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MITOGENOME REPORT

The complete mitochondrial genome of *Helicana japonica* (Crustacea, Decapoda, Varunidae) from South Korea

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ABSTRACT

Helicana japonica mainly inhabits burrowed holes in the mudflats and intertidal zones. Specimens from the Republic of Korea were collected and whole genomic DNA from the cheliped muscle tissue was extracted. We determined the complete mitochondrial genome using Illumina HiSeq X Ten. The mitogenome is 16,535 bp in length and consists of 13 protein-coding genes, 2 rRNA genes, and 22 tRNA genes. A phylogenetic tree was reconstructed using the maximum-likelihood of phylogeny methods. *H. japonica* formed a sister clade with *Helicana wuana*, which is another *Helicana* species.

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Varunidae; mitochondrial genome; *Helicana japonica*; phylogeny

Introduction

Helicana japonica (Sakai and Yatsuzuka 1980) (Crustacea: Decapoda: Varunidae) inhabits the muddy banks of estuaries and salt marshes from southern Japan to Korea and Taiwan (Irawan and Kijima, 1994; Omori et al. 2006; Shih and Suzuki 2008) and *Helicana wuana* inhabits a similar environment. However, the morphological differences between the two *Helicana* species were unclear. Therefore, it is necessary to study the genetic characteristics that distinguish the two species. This study reported the first complete mitogenome of *H. japonica* and will provide genetic information for improving the taxonomy of *Helicana*.

Materials and methods

H. japonica specimens were collected from Daesan-eup, Seosan-si, Chungcheongnam-do, Republic of Korea (36°58′6.67″N, 126°20′14.80″E). Total genomic DNA was extracted from the specimens using a DNeasy Blood & Tissue DNA Kit (Qiagen, Hilden, Germany). A genomic library was constructed using the QIAseq FX Single Cell DNA Library kit (Qiagen, Hilden, Germany) with paired-end reads. Next-generation sequencing analysis was performed using Illumina HiSeq X Ten (Illumina Inc., San Diego, CA, USA). The quality of all sequence was checked using FastQC v. 0.11.5 (Andrews, 2010) to obtain clean data, and reads were trimmed with Trimmomatic v. 0.36 (Bolger et al. 2014). De novo assembly was performed using SPAdes 3.13.0. (Bankevich et al. 2012). Mitochondrial genes were predicted and annotated using MitoZ v. 2.3 (Meng et al. 2019). The definitive species information was genetically identified by cytochrome oxidase subunit *I* (*COI*) from the mitochondrial genome results. For species identification, *COI* sequences of the related species were aligned using Geneious Prime v. 2022.1.1 (Biomatters, Auckland, New Zealand), and calculated genetic variations using Mega X (Kumar et al. 2018) with the Kimura two-parameter model (Kimura, 1980). To investigate the phylogenetic status of *Helicana japonica* in Varunidae, combined 10,795 bp sequences of the 12 protein-coding genes (PCGs) from 11 Brachyura species and an outgroup species were compared. The species sequence data were downloaded from NCBI GenBank, and PCG *ND6* was excluded because of its loss in some species. The PCGs sequences were aligned by MAFFT v.7.490 (Katoh and Standley, 2013), a maximum-likelihood phylogenetic tree was reconstructed using Phy ML 3.0 (Guindon et al. 2010) with 1000 bootstraps. The best model selected using PAUP* v.4a168 (Swofford, 2003) was the GTR + G + I model. The specimens were preserved in ethanol and deposited in the National Marine Biodiversity Institute of Korea (https://www.mabik.re.kr, Ji Min Kim, and jiminkim@mabik.re.kr, Voucher No. MABIK CR00248070).

Results and discussion

The morphological identification of *H. japonica* was based on descriptions by Kim (1973) and Sakai et al. (2006) (Figure 1). As described in the references, the suborbital ridge of the male consisted of a row of 10 tubercles, and the last tooth

