Genetics and Molecular Research

Characterization of the complete mitochondrial genome and phylogenetic relationships of the three-spot swimming crab (*Portunus sanguinolentus*)

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Genet. Mol. Res. 15 (3): gmr.15038580
Received February 29, 2016
Accepted May 17, 2016
Published August 29, 2016
DOI http://dx.doi.org/10.4238/gmr.15038580

ABSTRACT. In this study, we determined the whole mitochondrial genome profile of the three-spot swimming crab (*Portunus sanguinolentus*) and elucidated phylogenetic relationships between representative species in the order Decapoda. The mitochondrial genome was 16,024 bp in length and consisted of 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and a putative control region. Of the 37 genes, 23 were encoded by the heavy strand while 14 were encoded by the light strand. Four types of start codons were identified; ATG initiated nine genes, ATT initiated two genes, and

ATC and GTG each started one gene. Nine protein-coding genes ended with a complete TAA or TAG stop codon, and four genes ended with an incomplete T or TA codon. Fourteen non-coding regions were found, which ranged from 1 to 34 bp in length. Nine overlaps were observed, with lengths between 1 and 7 bp. Phylogenetic analysis suggested that *P. sanguinolentus* is genetically closest to *P. trituberculatus* and *P. pelagicus*. *Charybdis feriata*, *C. japonica*, and *Thalamita crenata* formed a single cluster, and were close to the genera *Callinectes* and *Portunus*. Therefore, the genera *Charybdis* and *Thalamita* should be classified into the subfamily Portuninae.

Key words: *Portunus sanguinolentus*; Mitochondrion; Gene order; Phylogenetic relationship

INTRODUCTION

The three-spot swimming crab (*Portunus sanguinolentus*, Herbst, 1783) is a commercially important crab species that is mainly found in the East and South China Seas, but also inhabits oceanic waters from East Africa through the Indo-Pacific region to the Hawaiian Islands (Stephenson and Campbell, 1959). Juveniles and adult males usually inhabit sandy or muddy coastal sea beds at 10 to 30 m in depth (Sumpton et al., 1989). In contrast, females are abundant at depths of 40 to 80 m (Campbell and Fielder, 1986), and in the reproductive season, berried females migrate to deeper waters for spawning.

Crab fishing in China has rapidly increased in recent decades, and there is an increasing demand for crab meat from both national and international markets. Due to over-catching and seawater deterioration, yields of this crab have substantially decreased year on year (Lee and Hsu, 2003; Yang et al., 2014). In order to assess the stock and effectively manage crab fisheries, a large amount of researches concerning the biology of *P. sanguinolentus* have been conducted, e.g., body size at sexual maturity, breeding season, and fecundity (Rasheed and Mustaquim, 2010). Females prefer large males to small males, and two-chela males to single-chela males (Dinakaran and Soundarapandian, 2009). To date, most studies on *P. sanguinolentus* have focused on morphology, and molecular techniques have been rarely applied to investigate the species' biology, evolution, and stock management.

Molecular markers are ideal genetic tools, because they can be used to investigate phylogenetic relationships, population genetic structures, and resource conservation. Mitochondrial markers are currently one of the most popular molecular markers, particularly in studies of molecular evolution, species identification, and genetic diversity (Yu et al., 2012; Sahoo et al., 2015; Korkmaz et al., 2016). The mitochondrial genome is usually a closed circular molecule, and contains 13 protein-coding genes, 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes, and a control region. The molecular phylogenetic position of *P. sanguinolentus* has been studied based on a single gene sequence (*16S rRNA*) of the mitochondrial genome, which suggested a close relationship with *P. pelagicus* and *P. trituberculatus* (Mantelatto et al., 2007). Although the whole mitochondrial genome of *P. sanguinolentus* has been recently reported (Meng et al., 2016a), a detailed comparative characterization of this genome with those of other crustaceans has not been conducted. In addition, several key issues, such as species identification and the taxonomic status of crab

species within the family Portunidae, need to be resolved.

