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Research paper

The first complete mitochondrial genome of a Belostomatidae species, *Lethocerus indicus*, the giant water bug: An important edible insect

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ABSTRACT

Lethocerus indicus of the family Belostomatidae is one of the most preferred and delicious edible insects in different parts of South-East Asia including North-East, India. The mitogenome of *L. indicus* represents the first complete mitogenome sequence of a Belostomatidae species in Heteroptera order. The mitogenome of *L. indicus* is 16,251 bp and contains 37 genes including 13 protein coding genes (PCGs), 22 tRNA genes, two rRNA genes, and a large non-coding region. The genome has a typical gene order which is identical to other Heteroptera species. All tRNAs exhibit the classic cloverleaf secondary structure except tRNA*Ser* (AGN). All the PCGs employ a complete translation termination codon either TAA or TAG except *COII*. The nucleotide composition showed heavy biased toward AT accounting to 70.9% of total mitogenome. The overall A + T content of *L. indicus* mitogenome was comparatively lower than some other Heteropteran bugs mitogenomes. The control region is divided into seven different parts which includes the putative stem loop, repeats, tandem repeats, GC and AT rich regions. The phylogenetic relationship based on maximum-likelihood method using all protein coding genes was congruent with the traditional morphological classification that Belostomatidae is closely related to Nepidae. The complete mitogenome sequence of *L. indicus* provides fundamental data useful in conservation genetics and aquaculture diversification.

1. Introduction

Lethocerus indicus (Lepeletier and Serville) of the family Belostomatidae is the largest insect of the order Heteroptera and is commonly known as giant water bug. It is an important edible insect in South-East Asia including North eastern part of India, Manipur in particular, and contributes a bulk share in entomophagy (Meyer-Rochow and Changkija, 1997; Chakravorty et al., 2011). It is used as flavoring and aromatic ingredient in various indigenous food and traditional recipes. The odorants present in the scent gland of *L. indicus* are lipid derivative compounds including the most abundant volatile components, (E)-2-hexenyl acetate and (E)-2-hexenyl butanoate, which give rise to banana-like odors (Kiatbenjakul et al., 2015). This particular insect species contains rich nutrient profile with good proportion of minerals like, calcium, sodium, potassium and iron (Shantibala et al., 2014) which is comparable to that of fish and other animal meat (Gopi and Prasad, 1983). Apart from its use as food, it is also used in traditional medicine. The salivary venom of *L. indicus* is used for treatment of benign tumour on skin (Debaraj et al., 2014). It is also used as an effective biocontrol agent in malaria control against mosquito larvae of *Culex quinquefasciatus* (Wongsiri, 1982).

Encompassing its value as food and medicine, the demand of *L. indicus* is gradually increasing in different parts of North-East India and is exhaustively being captured from its natural habitat. Due to its increasing harvest from nature coupled with unsuccessful captive breeding, the population of *L. indicus* is gradually declining (Wisoram et al., 2013). This poses a serious threat on the existence of this valuable insect unless certain effective measures are taken up to conserve the species. In order to achieve captive breeding as well as to elucidate genetic population relationship, there is a need to infer the phylogenetics of the species. However, as of now, no DNA barcode as well as mitochondrial genome constitution (mitogenome) of *L. indicus* is available that impedes any comprehensive study on the phylogenetic relationship of the species in the order Heteroptera. Currently, only one partial mitogenome of a closely related species (*Diplonychus rusticus*) belonging to Belostomatidae is available (Hua et al., 2009).

Abbreviations: A, adenine; C, cytosine; DNA, deoxyribonucleicacid; EDTA, ethylenediaminetetraacetic acid; G, guanine; HCl, hydrochloric acid; M, molar; mM, millimolar; ML, maximum-likelihood; PCG, protein coding genes; RNA, ribonucleicacid; rRNA, ribosomal RNA; tRNA, transfer RNA; T, thymine.

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Table 1
Organization of *Lethocerus indicus* mitochondrial genome.

