Characterization of the complete mitochondrial genome of *Portunus pelagicus* with implications for phylogenomics

C.Y. Ma, H.Y. Ma, G.J. Ren, W. Wang, W. Chen, J.X. Lu, X. Zou and L.B. Ma

East China Sea Fisheries Research Institute,
Chinese Academy of Fishery Sciences, Shanghai, China

Corresponding author: L.B. Ma
E-mail: malingbo@vip.sina.com

Genet. Mol. Res. 15 (3): gmr.15038719
Received April 20, 2016
Accepted July 6, 2016
Published August 18, 2016
DOI http://dx.doi.org/10.4238/gmr.15038719

ABSTRACT. This study determined the mitochondrial genome structure of the blue swimming crab (*Portunus pelagicus*), and elucidated its phylogenetic relationships among the species within the order Decapoda. The complete mitochondrial genome was 16,155 bp long, and contained 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, and 1 DNA control region. The gene order of the genome was the same as that found within the family Portunidae. Twenty-three genes were on the heavy strand and 14 were on the light strand. Almost all of the protein-coding genes were initiated by an ATG codon, except for three genes (*ATP6*, *ND1*, and *ND3*) that started with a rare ATT codon. Of the 13 protein-coding genes, 10 ended with complete TAA or TAG stop codons and three ended with an incomplete T codon. Thirteen non-coding regions were identified that ranged from 1 to 30 bp in length. Nine overlaps were found, which ranged from

1 to 7 bp in length. Phylogenetic analyses based on 12 concatenated protein-coding genes revealed that *P. pelagicus* formed a monophyletic group with *Portunus trituberculatus*, which were in a larger group with *Callinectes sapidus*, while the genera *Charybdis* and *Thalamita* formed another group. These two groups clustered together and grouped with the genus *Scylla*. The phylogenetic analysis supported the inclusion of *Charybdis* in subfamily Portuninae of the family Portunidae, and revealed a close relationship between *Charybdis* and *Thalamita*. We suggest that *Thalamita* should also be classified into the subfamily Portuninae. The results can be used in the study of phylogenetic, population genetic and conservation genetics of *P. pelagicus*.

Key words: *Portunus pelagicus*; Mitochondrial genome; Phylogenetic relationship

INTRODUCTION

The blue crab, *Portunus pelagicus*, which is also known as the flower crab, is a large crab that is an excellent swimmer. In contrast with another portunid crab (*Scylla serrata*), this species cannot survive for long periods out of the water (Romano and Zeng, 2007a). *P. pelagicus* is found in the intertidal estuaries of the Indian and West Pacific Oceans, and is widely distributed in East Africa, Southeast Asia, East Asia, Australia, the Persian Gulf, and New Zealand (Kailola et al., 1993). It is commercially important, and is sold as a traditional hard-shell crab in the Indo-Pacific and a soft-shell crab in Asia.

Because of its growing popularity, the species has been heavily fished in recent years (Romano and Zeng, 2008; Lai et al., 2010), and is increasingly cultured. It has high potential for aquaculture because of its rapid growth rate (Josileen and Menon, 2004), ease of larviculture, high fecundity, and relatively high tolerance to nitrate and ammonia (Bryars and Havenhand, 2006; Romano and Zeng, 2007a,b,c, 2008). *P. pelagicus* is commercially cultivated in some areas, such as Thailand (Klinbunga et al., 2010).

Studies on this species have mainly focused on its reproductive biology (Zairion et al., 2015), embryonic and larval development, and artificial seeding (Andrés et al., 2010). Knowledge of the genetic diversity of *P. pelagicus* is essential for the conservation and aquaculture of this important crab resource, in order to construct an appropriate management scheme (Bryars and Adams, 1999). The genetic population structure of *P. pelagicus* has been investigated based on allozymes (Bryars and Adams, 1999), cytochrome oxidase subunit I (*COI*), microsatellite markers (Sezmis, 2004), amplified fragment length polymorphisms (Praipue et al., 2010), and random amplification of polymorphic DNA (Klinbunga et al., 2010).

The mitochondrial genome is typically a closed-circular molecule, and is approximately 14 to 18 kb in size. Complete mitochondrial genome sequence information is useful in studying genome-level characteristics and phylogenetic relationships (Knudsen et al., 2006), because of its high mutation rate, simple structure, abundant distribution, and maternal inheritance (Yu et al., 2012; Ma et al., 2013; Baek et al., 2014). With the development of molecular techniques, it has become easier to obtain complete mitochondrial genome sequences (Wang et al., 2015), and they have been reported in many crustacean species (Shen et al., 2007; Liu and Cui, 2010; Jondeung et al., 2012; Ma et al., 2013).

The purpose of this study was to report the complete mitochondrial genome of *P. pelagicus*, elucidate its evolutionary status, and estimate its population genetic diversity and differentiation. The results of this study should further our understanding of the evolutionary status and population genetic diversity of *P. pelagicus* and other related crustacean species.