Therefore, the aims of the present study were to determine and characterize the complete mitochondrial genome of *P. sanguinolentus* and elucidate the evolutionary status of representative species in the order Decapoda. This will improve our understanding of the species' evolutionary status, and facilitate research into its population genetic diversity and that of related crustacean species.

MATERIAL AND METHODS

Sample collection and DNA preparation

A *P. sanguinolentus* specimen was purchased from Qinglan Port Market, Hainan Island, China. The animal was killed by a lethal dose of tricaine mesylate. Muscle tissue was obtained and stored at -20°C, and genomic DNA was prepared using the traditional proteinase K and phenol-chloroform extraction protocol, as described by Ma et al. (2009).

Polymerase chain reaction (PCR) amplification

PCR primers were designed based on the published complete mitochondrial genome sequences of the crab species *Charybdis feriata* (Ma et al., 2015a), *Scylla paramamosain* (Ma et al., 2013), and *Portunus trituberculatus* (Yamauchi et al., 2003). PCR amplification was conducted in a 25-μL volume that contained 0.4 μM each primer, 0.2 mM each dNTP, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.75 U *Taq* polymerase, and approximately 50 ng DNA under the following conditions: one cycle of denaturation at 94°C for 4 min; 35 cycles of 30 s at 94°C, 50 s at a primer-specific annealing temperature, and 50 s at 72°C. Finally, the products were extended for 7 min at 72°C. The PCR products were separated on 1.0% agarose gels and directly sequenced in both directions using an ABI PRISM® 3730 DNA sequencer (Applera Corp., Norwalk, CT, USA). DNA sequences were edited and assembled using the EditSeq and SeqMan (DNASTAR) software.

Genome profile analysis

The whole genome of *P. sanguinolentus* was drawn using OrganellarGenomeDRAW (http://ogdraw.mpimp-golm.mpg.de/) (Lohse et al., 2007). The genomic structure of the mitochondrion was determined based on the published mitochondrial genomes of *S. paramamosain* and *P. trituberculatus*. tRNAs were identified using the web-based tRNA-scan SE 1.21 program (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe and Eddy, 1997). The codon usage of protein-coding genes and the nucleotide composition were determined using MEGA 4.0 (Kumar et al., 2008). The whole genome was deposited into the National Center for Biotechnology Information GenBank database using the Sequin 12.30 software (http://www.ncbi.nlm.nih.gov/Sequin/).

Phylogenetic analysis

A total of 22 mitochondrial genomes from the following related species were downloaded from GenBank: *Thalamita crenata* (LK391945), *Portunus pelagicus* (KT382858), *C. feriata*

(KF386147), *Charybdis japonica* (FJ460517), *Callinectes sapidus* (NC_006281), *Eriocheir hepuensis* (NC_011598), *Eriocheir japonica* (NC_011597), *Eriocheir sinensis* (NC_006992), *Litopenaeus vannamei* (DQ534543), *Geothelphusa dehaani* (NC_007379), *Fenneropenaeus chinensis* (DQ518969), *Pagurus longicarpus* (NC_003058), *Macrobrachium rosenbergii* (NC_006880), *Panulirus japonicus* (NC_004251), *Pseudocarcinus gigas* (NC_006891), *Marsupenaeus japonicus* (NC_007010), *Penaeus monodon* (NC_002184), *P. trituberculatus* (AB093006), *Scylla serrata* (NC_012565), *Scylla tranquebarica* (NC_012567), *Scylla olivacea* (NC_012569), and *S. paramamosain* (JX457150). *Harpiosquilla harpax* (NC_006916) was used as an outgroup in the phylogenetic tree.

All 13 protein-coding genes (except *ND6*) were concatenated to a single multiple sequence. The multiple sequence alignment was formatted and analyzed using the RAxML webserver (http://embnet.vital-it.ch/raxml-bb/index.php) (Stamatakis et al., 2008). The evolutionary rates of the different protein-coding genes were estimated using the CAT model, and a maximum likelihood search was conducted after bootstrapping. The phylogenetic tree was drawn using FigTree v1.4.2.