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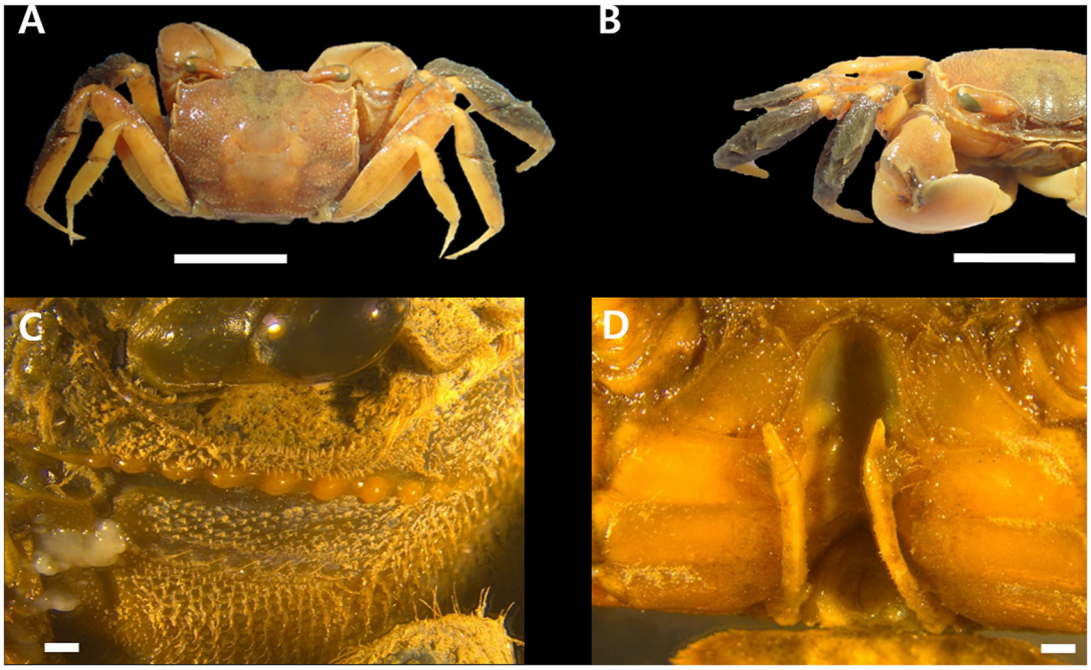

Figure 1. *Helicana japonica* male–Scale 10 mm (A, B); 1 mm (C, D). (A) dorsal over view; (B) Ambulatory legs; (C) Suborbital ridge; (D) First pleopods. (photos taken by Ji Min Kim at MABIK).

Table 1. Pairwise genetic distances based on 658 bp size of *COI* genes from *H. japonica*. (*Outgroup).

Species	Location	No.	Accession No.	1	2	3	4	5	6	7	8	9	Data source
Helicana japonica	South Korea	1	ON646695										Present study
		2	JX502897	0.002									Unpublished
	Japan	3	AB334553	0.000	0.002								Shih and Suzuki, 2008
Helicana wuana	South Korea	4	JX502899	0.115	0.117	0.115							Unpublished
	Japan	5	AB334551	0.114	0.115	0.114	0.002						Shih and Suzuki, 2008
	China	6	MH593562	0.110	0.112	0.110	0.015	0.014					Yuan et al. 2018
Helicana doerjesi	Taiwan	7	AB334554	0.059	0.061	0.059	0.119	0.117	0.058				Shih and Suzuki, 2008
Helice tridens	Japan	8	AB334548	0.166	0.168	0.166	0.191	0.189	0.164	0.177			
Pseudohelice subquadrata*	Japan	9	AB334557	0.186	0.188	0.186	0.215	0.213	0.184	0.202	0.188		

hardly developed (Figure 1(B and C)). The first and second ambulatory legs comprised a thick pelt of setae, surfaces of the third ambulatory leg with setae, and the fourth ambulatory leg without setal tufts (Figure 1(B)). The first pleopods were slender, stem incurved distally, and swollen in the middle (Figure 1(D)).

In the 658 bp *COI* comparison for molecular identification, the *H. japonica* presented in this study was 100% identical with the Japanese *Helicana japonica* (AB334553) of (Shih & Suzuki (2008) and 99.8% identical with the Korean *H. japonica* (JX502897) (intraspecific variation 0.000 ~ 0.002). Furthermore, comparison with allied species in related genera confirmed a genetic differences (interspecific variation 0.058 ~ 0.215; Table 1). During the blasting process for the molecular comparison, we found that a *Helicana wuana* sequence already registered in NCBI (NC034995/KX334898; Tang et al. 2018) matched ours. However, this data was consequently excluded from the present comparative analysis due to taxonomic uncertainty because: (1) it showed genetic differences from other *H.wuana* sequences (MH593562, JX502899, AB334551); and (2) no morphological description of the specimens was provided.

The complete mitochondrial gene sequence of *H. japonica* is a circular molecule of 16,535 bp consisting of 13 protein-coding genes (PCGs), 2 ribosomal RNA (rRNA) genes, and 22 tRNA genes (Figure 2). The full-length mitochondrial genome sequence was deposited in GenBank accession number ON646695. The overall nucleotide composition was A (33.0%), T (35.6%), C (19.8%), G (11.6%) and GC content (31.4%). Most genes were encoded by the heavy strand, and only *ND4, ND4L, ND1, ND5, 16S rRNA, 12S rRNA tRNA-Gln, tRN-Cys, tRNA-Tyr, tRNA-Phe, tRNA-Pro, tRNA-Leu, tRNA-His, and tRNA-Val* were encoded by the light strand. There are four types of start codons: ATG (*ND4, ND4L, CYTB, COX1, COX2, ATP8, COX3, ND5*), ATT (*ND6, ATP6*), ATC (*ND2, ND3*), ATA (*ND1*). There are also two types of stop codons: TAA (*ND4L, ND6, COX1, ATP8, ATP6, COX3, ND3, ND5*), TAG (*ND4, CYTB, ND2, COX2, ND1*).