MATERIAL AND METHODS

Sample collection and DNA extraction

A wild *P. pelagicus* individual was sampled from the southeastern coast of Guangxi, China. The crab was killed by a lethal dose of MS-222. Muscle tissues were collected from the claws and fixed in 99% ethanol at room temperature. Genomic DNA was extracted according to the protocol described by Ma et al. (2009).

Primers, polymerase chain reaction (PCR), and sequencing

First, the partial sequences of four *P. pelagicus* genes (*12S rRNA*, *16S rRNA*, *Cytb*, and *COI*) and the complete mitochondrial genomes of three other crab species (*Charybdis japonica*, *Scylla paramamosain*, and *Portunus trituberculatus*) were downloaded from the GenBank database. The four genes were amplified, resequenced, and confirmed. The complete mitochondrial genome of *P. pelagicus* was then obtained by overlapping PCR and sequencing.

The total PCR volume was 25 μL, which 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 100 ng template DNA. The thermocycler conditions were as follows: an initial denaturation at 94°C for 4 min; then 37 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 amplification products were separated on 1.5% agarose gels (TaKaRa, China), and directly sequenced in both directions using an ABI PRISM® 3730 Automated DNA Sequencer (PE Corporation). The sequences obtained were edited and assembled by EditSeq and SeqMan (DNASTAR).

Complete mitochondrial genome analysis

A graphical map of the complete mitochondrial genome was drawn using the online software OrganellarGenomeDRAW (http://ogdraw.mpimp-golm.mpg.de/) (Lohse et al., 2007). The genome structure of *P. pelagicus* was determined based on those of the closely related species *S. paramamosain* (Ma et al., 2013) and *C. japonica* (Liu and Cui, 2010). Transfer RNAs (tRNAs) were identified by their proposed clover-leaf secondary structure and their anticodons using the web-based tRNA-scanSE 1.21 program (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe and Eddy, 1997), with the default search mode set. MEGA 4.0 was used to determine the nucleotide composition of the mitochondrial genome, and translate protein-coding genes into amino acids according to codon usage (Kumar et al., 2008). Finally, the complete mitochondrial genome DNA sequence was submitted to the GenBank database using the software Sequin 12.30 (http://www.ncbi.nlm.nih.gov/Sequin/).

Phylogenomic analysis

The complete mitochondrial genomes of 22 Decapoda species were downloaded for

phylogenetic analysis from the GenBank database, and one species (*Harpiosquilla harpax*) was used as an outgroup taxon.

The 13 protein-coding genes were aligned by ClustalW in MEGA 4.0 with the default settings; however, the gene *ND6* was excluded because of its high heterogeneity, which may cause poor phylogenetic performance (Miya and Nishida, 2000). The remaining 12 protein-coding gene sequences were linked to construct sequence alignments. The sequence alignments were then formatted and analyzed using RAxML web servers (http://embnet.vital-it.ch/raxml-bb/index.php) (Stamatakis et al., 2008). The evolutionary rates of the 12 protein-coding genes were estimated using the CAT model, and a maximum likelihood search was conducted after bootstrapping. Finally, the phylogenetic tree was viewed and edited using the software FigTree v1.4.2.

RESULTS AND DISCUSSION

Genome organization

The *P. pelagicus* mitochondrial genome was a typical, circular molecule that was 16,155 bp in length (GenBank accession No. KT382858), and consisted of 13 protein-coding genes, 22 tRNA genes, 2 ribosomal RNA genes, and 1 DNA control region (Figure 1).