RESULTS AND DISCUSSION

Mitochondrial genome structure

The mitochondrial genome of *P. sanguinolentus* (GenBank accession No. KT438509) was a circular molecule that was similar to those reported in other crab species. It was 16,024 bp in length and consisted of the following 37 genes: 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes, and a control region (Figure 1).

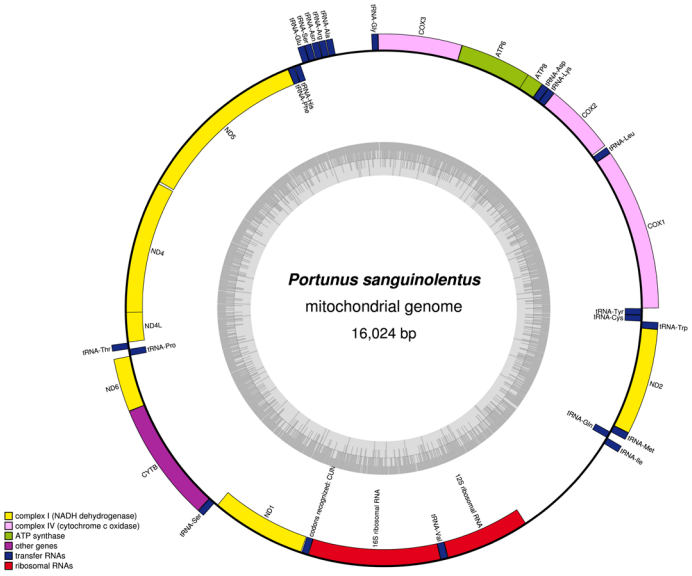

Figure 1. Complete mitochondrial genome structure of *Portunus sanguinolentus*. Genes located on the heavy strand are shown outside the circle and those on the light strand are shown inside the circle. The inner ring shows the GC content of the genome.

The gene arrangement and order were the same as those found in most metazoan species, including *S. paramamosain* (Ma et al., 2013), *P. pelagicus* (Meng et al., 2016b), and *C. feriata* (Ma et al., 2015a); however, they differed to those observed in the fish species *Caranx tille* (Ma et al., 2015b) and *Macropodus opercularis* (Mu et al., 2015). The genome was shorter than those in *P. pelagicus* (KT382858), *C. sapidus* (NC_006281), and *E. sinensis* (NC_006992), but longer than those in *C. feriata* (KF386147), *C. japonica* (FJ460517), *T. crenata* (NC_024438), and *S. tranquebarica* (NC_012567). The differences in genome length were mainly related to size variations in the control region (Table 1). The lengths of other regions of the genome were approximately equal among the above-mentioned crab species. Of the 37 genes in the genome, 23 were on the heavy strand (H-strand) and 14 were on the light strand (L-strand) (Table 2). Table 2 shows that $tRNA^{His}$ was between $tRNA^{Glu}$ and $tRNA^{Phe}$, which is different to that observed in most arthropods, in which $tRNA^{His}$ is between *NAD4* and *NAD5*. Gene rearrangements in the mitochondrial genome are relatively common events in crustacean species (Shen et al., 2007). The tandem duplication of gene regions is the most probable mechanism of mitochondrial gene rearrangement; in this case, a slipped-strand mispairing occurred first and was followed by gene deletions (Yamauchi et al., 2003).

Fourteen small, non-coding segments were identified in the genome, which ranged in size from 1 to 34 bp; most of them have been previously reported in the mitochondrial genomes of other crab species. Nine overlaps were detected among the 37 genes, which ranged from 1 to 7 bp in length. The total lengths of the non-coding regions and overlaps were 118 and 24 bp, respectively. The overall base composition of the mitochondrial genome was 31.59% for A, 12.91% for G, 34.01% for T, and 21.50% for C. The total A + T content (65.60%) was considerably higher than the G + C content (34.41%), but is the lowest among so far reported in crab species (Table 1). The control region is considered to have the highest A + T content; however, in this study, the highest A + T content was observed in the tRNA genes (71.41%). The control region had the lowest A + T content (69.73%) of all the crab species investigated (Table 1). In addition, the genome was slightly shorter than that observed in Meng's study (Meng et al., 2016a, 16,027 bp) with a total of 68 variable sites, which suggests that *P. sanguinolentus* has a moderate level of genetic diversity.

