trnM* to between the CR and *trnI* could also be explained similarly (*Figure 4C*).

**Gene Order of Crabs From Other Families**

The gene orders of all species are shown in *Figure 5*. The gene sequences within 13 families were the same. The gene order pattern of *Macrophthalmus japonicus* (Ocypodoidea, Macrophthalmidae) was identical to that of other Varunidae. The gene orders of *Damithrax spinosissimus* (Majoidea, Mithracidae) and *Dynomene pilumnoides* (Dromioidea, Dynomenidae) were different, as were those of two Xenograpsidae crabs (*Xenograpsus testudinatus* and *X. ngatama*). However, two Majidae crabs (*Maja*...
**FIGURE 3** Amino acid composition of the *P. bidens* mitogenome.

**FIGURE 4** Generation of the *P. bidens* mitochondrial gene arrangement. The duplication/random loss, recombination, and duplication/non-random loss models were used to explain the principle of gene rearrangement. (A) Comparison of gene order in mitogenome of Perisesarma bidens and Pancrustacean ground pattern. tRNA genes are indicated by the singler letter IUPAC-IUB abbreviation with S1 = AGN, S2 = UCN, L1 = CUN, and L2 = UUR, where as protein and rRNA genes are labeled with three letter codes. (B) Gene duplication occurred in trnF, nad5, and trnH, changing the arrangement of trnF-nad5-trnH to trnF-nad5-trnH-trnF-nad5-trnH. Then, the redundant trnF, nad5, and trnH genes were lost at random. Finally, the new gene order of trnH-trnF-nad5 was formed. (C) Gene duplication occurred in trnI, trnQ, and trnM, changing the arrangement of trnI-trnQ-trnM to trnI-trnQ-trnM-trnI. Then, the redundant trnI, trnM, and trnQ genes were lost at random. Finally, the new gene order of trnQ-trnI-trnM was formed.

*squinado* and *M. crispata*) had the same gene order. Interestingly, although there were only four species of Potamoide, each showed a different gene order.

**Phylogenetic Analyses**

The phylogenetic trees were constructed based on 13 PCGs under ML and BI methods, which resulted in congruent tree topologies, except for minor differences within “Grapsoidea + Ocypodoidea” (Figure 6). As shown in Figure 6, *P. bidens* formed a well-supported clade with *Parasesarmops tripectinis* (BP = 100; BPP = 1). (*P. bidens + P. tripectinis*) clade, (*S. sinensis + S. neglectum*) clade, (C. sinensis + *M. depressus*) clade were well supported with each other; these results were in accordance with the information provided by the same genes orders of...
P. bidens, P. tripectinis, S. sinensis, S. neglectum, C. sinensis, and M. depressus. Moreover, S. neglectum, M. depressus, and P. tripectinis all belonged to Sesarmidae (Park et al., 2018). Therefore, P. bidens, S. sinensis, and C. sinensis should belong to Sesarmidae rather than to Sesarminae. The species originally belonging to the Sesarminae should belong to the Sesarmidae. These results agree with previous analyses using the mitogenome of one species (Tang et al., 2017; Xin et al., 2017a,b).

In our study, two families (Potamidae and Parathelphusidae) were primarily freshwater crabs and were recognized as true heterotremes (Guinot et al., 2013). The systemic status of primary freshwater crabs had stimulated interest because of their high value and diversity (Cumberlidge et al., 2009; Klaus et al., 2010). The monophyly of Potamidae and Parathelphusidae was confirmed based on morphological and molecular analyses. However, there still were uncertainties regarding the phylogenetic placement of Potamidae and Parathelphusidae (Xing et al., 2017). Von Sternberg and Cumberlidge (2001) suggested that these two families Potamidae and Parathelphusidae should be placed in Thoracotremata. Here, the Thoracotremata contained Grapsoidea and Ocypodoidea crabs. Our results showed that four heterotreme crabs (Geothelphusa dehaani, Longpotamon xiushuiense, Huananpotamon lichuanense, and Somanniathelphusa boyangensis) were actually more closely associated with thoracotreme crabs, showing that Heterotremata was not monophyletic; this result was in accordance with that inferred from 23 brachyuran crabs, in which the author use the two mitogenomes (Ji et al., 2014). Within Podotremata, the clade was monophyletic. The six crabs formed a robust clade [(Homolidae + Dynomenidae) + Raninidae]. Within Heterotremata, the phylogenetic relationships were clear, with
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FIGURE 6 | Phylogenetic trees were constructed using BI and ML methods based on NT dataset. Bootstrap values (BP) (IQ-Tree) and Bayesian posterior probability (BPP) of each node are shown as BP based on NT dataset/BPP based on NT dataset. C. destructor, C. similis, N. maculatus, P. camtschaticus, and P. longicarpus were used as outgroups. The supermatrix underlying this figure is as a Supplementary File.

the exception of the four potamid crabs, which were outside of the heterotreme crabs.

DATA AVAILABILITY STATEMENT
The datasets generated for this study can be found in the GenBank accession no. KY808394.

AUTHOR CONTRIBUTIONS
Q-NL, B-PT, and X-MY conceived and designed the study. Z-ZX, Q-NL, Y-YT, and T-TY conducted the molecular work and data analysis. Z-ZX and Y-TL drafted the manuscript. Z-ZX, Q-NL, Y-YT, YS, D-ZZ, C-LZ, and T-TY prepared all figures and tables. Z-ZX and Q-NL performed the phylogenetic analyses. Z-ZX, Q-NL, B-PT, and X-MY contributed to drafting the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2020.536640/full#supplementary-material
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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