Genomics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Characterisation of the complete mitochondrial genome of *Helice wuana* (Grapsoidea: Varunidae) and comparison with other Brachyuran crabs

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ARTICLE INFO

Keywords:
Brachyura
Varunidae
Helice wuana
Mitogenome
Phylogeny

ABSTRACT

The mitochondrial genome (mitogenome) provides important information for phylogenetic analysis and understanding evolutionary origins. Herein, we sequenced, annotated, and characterised the mitogenome of the crab *Helice wuana* to better understand its molecular evolution and phylogeny. The 16,359 bp mitogenome includes 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes and one control region. The genome composition is highly A + T biased 68.42%, and exhibits a negative AT–skew (− 0.036) and GC–skew (− 0.269) among Brachyura crabs. Gene rearrangements were detected, as was tandem duplication followed by random loss, which explains the translocation of mitochondrial genes. Phylogenetic analysis showed that *H. wuana* and *H. tientsinensis* clustered on one branch with high nodal support values. These results confirm that the placement of *H. wuana* within the Varunidae family of Thoracotrematan crabs. This study will provided a better understanding for gene rearrangements and crab evolution in the further.

1. Introduction

Decapoda is an economically important order of crustaceans comprising 18,000 living and extinct species [1], such as freshwater crayfish, lobsters, hermit crabs, shrimps, and 'true' crab [2]. Decapoda is the most species-rich, abundant, visible, popular, and economically important order of crustaceans, supporting seafood and marine industries worth billions of dollars each year to the world economy. Traditionally, Decapoda are divided into Dendrobranchiata and Pleocyemata sub-orders, and the Brachyura infra-order, belonging to Pleocyemata, is among the most diverse group, with over 7000 described species in 98 families inhabiting marine, freshwater, and terrestrial habitats [3–6]. Brachyura, the true crabs, is the largest clade Decapod Crustacea [7,8]. Due to the extreme morphological and ecological diversity, phylogenetic relationships within Brachyura are complicated [9]. The Grapsoidea crab, belonging to the higher taxa in the classification system, appeared late in the evolution of crustaceans, multiplied in the Tertiary period of the Cenozoic era, and gradually became dominant. *Helice wuana* is a mudflat crab inhabiting Chinese coastal regions including Guangdong, Fujian, Jiangsu, and Shandong peninsulas, and the Gulf of Bohai and Liaotung [2,10]. It is also common in North Korea and Japan. *H. wuana* burrows in intertidal mudflats, swamps, salt marshes and estuaries, especially in the high intertidal and supralittoral zones [10,11]. It is semi-terrestrial, returning to sea to spawn [12]. *H. wuana* also has a salinity requirement which lies between those of two other estuarine crabs in Japan, *Helicana japonica* and *Chiromantes dehaani* [13]. *H. wuana* is one of the most economically important crustacean species in aquaculture in China.

In metazoans, the mitochondrial genome (mitogenome) is usually small (~14–16 kb) and contains 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (*rrnS* and *rrnL*) and a control region [14]. In animals, the mitochondrial control region is the only non-coding sequence in the mitogenome, and it is called D-loop region in vertebrates and the A + T–rich region in invertebrate [15,16]. The control region contains initiation sites for transcription and replication. With the development of sequencing technologies, complete mitogenomes can now be rapidly sequenced to investigate phylogenetic matters. Besides being used for reconstruction of phylogenetic relationships, complete mitogenomes can also provide information on gene rearrangements and nucleotide bias [17]. Mitogenome sequences are now widely used in population genetics, comparative and evolutionary genomics, reconstruction of phylogenetic relationships, and molecular evolutionary research [18–21]. Numerous analyses of Decapoda have been published based on molecular from the

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http://dx.doi.org/10.1016/j.ygeno.2017.10.001
Received 28 July 2017; Received in revised form 29 September 2017; Accepted 2 October 2017
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Table 1
Details of the primers used to amplify the mitogenome of *H. wuana*.

Primer	Sequence (5′ − 3′)	Annealing temperature	Location	Length (bp)
F1	GGTCAACAAATCATAAAGATATTGG	55 °C	*cox1*	698
R1	TAAACTTCAGGGTGACCAAAAAATCA		*cox1*	
F2	TAGTWATHAANGGHCTACGVTGRGG	50 °C	*cox3*	388
R2	AAGTCCRTGRAAYCCDGTDGCHAC		*cox3*	
F3	GTGCCAGCCGCCGCGGTTA	52 °C	*rrnS*	568
R3	ATGCACTTTCCAGTACATCTA		*rrnS*	
F4	CCHTGAGGHCAAATRTCHTTYTGAGG	49 °C	*cob*	477
R4	GCDATWACTCCTCCTARTTTRTTRGG		*cob*	
F5	AGTAGAAAGAGGAGTAGGCACA	57 °C	*cox1 - cox3*	3421
R5	CTGAATCGGCAATAGTAAAA		*cox1 - cox3*	
F6	TTACTATTGCCGATTCAGTT	56 °C	*cox3 - rrnS*	3788
R6	GAATCTTACTTGCCTTTGTA		*cox3 - rrnS*	
F7	TACAAAGGCAAGTAAGATTC	60 °C	*rrnS - cob*	7012
R7	AAAGTAAAGTAAGGGTGGAA		*rrnS - cob*	
F8	CCCATTTATCGGGACTGACTTG	58 °C	*cob - cox1*	2632
R8	GTAGTAAGAGGATGGCGGTAAT		*cob - cox1*	

Table 2
List of 49 Brachyura species analyzed in this paper with their GenBank accession numbers.