Protein-coding genes

Of the 13 protein-coding genes identified in this study, nine were encoded by the H-strand (*COI*, *COII*, *COIII*, *APT6*, *ATP8*, *ND2*, *ND3*, *ND6*, and *Cytb*) and four were encoded by the L-strand (*ND1*, *ND4*, *ND4L*, and *ND5*). These genes coded 3715 amino acids, which is slightly longer than that in *G. dehaani* and *C. sapidus* but shorter than that in *C. feriata* and *S. tranquebarica*. Four types of start codons were observed in the 13 protein-coding genes; ATG initiated nine genes, ATT initiated two genes, ATC initiated one gene, and GTG initiated one gene. In addition, nine genes ended with a complete TAG or TAA stop codon and four ended with an incomplete TA- or T- stop codon. In contrast to nuclear genes, variable start codons and stop codons are often detected in mitochondrial genomes. Four types of start codons (ATT, ATG, ATA, and ACG) were found in the mitochondrial genome of *Myrmeleon immanis* (Yan et al., 2014), and one and two incomplete stop codons were found in the mitochondrial genomes of *S. paramamosain* and *Lutjanus russellii*, respectively (Guo et al., 2008; Ma et al., 2013). In the case of incomplete stop codons, missed nucleotides could be added by post-transcriptional polyadenylation (Ojala et al., 1981). The base composition of the 13 protein-coding genes was 30.71% for A, 13.37% for G, 33.09% for T, and 22.81% for C (Table 3). *ATP8* had the highest A + T content (69.14%) and *COI* the lowest (59.84%).

Table 1. Comparison of the mitochondrial genome structure of *Portunus sanguinolentus* with that of other crab species.

Species	GenBank accession No.	Heavy strand		13 protein-coding genes		16S rRNA		12S rRNA		22 tRNA genes		Control region	
		Length (bp)	A + T (%)	No. of amino acids	A + T (%)	Length (bp)	A + T (%)	Length (bp)	A + T (%)	Length (bp)	A + T (%)	Length (bp)	A + T (%)
Portunus sanguinolentus	KT438509	16,024	65.60	3,715	63.80	1,327	69.03	847	67.30	1,468	71.41	1,110	69.73
Portunus pelagicus	KT382858	16,155	68.72	3,715	66.79	1,330	73.31	846	69.62	1,467	71.57	1,245	76.22
Portunus trituberculatus	AB093006	16,026	70.20	3,715	68.80	1,332	73.80	840	70.10	1,468	72.00	1,104	76.30
Charybdis feriata	KF386147	15,660	70.15	3,716	68.60	1,321	74.26	843	71.89	1,473	71.76	762	78.74
Charybdis japonica	FJ460517	15,738	69.20	3,712	67.80	1,317	74.20	834	70.30	1,458	70.90	863	74.70
Thalamita crenata	NC_024438	15,787	69.66	3,722	67.82	1,359	73.07	834	72.90	1,472	72.08	897	79.26
Scylla tranquebarica	NC_012567	15,833	73.80	3,716	72.00	1,339	77.10	869	75.90	1,486	74.40	854	86.50
Scylla olivacea	NC_012569	15,723	69.40	3,715	67.30	1,337	74.40	852	72.40	1,482	72.30	778	79.00
Scylla serrata	HM590866	15,721	69.22	3,714	69.20	1,337	74.50	839	71.80	1,478	72.26	788	79.10
Scylla paramamosain	JX457150	15,824	73.04	3,715	70.88	1,340	77.46	869	75.72	1,482	74.56	833	86.67
Callinectes sapidus	NC_006281	16,263	69.10	3,712	67.00	1,323	71.80	785	70.30	1,463	71.60	1,435	78.20
Eriocheir sinensis	NC_006992	16,354	71.70	3,718	68.90	1,311	77.40	899	76.60	1,473	72.40	896	83.10
Pseudocarcinus gigas	NC_006891	15,515	70.50	3,734	68.80	1,324	74.90	821	73.80	1,460	73.20	593	80.30
Geothelphusa dehaani	NC_007379	18,197	74.90	3,711	71.50	1,315	77.10	821	76.40	1,519	75.80	514	87.20

Table 2. Mitochondrial genome structure of *Portunus sanguinolentus*.

