The Complete Mitochondrial Genome of *Limnoria quadripunctata* Holthuis (Isopoda: Limnoriidae)

Rhiannon E Lloyd ^{1*}

Email: rhiannon.lloyd@port.ac.uk

Simon D Streeter ²

Email: simon.streeter@port.ac.uk

Peter G Foster ³

Email: p.foster2@gmail.com

D Timothy J Littlewood ³

Email: t.littlewood@nhm.ac.uk

Jim Huntley ⁴

Email: jim.huntley@colorado.edu

Gregg T Beckham⁶

Email: Gregg.Beckham@nrel.gov

Michael E Himmel ⁵

Email: mike.himmel@nrel.gov

Simon M Cragg ²

Email: simon.cragg@port.ac.uk

¹ Institute of Biomedical and Biomolecular Sciences, University of Portsmouth, Portsmouth PO1 2DT, UK

² School of Biological Sciences, University of Portsmouth, Portsmouth PO1 2DY, UK

³ Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK

⁴ University of Colorado BioFrontiers Institute, Boulder CO, 80309, USA

⁵ Biosciences Center and ⁶ National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO 80401, USA

* Corresponding author. Institute of Biomedical and Biomolecular Sciences, University of Portsmouth, Portsmouth PO1 2DT, UK

Abstract

The complete mitochondrial genome of *Limnoria quadripunctata*, a marine wood-eating isopod crustacean, was determined from whole genome sequence data. The mitogenome is 16 503 base pair in length and contains 39 genes: 13 protein-coding, 2 ribosomal RNA, 22 tRNA, two of which are repeated, and a control region. The start codon most commonly used by the *Limnoria* protein-coding genes is ATN, as is the case in the two other available complete isopod mitogenomes. The gene arrangement differs among these complete isopod mitogenomes, as does the AT-content of H-strand protein-coding genes. The latter observations, coupled with the considerable nucleotide diversity observed between the isopod mitogenomes, support the idea that each isopod species belongs to a distinct lineage as implied by their current placement in separate suborders.

Keywords

gribble, mitogenome, Crustacea

Main text

Limnoria quadripunctata (*Lq*) is a small crustacean that feeds on wood and is a major pest of maritime timber structures. Wood digestion is promoted by endogenous lignocellulose-degrading enzymes that may have properties of value in the biofuel industry (Himmel et al 2007; Kern et al. 2013). The entire *Lq* genome is being sequenced, one aim being to clarify the control mechanisms of this wood digestion system. The complete mitochondrial genome of *Lq* has been assembled from the first 2 GB of sequence data generated (GenBank accession no. KF704000). The average base and read quality scores were >30, with an average depth of coverage of 100 across the entire mitogenome. The size was 16 503-bp (Table 1), more similar to *Ligia ocellata* (*Lo*: the common sea slater) than to *Eophreata* sp. (*Esp*: an Australian freshwater isopod), which were 15 289-bp and 14 994-bp, respectively; the only other two complete isopod mitogenomes (Kilpert et al. 2006, 2010). These mitogenomes all contain the typical metazoan content of 13 protein-coding genes, 2 ribosomal RNA genes and a control region. Furthermore, *Lq* mtDNA has 24 transfer RNAs, as trnV and trnE are repeated, whereas *Lo* has 21 tRNAs and *Esp* has 22 tRNAs (Table 1, Kilpert et al. 2006, 2010). In *Lq*, the protein-coding genes, *cox1*-3, *nad4L*+4, *cytb*, and *nad2*, are predicted to be transcribed from the H-strand; whereas, the genes, *atp6*+8, *nad3*+6, and *nad5*+1, are predicted to be transcribed from the L-strand (Table 1). This arrangement differs from *Lo* (H-strand: *cox1*+2, *atp8*+6, *cox3*, *nad3*+5; L-strand: *cytb*, *nad4*+4L and *nad1*) and *Esp* (H-strand: *cox2*, *atp8*+6, *cox3*, *nad3*+5 and *nad6*, and L-strand: *cox1*, *nad2*+1, *cytb* and *nad4*+4L) (Kilpert et al. 2006, 2010). The AT-content of *Lq* H-strand protein-coding genes is 64.56%, which can be compared to 60.04% in *Lo* and 70.33% in *Esp*. Such positive AT skews are common among Metazoa (Castellana et al. 2011), and suggest that H-strand protein-coding genes of different lineages of isopods mutate at different rates; as is the case for mammals (Reyes et al. 1998). All the isopod protein-coding genes start with ATN codons, except for *cox1* in *Lq* and *atp8* in *Lo*, which use GTG. Other exceptions are *cox1* and *nad6* in *Esp*, which use ACG and TTG, respectively. Protein-coding genes in *Lq*, *Lo* and *Esp* use TAA, TAG or T/TA as termination codons (Kilpert et al. 2006, 2010; Table 1). Nucleotide similarity was variable across the isopod protein-coding genes, with the lowest in *nad6* (43.77%) and highest in *cox1*-3 (ranging from 64.24 to 69.59%), suggesting the latter might be under strong, negative selection and important in mitochondrial respiratory chain function. More nucleotide similarity was observed between *Lo* and *Esp* protein-coding

genes (58.87%), than between *Lq* and *Lo* (55.69%) or between *Lq* and *Esp* (54.93%). The differences in the mtDNA gene content, order and nucleotide similarity supports the current phylogeny that places Limnoriidae in a suborder distinct from the other two isopods (Poore and Bruce 2012).