Species	Taxonomic position	Size (bp)	GenBank No.
Helice wuana	**Grapsoidea;Varunidae**	**16,359**	**KX344898**
Helice tientsinensis	Grapsoidea;Varunidae	16,212	KR336555
Cyclograpsus granulosus	Grapsoidea;Varunidae	16,300	LN624373
Eriocheir japonica sinensis	Grapsoidea;Varunidae	16,378	KM516908
Eriocheir japonica hepuensis	Grapsoidea;Varunidae	16,335	FJ455506
Eriocheir japonica japonica	Grapsoidea;Varunidae	16,352	FJ455505
Sesarmops sinensis	Grapsoidea;Sesarmidae	15,905	KR336554
Sesarma neglectum	Grapsoidea;Sesarmidae	15,920	KX156954
Parasesarma tripectinis	Grapsoidea;Sesarmidae	15,612	KU343209
Metopaulias depressus	Grapsoidea;Sesarmidae	15,765	KX118277
Clistocoeloma sinensis	Grapsoidea;Sesarmidae	15,706	KU589292
Pachygrapsus crassipes	Grapsoidea;Grapsidae	15,652	KC878511
Grapsus tenuicrustatus	Grapsoidea;Grapsidae	15,858	KT878721
Xenograpsus testudinatus	Xenograpsoidea;Xenograpsidae	15,798	EU727203
Pseudocarcinus gigas	Xanthoidea;Eriphiidae	15,515	AY562127
Myomenippe fornasinii	Xanthoidea;Menippidae	15,658	LK391943
Leptodius sanguineus	Xanthoidea;Xanthidae	15,480	KT896744
Callinectes sapidus	Portunoidea;Portunidae	16,263	AY363392
Portunus trituberculatus	Portunoidea;Portunidae	16,026	AB093006
Portunus sanguinolentus	Portunoidea;Portunidae	16,024	KT438509
Charybdis japonica	Portunoidea;Portunidae	15,738	FJ460517
Scylla paramamosain	Portunoidea;Portunidae	15,824	JX457150
Scylla olivacea	Portunoidea;Portunidae	15,723	FJ827760
Scylla tranquebarica	Portunoidea;Portunidae	15,833	FJ827759
Scylla serrata	Portunoidea;Portunidae	15,775	FJ827758
Charybdis feriata	Portunoidea;Portunidae	15,660	KF386147
Thalamita crenata	Portunoidea;Portunidae	15,787	LK391945
Portunus pelagicus	Portunoidea;Portunidae	16,157	KM977882
Chaceon granulatus	Portunoidea;Geryonidae	16,135	AB769383
Chaceon sp.	Portunoidea;Geryonidae	16,126	KU507298
Lyreidus brevifrons	Raninoidea;Raninidae	16,112	KM983394
Umalia orientalis	Raninoidea;Raninidae	15,466	KM365084
Ranina ranina	Raninoidea;Raninidae	15,563	KM189817
Gandalfus yunohana	Bythograeoidea;Bythograeidae	15,567	EU647222
Gandalfus puia	Bythograeoidea;Bythograeidae	15,548	KR002727
Austinograea alayseae	Bythograeoidea;Bythograeidae	15,620	JQ035660
Austinograea rodriguezensis	Bythograeoidea;Bythograeidae	15,611	JQ035658
Ocypode cordimanus	Ocypodoidea;Ocypodidae	15,604	KT896743
Ocypode ceratophthalmus	Ocypodoidea;Ocypodidae	15,564	LN611669
Macrophthalmus japonicus	Ocypodoidea;Macrophthalmidae	16,170	KU343211
Geothelphusa dehaani	Potamoidea;Potamidae	18,197	AB187570
Sinopotamon xiushuiense	Potamoidea;Potamidae	18,460	KU042041
Huananpotamon lichuanense	Potamoidea;Potamidae	15,380	KX639824
Somanniathelphusa boyangensis	Potamoidea;Parathelphusidae	17,032	KU042042
Damithrax spinosissimus	Majoidea;Mithracidae	15,817	KM405516
Chionoecetes japonicus pacificus	Majoidea;Majidae	15,341	AB735678
Homologenus malayensis	Homoloidea;Homolidae	15,793	KJ612407
Moloha majora	Homoloidea;Homolidae	15,903	KT182069
Dynomene pilumnoides	Dromioidea;Dynomenidae	16,475	KT182070

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