Gene	Position		Size (bp)	Codon			Intergenic nucleotide (bp)	Strand
	From	To		Amino acid	Start	Stop		
COI	1	1,534	1,534	511	ATG	T-	0	H
tRNA$^{Leu\ (UUR)}$	1,535	1,599	65				16	H
COII	1,616	2,300	685	228	ATG	T-	0	H
tRNA^{Lys}	2,301	2,367	67				0	H
tRNA^{Asp}	2,368	2,435	68				0	H
ATP8	2,436	2,597	162	53	ATG	TAG	-7	H
ATP6	2,591	3,268	678	225	ATT	TAA	-1	H
COIII	3,268	4,059	792	263	ATG	TAA	-1	H
tRNA^{Gly}	4,059	4,122	64				0	H
ND3	4,123	4,476	354	117	ATC	TAA	8	H
tRNA^{Ala}	4,485	4,549	65				3	H
tRNA^{Arg}	4,553	4,617	65				1	H
tRNA^{Asn}	4,619	4,684	66				2	H
tRNA$^{Ser\ (AGN)}$	4,687	4,753	67				0	H
tRNA^{Glu}	4,754	4,820	67				25	H
tRNA^{His}	4,846	4,910	65				1	L
tRNA^{Phe}	4,912	4,976	65				-1	L
ND5	4,976	6,702	1,727	575	ATG	TA-	17	L
ND4	6,720	8,054	1,335	444	ATG	TAG	-7	L
ND4L	8,048	8,350	303	100	ATG	TAA	2	L
tRNA^{Thr}	8,353	8,415	63				0	H
tRNA^{Pro}	8,416	8,483	68				2	L
ND6	8,486	8,992	507	168	ATG	TAA	-1	H
Cytb	8,992	10,126	1,135	378	ATG	T--	0	H
tRNA$^{Ser(UCN)}$	10,127	10,193	67				34	H
ND1	10,228	11,184	957	318	ATT	TAA	5	L
tRNA$^{Leu(CUN)}$	11,190	11,258	68				1	L
16 S rRNA	11,260	12,586	1,327				0	L
tRNA^{Val}	12,587	12,660	74				0	L
12 S rRNA	12,661	13,507	847				0	L
Control region	13,508	14,617	1,110				0	
tRNA^{Ile}	14,618	14,684	67				-3	H
tRNA^{Gln}	14,682	14,750	69				1	L
tRNA^{Met}	14,752	14,821	70				0	H
ND2	14,822	15,829	1,008	335	GTG	TAG	-2	H
tRNA^{Trp}	15,828	15,895	68				-1	H
tRNA^{Cys}	15,895	15,958	64				0	L
tRNA^{Tyr}	15,959	16,024	66				0	L

tRNA and rRNA genes

A total of 22 tRNA genes were identified in the mitochondrial genome of *P. sanguinolentus*, with lengths ranging from 63 to 74 bp. The total length of tRNAs was 1468 bp, which is equal to that of *P. trituberculatus*, larger than those of *C. japonica* and *Pseudocarcinus gigas*, but smaller than those of *G. dehaani* and *S. tranquebarica*. In the closely related crab species *C. japonica* and *S. paramamosain*, 21 tRNAs can fold into a cloverleaf secondary structure, but one [*tRNA$^{Ser\ (AGN)}$*] cannot form this structure because it lacks dihydrouracil (DHU) arms (Liu and Cui, 2010; Ma et al., 2013). In *P. sanguinolentus*, all of the tRNAs were capable of folding into a typical cloverleaf secondary structure (Figure 2). The same phenomenon has also been reported in *C. feriata* (Ma et al., 2015a). Of the 22 tRNAs, 14 were located on the H-strand and 8 on the L-strand. All of the tRNA genes had a common length of 7 bp for the aminoacyl stem and an invariable length of 7 bp for the anticodon loop. Variable tRNA nucleotide lengths were found at the DHU, TΨC, and anticodon arms.