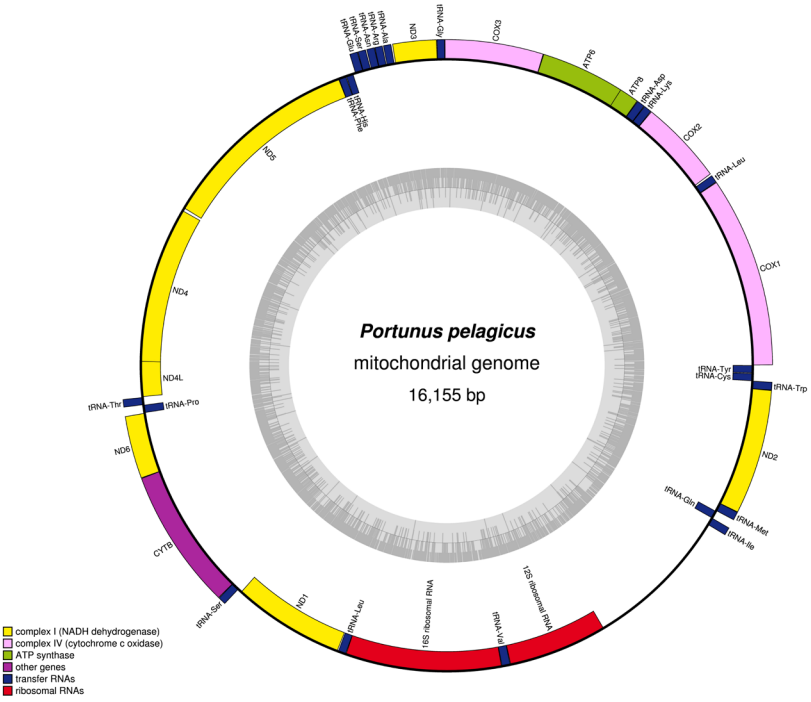

Figure 1. Complete mitochondrial genome of *Portunus pelagicus*. Genes encoded by the heavy strand are shown outside the circle and those encoded by the light strand are shown inside the circle. The inner ring shows the GC content of the genome.

Twenty-three genes were located on the heavy strand and 14 were on the light strand. All of the protein-coding genes were initiated by an ATG codon, except three genes (*ATP6*, *ND1*, and *ND3*), which started with a rare ATT codon. Of the 13 protein-coding genes, 10 ended with complete TAA or TAG stop codons and three ended with an incomplete T codon (Table 1). The lengths of the control regions in the 12 species ranged from 514 to 1435 bp.

Table 1. Genetic structure of the mitochondrial genome of *Portunus pelagicus*.

Gene	Position		Size (bp)	Codon			Intergenic nucleotide (bp)[b]	Strand[c]
	From	To		Amino acid	Start	Stop[a]		
COI	1	1,534	1,534	511	ATG	T-	0	H
tRNA$^{Leu\ (UUR)}$	1,535	1,599	65				12	H
COII	1,612	2,296	685	228	ATG	T-	0	H
tRNA^{Lys}	2,297	2,363	67				0	H
tRNA^{Asp}	2,364	2,431	68				0	H
ATP8	2,432	2,593	162	53	ATG	TAG	-7	H
ATP6	2,587	3,264	678	225	ATT	TAA	-1	H
COIII	3,264	4,055	792	263	ATG	TAA	-1	H
tRNA^{Gly}	4,055	4,118	64				0	H
ND3	4,119	4,472	354	117	ATT	TAG	8	H
tRNA^{Ala}	4,481	4,545	65				4	H
tRNA^{Arg}	4,550	4,614	65				0	H
tRNA^{Asn}	4,615	4,680	66				5	H
tRNA$^{Ser\ (AGN)}$	4,686	4,752	67				0	H
tRNA^{Glu}	4,753	4,819	67				19	H
tRNA^{His}	4,839	4,901	63				1	L
tRNA^{Phe}	4,903	4,966	64				-1	L
ND5	4,966	6,693	1,728	575	ATG	TAA	20	L
ND4	6,714	8,048	1,335	444	ATG	TAG	-7	L
ND4L	8,042	8,344	303	100	ATG	TAA	2	L
tRNA^{Thr}	8,347	8,411	65				0	H
tRNA^{Pro}	8,412	8,479	68				2	L
ND6	8,482	8,988	507	168	ATG	TAA	-1	H
Cytb	8,988	10,122	1,135	378	ATG	T-	0	H
tRNA$^{Ser(UCN)}$	10,123	10,189	67				30	H
ND1	10,220	11,176	957	318	ATT	TAA	5	L
tRNA$^{Leu(CUN)}$	11,182	11,250	69				0	L
16S rRNA	11,251	12,580	1,330				0	L
tRNA^{Val}	12,581	12,654	74				0	L
12S rRNA	12,655	13,500	846				0	L
Control region	13,501	14,745	1,245				0	
tRNA^{Ile}	14,746	14,812	67				-3	H
tRNA^{Gln}	14,810	14,878	69				3	L
tRNA^{Met}	14,882	14,950	69				0	H
ND2	14,951	15,958	1,008	335	ATG	TAG	-2	H
tRNA^{Trp}	15,957	16,023	67				-1	H
tRNA^{Cys}	16,023	16,087	65				2	L
tRNA^{Tyr}	16,090	16,155	66				0	L

[a]T- represents incomplete stop codons; [b]Numbers correspond to the nucleotides separating adjacent genes. Negative numbers indicate overlapping nucleotides; [c]H or L indicates that the gene was encoded by the heavy or light strand, respectively.

The length of the control region was 1245 bp, which was shorter than that in *Callinectes sapidus* but longer than that in other crab species under Decapoda (Table 2).

The lengths of other regions of the mitochondrial genome were approximately the same among these species. The mitochondrial genome lengths of most crab species sequenced in the Decapoda were slightly different, mainly due to the differing sizes of the control region. The gene arrangement and order was the same as that found within the family Portunidae, such as that in *C. japonica* (Liu and Cui, 2010) and *S. paramamosain* (Ma et al., 2013). However, *tRNA^{His}* was between *NAD4* and *NAD5*, which is different to that in *Charybdis feriata* but the

same as that in most arthropods (Table 1). Different gene rearrangements in the mitochondrial genome is relatively common in crustacean species (Shen et al., 2007), and are probably caused by slipped-strand mispair and gene deletions (Yamauchi et al., 2003).