The phylogenetic analysis result showed that the family Varunidae is a monophyly group. Within the clade, Varunidae species were subdivided into five clades: *Helice*, *Helicana*, *Eriocheir*, *Chasmagnathus*, and *Pseudohelice*. Similar to the findings of Shih and Suzuki (2008), the phylogenetic tree revealed that *H. japonica* formed a sister clade with *H. wuana*, another *Helicana* species (Figure 3).

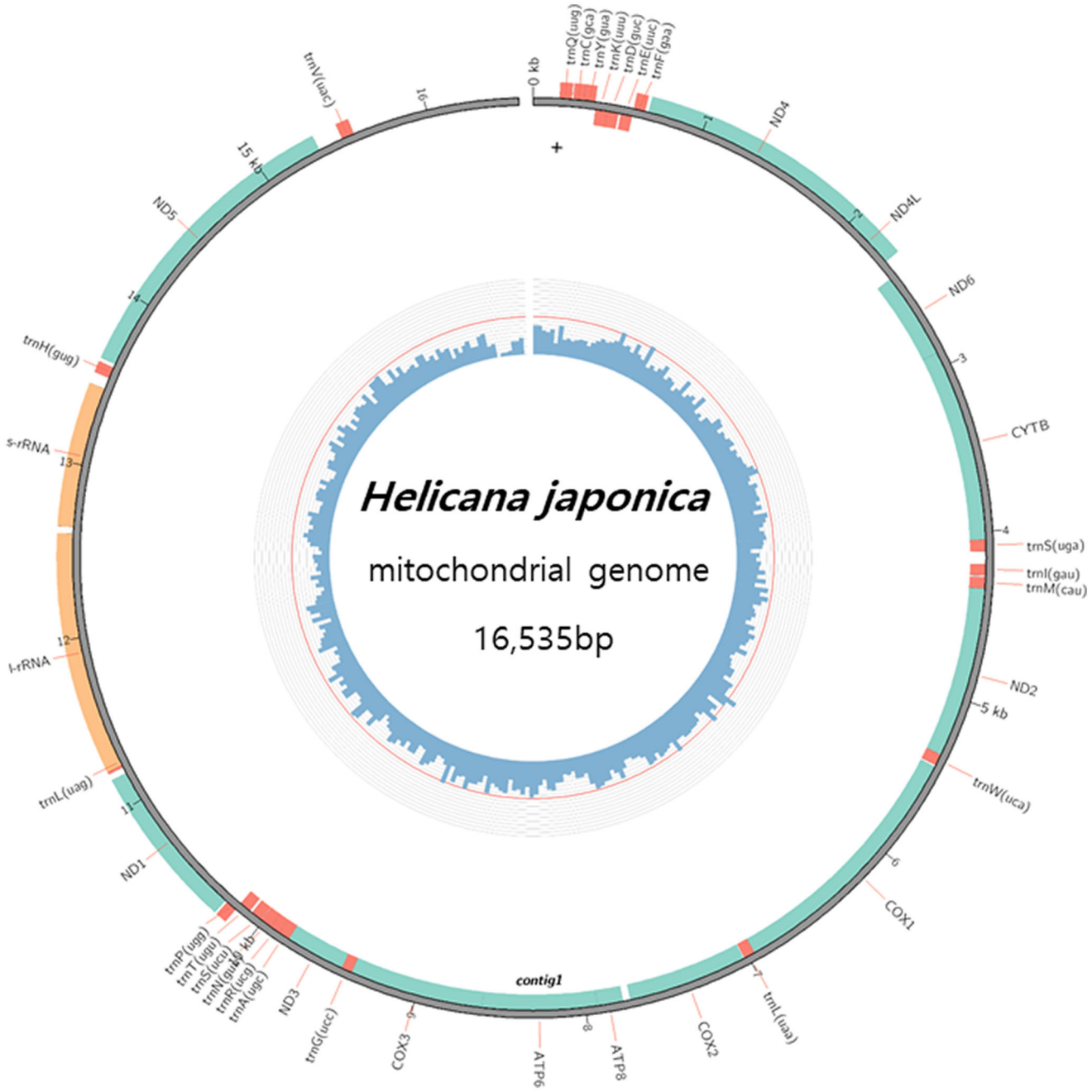

Figure 2. Complete mitochondrial genome circle map of *Helicana japonica*. The genome is 16,535 bp consisting of 13 PCGs, 2 rRNA, and 22 tRNA genes.