Table 3. Base composition of different regions of the mitochondrial genome of *Portunus sanguinolentus*.

Region	Base composition (%)				A + T content (%)
	A	G	T	C	
Protein-coding genes					
COI	24.25	17.47	35.59	22.69	59.84
COII	27.45	15.47	33.58	23.50	61.02
ATP8	27.16	12.35	41.98	18.52	69.14
ATP6	27.73	12.54	36.43	23.30	64.16
COIII	26.01	15.28	34.85	23.86	60.86
ND3	24.86	14.41	37.01	23.73	61.86
ND5	37.46	12.33	28.89	21.31	66.36
ND4	39.85	11.69	26.44	22.02	66.29
ND4L	42.24	10.23	23.10	24.42	65.35
ND6	23.87	10.85	41.42	23.87	65.29
Cytb	25.55	14.36	36.12	23.96	61.67
ND1	40.44	12.33	26.65	20.59	67.08
ND2	24.01	10.71	40.08	25.20	64.09
tRNA genes					
$tRNA^{Leu\ (UUR)}$	35.38	20.00	30.77	13.85	66.15
$tRNA^{Lys}$	28.36	20.90	31.34	19.40	59.70
$tRNA^{Asp}$	38.24	10.29	47.06	4.41	85.29
$tRNA^{Gly}$	42.19	10.94	37.50	9.38	79.69
$tRNA^{Ala}$	33.85	18.46	35.38	12.31	69.23
$tRNA^{Arg}$	32.31	13.85	32.31	21.54	64.62
$tRNA^{Asn}$	36.36	16.67	34.85	12.12	71.21
$tRNA^{Ser\ (AGN)}$	31.34	14.93	40.30	13.43	71.64
$tRNA^{Glu}$	29.85	13.43	43.28	13.43	73.13
$tRNA^{His}$	41.54	7.69	30.77	20.00	72.31
$tRNA^{Phe}$	27.69	10.77	38.46	23.08	66.15
$tRNA^{Thr}$	38.10	9.52	41.27	11.11	79.37
$tRNA^{Pro}$	38.24	8.82	35.29	17.65	73.53
$tRNA^{Ser\ (UCN)}$	41.79	13.43	38.81	5.97	80.60
$tRNA^{Leu\ (CUN)}$	37.68	8.70	37.68	15.94	75.36
$tRNA^{Val}$	29.73	13.51	37.84	18.92	67.57
$tRNA^{Ile}$	35.82	16.42	38.81	8.96	74.63
$tRNA^{Gln}$	33.33	10.14	31.88	24.64	65.22
$tRNA^{Met}$	35.71	12.86	32.86	18.57	68.57
$tRNA^{Trp}$	45.59	10.29	30.88	13.24	76.47
$tRNA^{Cys}$	31.25	14.06	34.38	20.31	65.62
$tRNA^{Tyr}$	30.30	12.12	34.85	22.73	65.15
rRNA genes					
16S rRNA	33.08	12.06	35.95	18.91	69.03
12S rRNA	32.59	11.69	34.71	21.02	67.30
Control region	33.69	10.45	36.04	19.82	69.73
Overall for protein-coding genes	30.71	13.37	33.09	22.81	63.80
Overall for tRNA genes	35.19	13.07	36.22	15.52	71.41
Overall for rRNA genes	32.89	11.91	35.46	19.73	68.35
Overall for the genome	31.59	12.91	34.01	21.50	65.60

tRNA, transfer RNA; rRNA, ribosomal RNA.