Table 2. Comparison of the mitochondrial genomes of crustacean species.

Species	GenBank accession No.	Heavy strand		13 protein-coding genes		*16S rRNA*		*12S rRNA*		22 transfer RNA 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 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
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

Nine 1- to 7-bp overlaps and 13 intergenic spacers that were between 1 and 30 bp in length were found, which in general is similar to that reported in other species' mitochondrial genomes (Ma et al., 2013; Baek et al., 2014; Wang et al., 2014). The A + T contents of the different regions ranged from 66.79% (protein-coding region) to 76.22% (control region), and the overall A + T content was 68.72%, which is similar to that in *C. japonica*, *Scylla olivacea*, and *S. serrata* (Table 2).

Protein-coding genes

A total of 13 protein-coding genes were identified, which were 11,178 bp in length and coded 3715 amino acids. *ATP8* had the highest A + T content (72.84%) and *COIII* the lowest (63.36%) (Table 2). Four protein-coding genes (*ND1*, *ND4*, *ND4L*, and *ND5*) were encoded by the light strand, and the other nine (*COI*, *COII*, *COIII*, *APT6*, *ATP8*, *ND2*, *ND3*, *ND6*, and *Cytb*) were encoded by the heavy strand. All 13 genes were initiated by two types of start codon (ATG and ATT) (Table 1). Nine protein-coding genes (*ATP6*, *ATP8*, *ND1*, *ND2*, *ND3*, *ND4*, *ND4L*, *ND5*, and *ND6*) ended with a typical stop codon (TAA or TAG), whereas an incomplete stop codon (T-) was detected in the remaining four genes (*COI*, *COII*, *COIII*, and *Cytb*). Variable start codons and incomplete stop codons exist in the mitochondrial genomes of many species (Ma et al., 2013, 2015). In the mitochondrial genome of *Myrmeleon immanis*, four types of start codons have been detected (Yan et al., 2014). Two incomplete stop codons have been found in the *C. feriata* mitochondrial genome (Ma et al., 2015), and one was detected in the *S. paramamosain* mitochondrial genome (Ma et al., 2013). Post-transcriptional polyadenylation may result in an incomplete stop codon (Ojala et al., 1981).

Transfer and ribosomal RNA genes

A total of 22 tRNA genes were identified, which ranged in size from 63 to 74 bp. The total length of the tRNA genes was 1467 bp. The average A + T content of the 22 tRNA genes was 71.57%, with the highest content in $tRNA^{Thr}$ (78.46%) and the lowest in $tRNA^{Lys}$ (59.70%). They could all be folded into a typical clover-leaf secondary structure, as in *C. feriata* (Figure 2). Because of a lack of dihydrouracil arms, $tRNA^{Ser\ (AGN)}$ cannot form a secondary structure in *C. japonica* and *S. paramamosain* (Liu and Cui, 2010; Ma et al., 2013). As in some closely related crabs, 14 tRNA genes were located on the heavy strand and the others were located on the light strand. The length of the aminoacyl stem in all of the tRNA genes was 7 bp. The

anticodon loop was 7 bp long in all of the tRNA genes, except for $tRNA^{Ser(UCN)}$. Seventeen unmatched base pairs were detected in the 22 tRNA genes, which is more than that found in the tRNA genes of *C. feriata* (Ma et al., 2015).