Table 1. Genes and other regions in the mitochondrial genome of *Limnoria quadripunctata*

Gene/region	Strand	Location	Length (bp)	Start/stop codon
<i>cox1</i>	H	1-1596	1596	GTG/TAA
<i>cox2</i>	H	1614-2273	660	ATC/TAA
<i>trnK</i>	H	2278-2342	65	
<i>cox3</i>	H	2345-3151	807	ATG/TAA
<i>trnV</i>	H	3151-3213	63	
<i>trnA</i>	H	3392-3453	62	
<i>trnE</i>	L	3517-3577	61	
Control region		3578-5102	1525	
<i>trnR</i>	L	5103-5164	62	
<i>rrnL</i>	L	5147-6513	1367	
<i>trnW</i>	L	6472-6527	56	
<i>trnM</i>	L	6546-6604	59	
<i>atp6</i>	L	6609-7271	663	ATG/TAA
<i>atp8</i>	L	7265-7420	156	ATC/TAA
<i>trnD</i>	L	7467-7526	60	
<i>nad3</i>	L	7553-7930	378	TTG/TAA
<i>trnT</i>	L	7907-7971	65	
<i>nad6</i>	L	8026-8535	510	ATT/TAA
<i>trnP</i>	H	8518-8580	63	
<i>nad4L</i>	H	8578-8880	303	ATA/TAA
<i>nad4</i>	H	8868-10195	1328	ATT/TA*
<i>trnH</i>	H	10192-10254	63	
<i>trnF</i>	L	10266-10325	60	
<i>nad5</i>	L	10326-12026	1703	ATA/TAA
<i>cytb</i>	H	12034-13188	1115	ATG/TAG
<i>trnS1</i>	L	13173-13235	63	
<i>trnN</i>	H	13179-13256	78	
<i>trnE</i>	L	13231-13293	63	
<i>trnV</i>	L	13247-13311	65	
<i>trnG</i>	H	13292-13348	57	
<i>nad1</i>	L	13351-14262	912	ATA/TAA
<i>trnL2</i>	L	14278-14341	64	
<i>trnI</i>	H	14394-14458	65	
<i>trnL1</i>	L	14459-14522	64	
<i>rrnS</i>	H	14523-15262	740	
<i>trnS2</i>	H	15269-15323	55	
<i>trnQ</i>	L	15318-15384	67	
<i>nad2</i>	H	15386-16363	978	ATT/TAA

<i>trnY</i>	L	16369-16432	64
<i>trnC</i>	L	16448-16503	56

*TAA inferred to be completed by polyadenylation during post-transcriptional modification

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- Castellana S, Vicario S, Saccone C. (2011). Evolutionary patterns of the mitochondrial genome in Metazoa: exploring the role of mutation and selection in mitochondrial protein coding genes. *Genome Biol Evol.* 3:1067–1079.
- Himmel ME, Ding S-Y, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD. (2007). Biomass recalcitrance: engineering plants and enzymes for biofuels production. *Science* 315(5813): 804–807.
- Kern M, McGeehan JE, Streeter SD, Martin RN, Besser K, Elias L, Eborall W, Malyon GP, Payne CM, Himmel ME, Schnorr K, Beckham GT, Cragg SM, Bruce NC, McQueen-Mason SJ. (2013). Structural characterization of a unique marine animal family 7 cellobiohydrolase suggests a mechanism of cellulase salt tolerance. *Proc Natl Acad Sci U S A.* 110(25):10189-94.
- Kilpert F, Podsiadlowski L. (2006). The complete mitochondrial genome of the common sea slater, *Ligia oceanica* (Crustacea, Isopoda) bears a novel gene order and unusual control region features. *BMC Genomics.* 20;7:241.
- Kilpert F, Podsiadlowski L. (2010). The Australian fresh water isopod (Phreatoicidea: Isopoda) allows insights into the early mitogenomic evolution of isopods. *Comp Biochem Physiol Part D Genomics Proteomics.* 5(1):36-44.
- Poore GC, Bruce NL. (2012). Global diversity of marine isopods (except Asellota and crustacean symbionts). *PLoS One.* 7(8):e43529.
- Reyes A, Gissi C, Pesole G, Saccone C. (1998). Asymmetrical directional mutation pressure in the mitochondrial genome of mammals. *Mol Biol Evol.* 15(8):957–966.