All 22 tRNA genes had the common anticodons of Decapod mitochondrial genomes, except that $tRNA^{Lys}$ and $tRNA^{Ser(AGN)}$ had the anticodons TTT and TCT rather than CTT and GCT, respectively. Thirteen unmatched base pairs were observed in the 22 tRNA genes, which is higher than those detected in tRNAs from *C. feriata* (Ma et al., 2015a). The base composition of the 22 tRNA genes was 35.19% for A, 13.07% for G, 36.22% for T, and 15.52% for C (Table 3). The overall A + T content of the tRNAs was 71.41%, with the highest content (85.29%) in $tRNA^{Asp}$ and the lowest (59.70%) in $tRNA^{Lys}$.

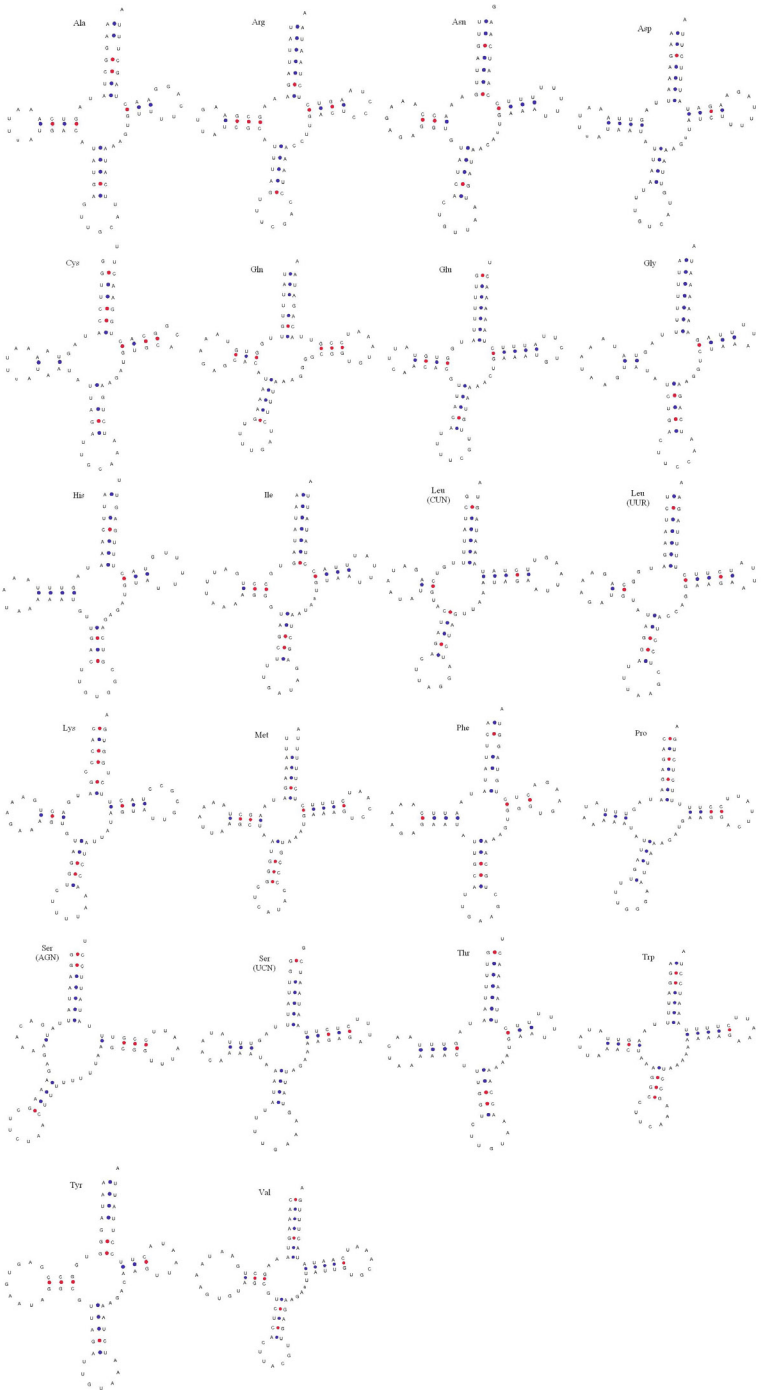

Figure 2. Predicted cloverleaf secondary structures of 22 transfer RNAs in the mitochondrial genome of *Portunus sanguinolentus*.