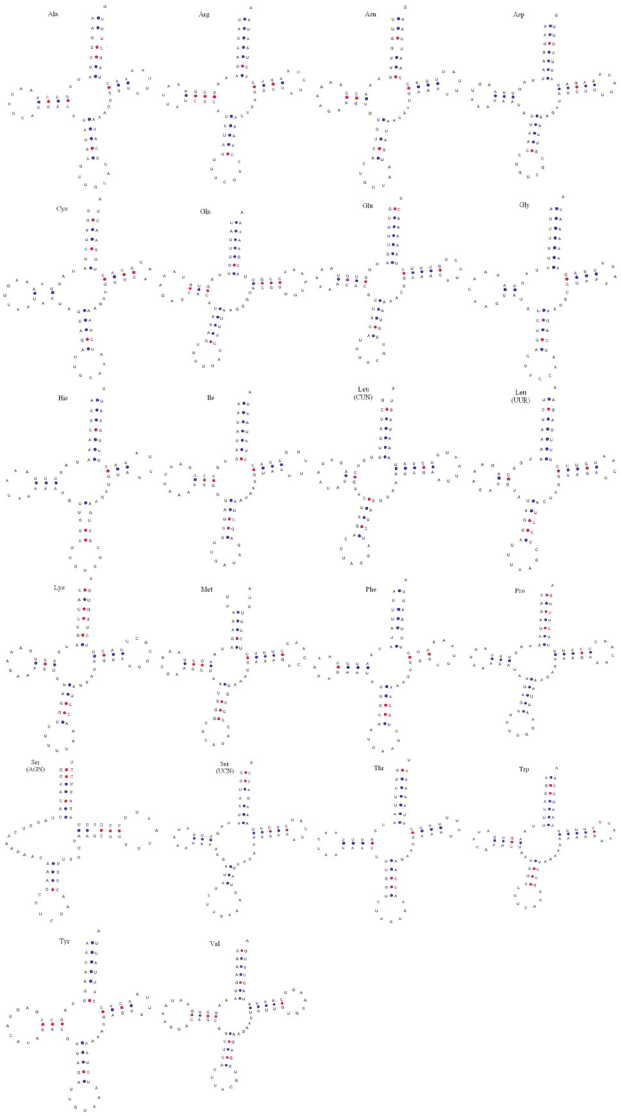

Figure 2. Putative secondary structures of 22 transfer RNAs detected in the mitochondrial genome of *Portunus pelagicus*.

As in related species, both *16S rRNA* and *12S rRNA* were located on the light strand of the mitochondrial genome. *16S rRNA* and *12S rRNA* were 1330 and 846 bp long, respectively, and their A + T contents were 73.31 and 69.62%, respectively. They were located between $tRNA^{Leu\ (CUN)}$ and the control region, and were separated by $tRNA^{Val}$.

Non-coding regions

The putative control region was located between *12S rRNA* and $tRNA^{Ile}$, and was the main non-coding region (1245 bp in length). The nucleotide composition of the control region was 42.08% for A, 9.80% for G, 34.14% for T, and 13.98% for C (Table 3). A and T were very abundant, which resulted in a high A + T content. The A + T content was higher (76.22%) than that of other regions in the mitochondrial genome, as in other crab species (Table 3). In addition, the microsatellite sequences $(TA)_3$, $(AT)_3$, $(TA)_4$, $(AT)_4$, and $(TA)_{11}$ were detected. Microsatellites have also been identified in the control region of the *Nymphes myrmeleonoides* mitochondrial genome (Yan et al., 2014). Twelve other non-coding regions were identified, which ranged from 1 to 30 bp in length.

Table 3. Base compositions of different regions of the mitochondrial genome of *Portunus pelagicus*.

Region*	Base composition (%)				A + T content (%)
	A	G	T	C	
Protein-coding genes					
COI	26.34	16.17	37.35	20.14	63.69
COII	29.34	15.33	34.01	21.31	63.36
ATP8	30.25	10.49	42.59	16.67	72.84
ATP6	28.17	12.24	38.50	21.09	66.67
COIII	27.27	14.65	37.12	20.96	64.39
ND3	27.68	12.71	37.29	22.32	64.97
ND5	39.58	11.05	29.92	19.44	69.50
ND4	40.82	11.24	29.21	18.73	70.04
ND4L	40.59	10.56	25.41	23.43	66.01
ND6	24.46	10.06	45.36	20.12	69.82
Cytb	25.99	14.36	38.41	21.23	64.41
ND1	42.53	11.39	24.45	21.63	66.98
ND2	26.39	10.42	41.37	21.83	67.76
tRNA genes					
$tRNA^{Leu\ (UUR)}$	36.92	18.46	30.77	13.85	67.69
$tRNA^{Lys}$	28.36	20.90	31.34	19.40	59.70
$tRNA^{Asp}$	38.24	13.24	39.71	8.82	77.94
$tRNA^{Gly}$	42.19	10.94	34.38	12.50	76.56
$tRNA^{Ala}$	33.85	16.92	36.92	12.31	70.77
$tRNA^{Arg}$	35.38	12.31	35.38	16.92	70.77
$tRNA^{Asn}$	36.36	18.18	34.85	10.61	71.21
$tRNA^{Ser\ (AGN)}$	31.34	14.93	40.30	13.43	71.64
$tRNA^{Glu}$	32.84	13.43	40.30	13.43	73.13
$tRNA^{His}$	38.10	7.94	33.33	20.63	71.43
$tRNA^{Phe}$	31.25	9.38	40.62	18.75	71.88
$tRNA^{Thr}$	36.92	10.77	41.54	10.77	78.46
$tRNA^{Pro}$	35.29	8.82	41.18	14.71	76.47
$tRNA^{Ser\ (UCN)}$	40.30	16.42	37.31	5.97	77.61
$tRNA^{Leu\ (CUN)}$	39.13	8.70	37.68	14.49	76.81
$tRNA^{Val}$	28.38	14.86	37.84	18.92	66.22
$tRNA^{Ile}$	34.33	17.91	38.81	8.96	73.13
$tRNA^{Gln}$	33.33	10.14	33.33	23.19	66.67
$tRNA^{Met}$	34.78	13.04	30.43	21.74	65.22
$tRNA^{Trp}$	46.27	10.45	28.36	14.93	74.63
$tRNA^{Cys}$	32.31	10.77	38.46	18.46	70.77
$tRNA^{Tyr}$	31.82	13.64	34.85	19.70	66.67
rRNA genes					
16S rRNA	35.79	10.98	37.52	15.71	73.31
12S rRNA	33.81	11.35	35.82	19.03	69.62
Control region	42.09	9.80	34.14	13.98	76.22
Overall of protein-coding	32.23	12.66	34.56	20.55	66.79
Overall of tRNA genes	35.31	13.29	36.26	15.13	71.57
Overall of rRNA genes	35.02	11.12	36.86	17.00	71.88
Overall of the genome	33.63	12.24	35.09	19.04	68.72