Authors' contributions

This study was designed and conceived of by JMK and CHY. JMK and HSK collected and identified the materials. JMK and CHY contributed significantly to phylogenetic analysis and manuscript preparation. HSK was involved in the interpretation of data and critically revised the manuscript for intellectual content. All authors approved the final version to be published and agreed to be accountable for all aspects of this work.

Ethical approval

The samples collected and used in this study did not include any marine organisms under protection, as determined by the Ordinance of the Ministry of Oceans and Fisheries in the Republic of Korea. Therefore, our study was exempted from ethical approval and did not require any permission to conduct it.

Disclosure statement

No potential conflict of interest was reported by the authors.

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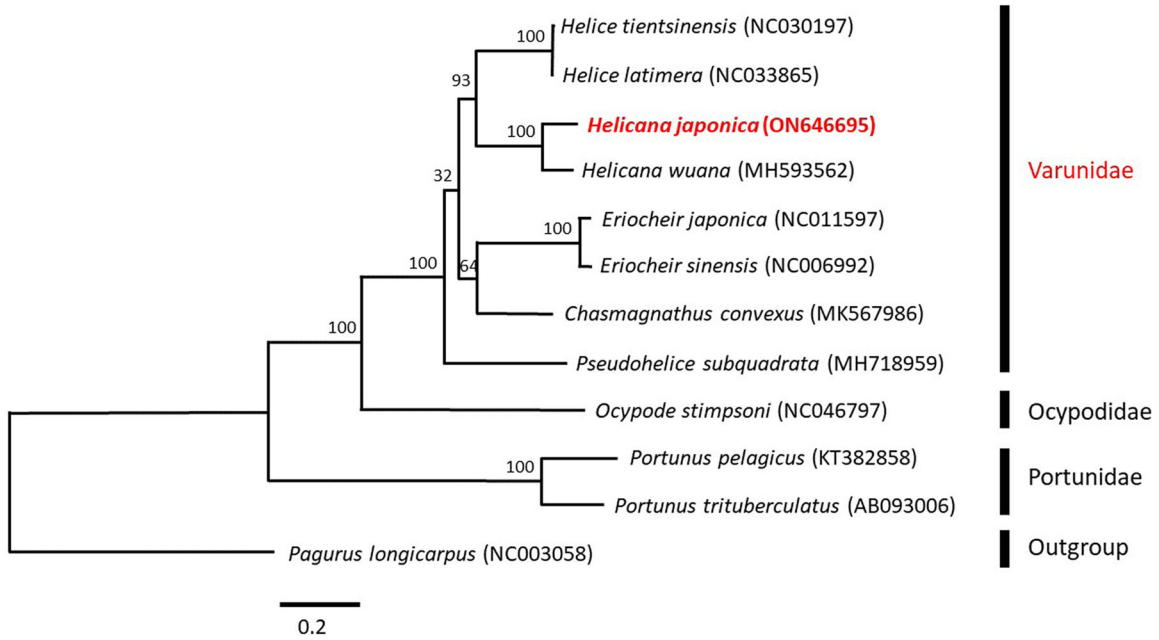

Figure 3. Phylogenetic tree of maximum-likelihood (ML) phylogenetic trees based on the mitochondrial 12 protein-coding genes (PCGs). the values of bootstrap support (ML) is shown the nodes. The following sequences were used: *Helice tientsinensis* NC030197 (identical to KR336555; Xin et al. 2017), *Helice latimera* NC033865 identical to KU589291; Tang et al. 2020), *Helicana wuana* MH593562 (Yuan et al. 2018), *Eriocheir japonica* NC011597 (identical to FJ455505; Wang et al. 2016), *Eriocheir sinensis* NC006992 (Sun et al. 2005), *Chasmagnathus convexus* MK567986 (Guo et al. 2019), *Pseudohelice subquadrata* MH718959 (Kim et al. 2019), *ocypode stimpsoni* NC046797 (identical to MN917464; Kim and Jung, 2020), *Portunus pelagicus* KT382858 (Koolkarnkhai et al. 2019), *Portunus trituberculatus* AB093006 (Yamauchi et al. 2003), and *pagurus longicarpus* NC003058 (identical to AF150756; Hickerson and Cunningham, 2000); (11 species sequence data were downloaded from NCBI).

Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank(https://www.ncbi.nlm.nih.gov/) under the accession no. ON646695. The associated BioProject, SRA, and Bio-Sample numbers were PRJNA785076, SRR17084632, and SAMN23526795, respectively.

Geolocation information

Daesan-eup, Seosan-si, Chungcheongnam-do, Republic of Korea (36°58′6.67″ N, 126°20′14.80″ E).

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