16S rRNA and *12S rRNA* were 1327 and 847 bp long, respectively, and their A + T contents were 69.03 and 67.30%, respectively. Both of them were encoded on the L-strand and were separated by $tRNA^{Val}$, and were co-located between $tRNA^{Leu\ (CUN)}$ and the control region.

Putative control region

The largest non-coding region was 1110 bp long, and was predicted to be the putative control region. This non-coding region was located between *12S rRNA* and $tRNA^{Ile}$, which is identical to that found in other reported crab species, such as *S. paramamosain* and *C. feriata*. The base composition of the control region was 33.69% for A, 10.45% for G, 36.04% for T, and 19.82% for C. The A + T content of the control region was high (69.73%), but lower than that observed in most crab species, in which the A + T content of the control region is higher than that of any other region of the mitochondrial genome. The high A + T content is probably due to the existence of A/T repeated motifs. In this study, TA and AT repeats were the most abundant motifs. The microsatellite repeat sequences $(AT)_6$, $(TA)_4$, $(TA)_3$, $(AT)_3$, and $(TA)_{22}$ were also detected. Microsatellite sequences have also been found in the control region of the mitochondrial genomes of *C. feriata* and *Nymphes myrmeleonides* (Yan et al., 2014; Ma et al., 2015a).

Phylogenetic relationships

In order to elucidate the molecular phylogeny of representative species in the family Portunidae, we constructed a phylogenetic tree using 23 species within the Decapoda. The tree topologies (Figure 3) revealed that *P. sanguinolentus* first grouped together with its sister species *P. trituberculatus* and *P. pelagicus*, and then formed a monophyletic group with *C. sapidus*. The molecular evidence presented here supports the species' traditional taxonomic status. The tree also showed that the genus *Charybdis* is genetically closest to the genus *Thalamita*; species within these two genera clustered as a monophyletic group. Furthermore, the genera *Charybdis* and *Thalamita* have a very close relationship with crab species in the subfamily Portuninae. Previous studies have shown that *Charybdis* is closer to the subfamily Portuninae than to the subfamily Thalamitinae (Liu and Cui, 2010; Ma et al., 2015a). Our results confirm that *Thalamita* is closest to *Charybdis*, and both should be classified into the subfamily Portuninae rather than the subfamily Thalamitinae.

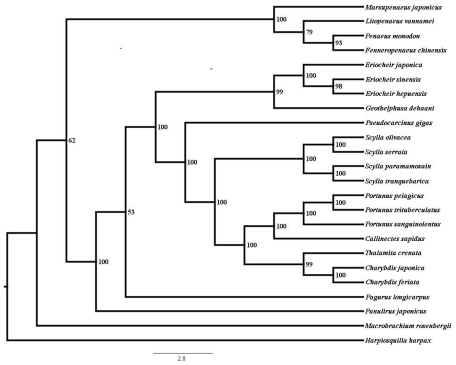

Figure 3. Molecular phylogenetic relationships between 23 species of Decapoda based on 12 protein-coding genes of the mitochondrial genome.

CONCLUSIONS

This study investigated the complete mitochondrial genome of the three-spot swimming crab (*P. sanguinolentus*), which contained a typical set of 37 genes and a putative control region. Molecular phylogenomic analysis based on 12 protein-coding genes demonstrated that *P. sanguinolentus* is genetically closest to its sister species, *P. trituberculatus* and *P. pelagicus*, and we suggest classifying the genera *Charybdis* and *Thalamita* into the subfamily Portuninae rather than the subfamily Thalamitinae. Our results will be useful for studies on phylogenetic relationships, population genetic diversity, and the conservation genetics of *P. sanguinolentus* and related crab species.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the National Program for Support of Top-Notch Young Professionals, and the National Basic Research Special Foundation of China (#2013FY110700).

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