*Genes that were encoded by the light strand were converted to complementary strand sequences. tRNA = transfer RNA; rRNA = ribosomal RNA.

Phylogenetic relationships

A phylogenetic tree was constructed based on 12 concatenated protein-coding genes from the mitochondrial genomes of 23 species (Figure 3). We estimated the evolutionary relationships of *P. pelagicus* within the Decapoda, and found that *P. pelagicus* and *P. trituberculatus* first clustered together and were the most closely related. They formed a monophyletic group with *C. sapidus*, and then clustered with another group formed by *C. feriata*, *C. japonica*, and *Thalamita crenata*. These species had a sister relationship with another four species of the genus *Scylla*. Therefore, our results support the inclusion of *Charybdis* in subfamily Portuninae of the family Portunidae, as proposed by Liu and Cui (2010).

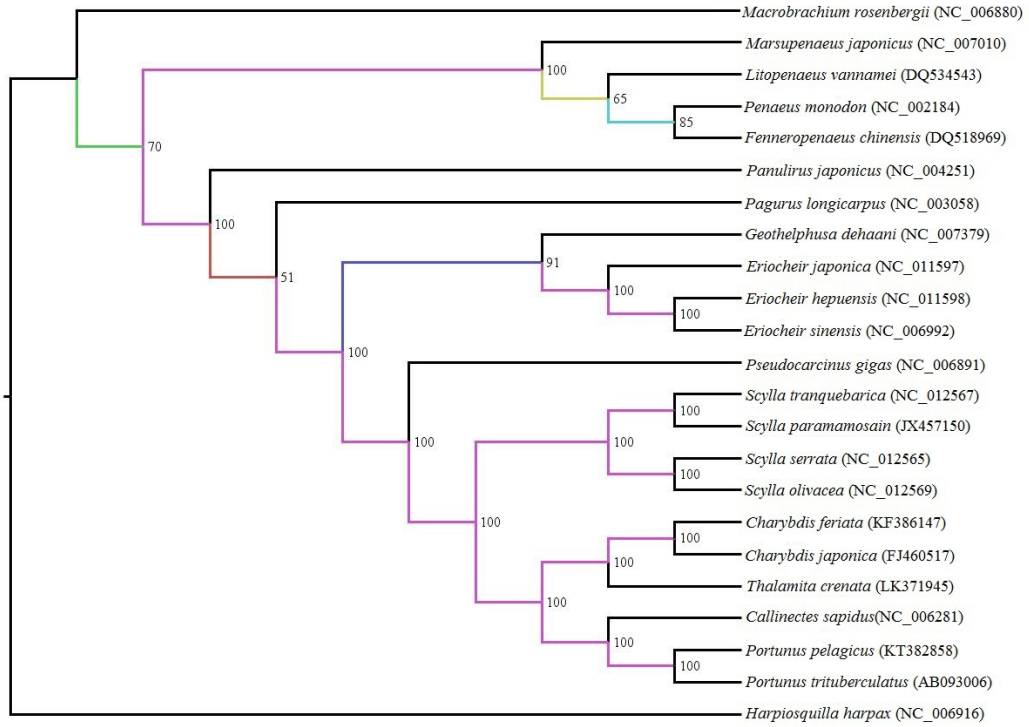

Figure 3. Phylogenetic relationships of Decapoda species based on 12 protein-coding.

CONCLUSIONS

We described the complete mitochondrial genome of *P. pelagicus*, which was 16,155 bp in length and contained 37 genes and a control region. Phylogenetic analysis based on 12 concatenated protein-coding genes supported the inclusion of *Charybdis* in subfamily Portuninae of the family Portunidae, and revealed a close relationship between *Charybdis* and *Thalamita*. We suggest that *Thalamita* should also be classified into the subfamily Portuninae. The results of this study can be used in phylogenetic, population genetic, and conservation genetic studies of *P. pelagicus*.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the National Basic Research Special Foundation of China (Grant #2013FY110700), the National Science and Technology Support Plan (Grant #2013BAD13B05), the National Program for Support of Top-Notch Young Professionals, and the National Infrastructure of Fishery Germplasm Resources.