Gene	Direction[a]	Location[b]	Size (bp)	Anticodon[c]	Codon		Intergenic nucleotides[d]
					Start	Stop	
tRNA*Ile*	F	505–568	64	GAT (534–536)			0
tRNA*Gln*	R	566–636	71	TTG (598–600)			−3
tRNA*Met*	F	636–704	69	CAT (666–668)			−1
ND2	F	678–1715	1038		ATA	TAA	−27
tRNA*Trp*	F	1714–1779	66	TCA (1745–1747)			−2
tRNA*Cys*	R	1772–1835	64	GCA (1802–1804)			−8
tRNA*Tyr*	R	1836–1899	64	GTA (1867–1869)			0
COI	F	1912–3438	1527		ATA	TAA	12
tRNA*Leu* (UUR)	F	3434–3498	65	TAA (3463–3465)			−5
COII	F	3475–4177	703		ATA	T	−24
tRNA*Lys*	F	4178–4247	70	CTT (4208–4210)			0
tRNA*Asp*	F	4248–4310	63	GTC (4278–4280)			0
ATP8	F	4312–4470	159		ATA	TAA	1
ATP6	F	4464–5144	681		ATG	TAA	−7
COIII	F	5131–5934	804		ATG	TAA	−14
tRNA*Gly*	F	5918–5979	62	TCC (5949–5951)			−17
ND3	F	5995–6345	351		ATA	TAA	15
tRNA*Ala*	F	6332–6395	64	TGC (6361–6363)			−14
tRNA*Arg*	F	6396–6461	66	TCG (6425–6427)			0
tRNA*Asn*	F	6462–6533	72	GTT (6492–6494)			0
tRNA*Ser* (AGN)	F	6534–6600	67	GCT (6560–6562)			0
tRNA*Glu*	F	6601–6663	63	TTC (6631–6633)			0
tRNA*Phe*	R	6662–6727	66	GAA (6694–6696)			−2
ND5	R	6728–8425	1698		ATG	TAA	0
tRNA*His*	R	8436–8499	64	GTG (8467–8469)			10
ND4	R	8500–9831	1332		ATG	TAG	0
ND4L	R	9825–10,055	231		ATA	TAA	−7
tRNA*Thr*	F	10,142–10,206	65	TGT (10,173–10,175)			86
tRNA*Pro*	R	10,207–10,272	68	TGG (10,238–10,240)			0
ND6	F	10,275–10,766	492		ATA	TAA	2
CytB	F	10,766–11,902	1137		ATG	TAG	−1
tRNA*Ser* (UCN)	F	11,901–11,969	69	TGA (11,931–11,933)			−2
ND1	R	12,005–12,946	942		ATA	TAG	35
tRNA*Leu* (CUN)	R	12,932–12,996	65	TAG (12,961–12,963)			−15
16S rRNA	R	12,997–14,256	1260				0
tRNA*Val*	R	14,267–14,336	70	TAC (14,298–14,300)			10
12S rRNA	R	14,337–15,130	794				0
Control region		15,131–16,251; 1–504	1625				0

[a] F: Forward direction along the J-strand; R: Reverse direction along the N-strand.
[b] Nucleotide positions in the major strand.
[c] Values within the parenthesis indicate the anticodon position in the major strand of the mitogenome.
[d] Negative value indicates the bases which are overlapped with the upstream gene.

Mitogenome is widely used to provide information on molecular evolution, phylogenetics and population genetics (Ingman et al., 2000; Miller et al., 2009). Mitogenome contains the largest set of homologous genes that can be compared across animal taxa to draw an effective data for resolving in-depth phylogenetic problems (Cameron et al., 2006; Dellaporta et al., 2006). In this study, we determined the complete mitogenome of *L. indicus* and compared with the mitogenome of other Heteropteran species with regard to genome organization. Phylogenetic analysis was conducted based on the protein coding genes (PCGs) of the mitogenome to gain insight on its phylogenetic status in the family Belostomatidae.

2. Materials and methods

2.1. Sample collection and mitochondrial DNA extraction

We collected 15 adult *L. indicus* from natural population at Khundrakpam (N24°54′, E93°58′), Manipur, India in April 2014. Tissue samples from the thigh region were used for mitochondrial genomic DNA extraction. Thigh tissue of about 30–40 mg was taken aseptically and homogenized in 1 ml homogenization medium (0.32 M Sucrose, 1 mM EDTA, 10 mM Tris HCl) in the Potter-Elvehjem tissue homogenizer (Labcorp, USA). The homogenized tissue was transferred to a sterile 15 ml centrifuge tube and centrifuged at 700 ×*g* for 5 min at 4 °C to remove nuclei and cell debris. The supernatant was collected in 1.5 ml centrifuge tubes and centrifuged at 12,000 ×*g* for 10 min at 4 °C to pellet intact mitochondria. The mitochondrial pellet was suspended in 500 μl of lysis buffer (50 mM Tris HCl, 25 mM of EDTA, 150 mM NaCl), and the lysis of mitochondria was carried out with the addition of 50 μl of 10% SDS and 10 μl of proteinase K (20 mg/ml) followed by incubation at 56 °C for 1 h and 30 min. Finally, the mitochondrial DNA was purified according to the standard phenol- chloroform method (Sambrook et al., 1989). The extracted DNA quality was assessed by electrophoresis in a 1% agarose gel containing ethidium bromide of final concentration 0.5 μg/ml and was quantified spectrophotometrically (Nanodrop ND-1000, NanoDrop Technologies, Inc., Rockland, USA).

2.2. Sequencing and genome annotation

Library construction and sequencing of mitogenome were carried out at Xcelris Genomic, Ahmedabad, India. For library construction, using Illumina TruSeq Nano DNA HT library preparation kit, 200 ng of DNA was used. After fragmenting by ultrasonication with a Covaris M220 (Covaris Inc., Woburn, MA, USA), purified fragments were A-tailed and ligated to sequencing indexed adapters. Fragments with an insert size around 450 bp were selected using sample purification beads supplied in the kits. Size-selected product was PCR amplified to enrich it. The amplified PCR library was loaded and analyzed in