REFERENCES

Andrés M, Rotllant G and Zeng C (2010). Survival, development and growth of larvae of the blue swimmer crab, *Portunus pelagicus*, cultured under different photoperiod conditions. *Aquaculture* 300: 218-222 http://dx.doi.org/10.1016/j.aquaculture.2009.12.026.

Baek SY, Choi EH, Jang KH, Ryu SH, et al. (2014). Complete mitochondrial genomes of *Carcinoscorpius rotundicauda* and *Tachypleus tridentatus* (Xiphosura, Arthropoda) and implications for chelicerate phylogenetic studies. *Int. J. Biol. Sci.* 10: 479-489 http://dx.doi.org/10.7150/ijbs.8739.

Bryars SR and Adams M (1999). An allozyme study of the blue swimming crab, *Portunus pelagicus* (Crustacea: Portunidae), in Australia: stock delineation in southern Australia and evidence for a cryptic species in northern waters. *Mar. Freshw. Res.* 50: 15-26. http://dx.doi.org/10.1071/MF98075

Bryars SR and Havenhand JN (2006). Effects of constant and varying temperatures on the development of blue swimmer crab (*Portunus pelagicus*) larvae: laboratory observations and field predictions for temperate coastal waters. *J. Exp. Mar. Biol. Ecol.* 329: 218-229 http://dx.doi.org/10.1016/j.jembe.2005.09.004.

Jondeung A, Karinthanyakit W and Kaewkhumsan J (2012). The complete mitochondrial genome of the black mud crab, *Scylla serrata* (Crustacea: Brachyura: Portunidae) and its phylogenetic position among (pan)crustaceans. *Mol. Biol. Rep.* 39: 10921-10937 http://dx.doi.org/10.1007%2Fs11033-012-1992-2. http://dx.doi.org/10.1007/s11033-012-1992-2

Josileen J and Menon NG (2004). Larval stages of the blue swimmer crab, *Portunus pelagicus* (Linnaeus, 1758) (Decapoda, Brachyura). *Crustaceana* 77: 785-803. http://dx.doi.org/10.1163/156854004774248681

Kailola PJ, Williams MJ, Stewart PC, Reichelt RE, et al. (1993). Australian fisheries resources. Bureau of Resource Sciences, Department of Primary Industries and Energy, and the Fisheries Research and Development Corporation, Canberra, Australia, 422.

Klinbunga S, Yuvanatemiya V, Wongphayak S, Khetpu K, et al. (2010). Genetic population differentiation of the blue swimming crab *Portunus pelagicus* (Portunidae) in Thai waters revealed by RAPD analysis. *Genet. Mol. Res.* 9: 1615-1624. http://dx.doi.org/10.4238/vol9-3gmr886

Knudsen B, Kohn AB, Nahir B, McFadden CS, et al. (2006). Complete DNA sequence of the mitochondrial genome of the sea-slug, *Aplysia californica*: conservation of the gene order in Euthyneura. *Mol. Phylogenet. Evol.* 38: 459-469 http://dx.doi.org/10.1016/j.ympev.2005.08.017.

Kumar S, Nei M, Dudley J and Tamura K (2008). MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Brief. Bioinform.* 9: 299-306. http://dx.doi.org/10.1093/bib/bbn017

Lai J, Ng PKL and Davie PJF (2010). A revision of the *Portunus pelagicus* (Linnaeus, 1758) species complex (Crustacea: Brachyura: Portunidae), with the recognition of four species. *Raffles Bull. Zool.* 58: 199-237.

Liu Y and Cui Z (2010). Complete mitochondrial genome of the Asian paddle crab *Charybdis japonica* (Crustacea: Decapoda: Portunidae): gene rearrangement of the marine brachyurans and phylogenetic considerations of the decapods. *Mol. Biol. Rep.* 37: 2559-2569 http://dx.doi.org/10.1007/s11033-009-9773-2.

Lohse M, Drechsel O and Bock R (2007). OrganellarGenomeDRAW (OGDRAW): a tool for the easy generation of high-quality custom graphical maps of plastid and mitochondrial genomes. *Curr. Genet.* 52: 267-274 http://dx.doi.org/10.1007/s00294-007-0161-y.

Lowe TM and Eddy SR (1997). tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25: 955-964. http://dx.doi.org/10.1093/nar/25.5.0955

Ma H, Ma C, Li X, Xu Z, et al. (2013). The complete mitochondrial genome sequence and gene organization of the mud crab (*Scylla paramamosain*) with phylogenetic consideration. *Gene* 519: 120-127 http://dx.doi.org/10.1016/j.gene.2013.01.028.

Ma H, Ma C, Li C, Lu J, et al. (2015). First mitochondrial genome for the red crab (*Charybdis feriata*) with implication of phylogenomics and population genetics. *Sci. Rep.* 5: 11524. http://dx.doi.org/10.1038/srep11524

Ma HY, Yang JF, Su PZ and Chen SL (2009). Genetic analysis of gynogenetic and common populations of *Verasper moseri* using SSR markers. *Wuhan Univ. J. Nat. Sci.* 14: 267-273. http://dx.doi.org/10.1007/s11859-009-0315-5

Miya M and Nishida M (2000). Use of mitogenomic information in teleostean molecular phylogenetics: a tree-based exploration under the maximum-parsimony optimality criterion. *Mol. Phylogenet. Evol.* 17: 437-455 http://dx.doi.org/10.1006/mpev.2000.0839.

Ojala D, Montoya J and Attardi G (1981). tRNA punctuation model of RNA processing in human mitochondria. *Nature* 290: 470-474. http://dx.doi.org/10.1038/290470a0

Praipue P, Klinbunga S and Jarayabhand P (2010). Genetic diversity of wild and domesticated stocks of Thai abalone, *Haliotis asinina* (Haliotidae), analyzed by single-strand conformational polymorphism of AFLP-derived markers. *Genet. Mol. Res.* 9: 1136-1152. http://dx.doi.org/10.4238/vol9-2gmr808

Romano N and Zeng C (2007a). Ontogenetic changes in tolerance to acute ammonia exposure and associated histological alterations of the gill structure through the early juvenile development of the blue swimmer crab, *Portunus pelagicus*. *Aquaculture* 266: 246-254. http://dx.doi.org/10.1016/j.aquaculture.2007.01.035

Romano N and Zeng C (2007b). Effects of potassium on nitrate mediated alterations of osmoregulation in marine crabs. *Aquat. Toxicol.* 85: 202-208. http://dx.doi.org/10.1016/j.aquatox.2007.09.004

Romano N and Zeng C (2007c). Acute toxicity of sodium nitrate, potassium nitrate, and potassium chloride and their effects on the hemolymph composition and gill structure of early juvenile blue swimmer crabs(*Portunus pelagicus* Linnaeus, 1758) (Decapoda, Brachyura, Portunidae). *Environ. Toxicol. Chem.* 26: 1955-1962 http://dx.doi.org/10.1897/07-144R.1.

Romano N and Zeng C (2008). Blue swimmer crabs- emerging species in Asia. *Global Aq. Adv.* 11: 34-36.

Sezmis E (2004). The population genetic structure of *Portunus pelagicus* in Australian waters. PhD thesis, Murdoch University, Perth.

Shen X, Ren J, Cui Z, Sha Z, et al. (2007). The complete mitochondrial genomes of two common shrimps (*Litopenaeus vannamei* and *Fenneropenaeus chinensis*) and their phylogenomic considerations. *Gene* 403: 98-109 http://dx.doi.org/10.1016/j.gene.2007.06.021.

Stamatakis A, Hoover P and Rougemont J (2008). A rapid bootstrap algorithm for the RAxML Web servers. *Syst. Biol.* 57: 758-771 http://dx.doi.org/10.1080/10635150802429642.

Wang QR, Xu C, Xu CR and Wang RJ (2015). Complete mitochondrial genome of the Southern catfish *(Silurus meridionalis* Chen) and Chinese catfish (*S. asotus* Linnaeus): Structure, phylogeny, and intraspecific variation. *Genet. Mol. Res.* 14: 18198-18209. http://dx.doi.org/10.4238/2015.December.23.7

Wang Y, Liu X and Yang D (2014). The first mitochondrial genome for caddisfly (insecta: Trichoptera) with phylogenetic implications. *Int. J. Biol. Sci.* 10: 53-63 http://dx.doi.org/10.7150/ijbs.7975.

Yamauchi MM, Miya MU and Nishida M (2003). Complete mitochondrial DNA sequence of the swimming crab, *Portunus trituberculatus* (Crustacea: Decapoda: Brachyura). *Gene* 311: 129-135 http://dx.doi.org/10.1016/S0378-1119(03)00582-1.

Yan Y, Wang Y, Liu X, Winterton SL, et al. (2014). The first mitochondrial genomes of antlion (Neuroptera: Myrmeleontidae) and split-footed lacewing (Neuroptera: Nymphidae), with phylogenetic implications of Myrmeleontiformia. *Int. J. Biol. Sci.* 10: 895-908 http://dx.doi.org/10.7150/ijbs.9454.

Yu JN, Azuma N and Abe S (2012). Genetic differentiation between collections of hatchery and wild masu salmon (*Oncorhynchus masou*) inferred from mitochondrial and microsatellite DNA analysis. *Environ. Biol. Fishes* 94: 259-271 http://dx.doi.org/10.1007/s10641-011-9869-0.

Zairion YW, Wardiatno Y, Boer M and Fahrudin A (2015). Reproductive biology of the blue swimming crab *Portunus pelagicus* (Brachyura: Portunidae) in East Lampung waters, Indonesia: fecundity and reproductive potential. *Trop. Life Sci. Res.* 26: 67-85.