

The complete mitochondrial genome of the stream snail *Clithon retropictus* (Neritimorpha: Neritidae)

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ABSTRACT

The complete mitochondrial genome of the stream neritid *Clithon retropictus* was determined by next-generation sequencing. The mitochondrial genome is 15,814 bp in length, comprising two ribosomal RNA genes, 13 protein-coding genes (PCGs) and 22 transfer RNA genes (tRNAs). Of these 37 genes identified, 7 PCGs and 8 tRNAs are encoded on the heavy strand and the other genes on the light strand. This gene order is consistent with the previously published mitochondrial genomes of the other neritid species. This is the first report of a complete mitochondrial genome sequence for the genus and the fourth for the gastropod subclass Neritimorpha.

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Snails and limpets of the family Neritidae (Neritimorpha: Neritoidea) are commonly found on rocky shores and in brackish to freshwater streams in tropical and subtropical regions worldwide (Kano et al. 2002). A recent phylogenetic reconstruction of the subclass Neritimorpha based on mitochondrial genome data shows that the superfamily Neritoidea is the sister clade of Hydrocenoidea (Uribe et al. 2016). However, relationships among subfamilies and genera within the Neritidae remain largely unresolved due to the scarcity of available molecular data. The neritid species *Clithon retropictus* (Martens, 1878) is found on hard substrates in coastal streams in Japan, South Korea and Taiwan (Noseworthy et al. 2013; Fukumori & Kano 2014, Supplementary material). In this study, we provide the complete mtDNA sequence of *C. retropictus*, which represents the first complete mitogenome for the genus and the fourth within the gastropod subclass Neritimorpha.

A single individual of *C. retropictus* was collected from Kiyose River on Chichijima Island, Ogasawara Islands, Japan (27°5'56"N, 142°11'52"E) in June 2015. The voucher specimen (YK#2717) was deposited in Benthos Laboratory, Atmosphere and Ocean Research Institute, The University of Tokyo. Genomic DNA was extracted from the foot tissue of the snail and shotgun sequencing was performed using a 454 GS Junior Plus System (454 Life Sciences, Roche, CT). A total of 116,318 reads were obtained from the sequencing. The reads associated with mitochondria were identified by a BLAST+ search (Camacho et al. 2009) and assembled by

SeqMan NGen ver. 12.3.1 (DNASTAR, Madison, WI). Four assembled contigs were obtained and the remaining gaps were closed by PCR-based techniques with Sanger sequencing. The complete mitogenome sequence was deposited in the DNA Data Bank of Japan (DDBJ) under the accession number LC127067.

This mtDNA sequence consists of 15,814 bp and contains two ribosomal RNA (12S and 16S) genes, 13 protein-coding genes (PCGs) and 22 transfer RNA genes (tRNAs). The gene order for *C. retropictus* is the same as the previously reported mitochondrial genomes of other neritids (Arquez et al. 2014; Uribe et al. 2016). Of the 37 genes on the mitogenome, seven PCGs and eight tRNAs are encoded on the heavy strand, and the remaining genes are encoded on the light strand. The 16S and 12S genes are located between tRNA^{Leu} and tRNA^{Met}, with respective lengths of 1,298 bp and 860 bp. All PCGs use ATG as the start codon, and TAA, TAG or T- as the stop codon. Gene overlaps are observed between two pairs of genes, NAD5-tRNA^{His} and NAD4-NAD4L, with the overlapped size of 1 and 7 bp, respectively. The length of 22 tRNAs ranges from 64 bp to 72 bp and the overall base composition is 33.9% for T, 14.9% for C, 31.0% for A and 20.2% for G with an AT-bias. The longest intergenic region is located between the tRNA^{Glu} and COIII genes and is considered to be a non-coding control region. Figure 1 shows the phylogenetic position of *C. retropictus* based on the present and previous mitogenome data.

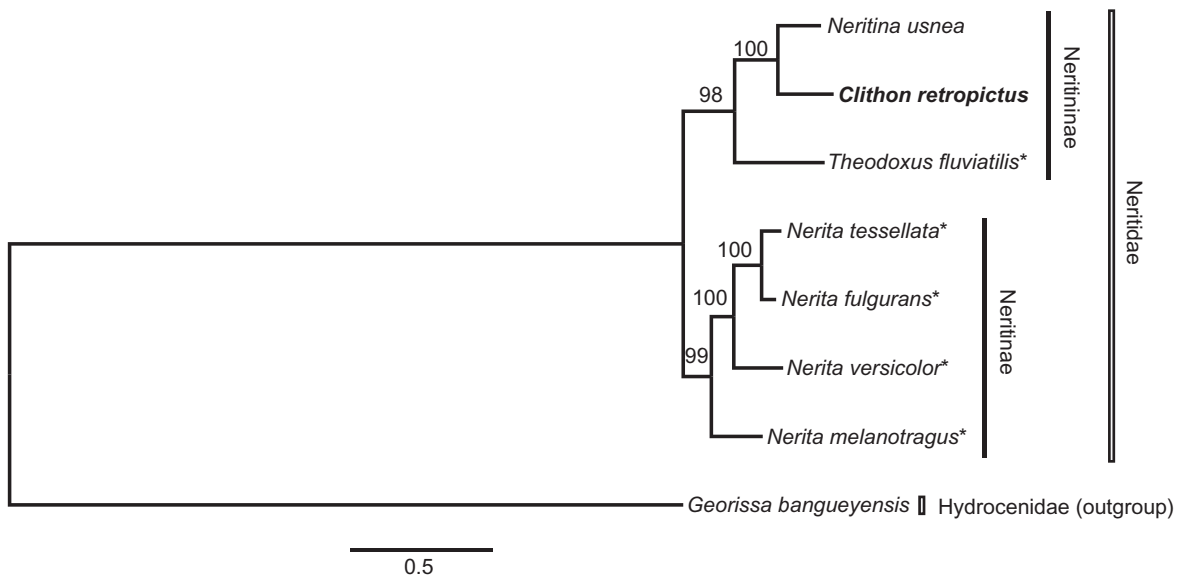


Figure 1. Maximum likelihood phylogeny of Neritidae based on mitochondrial DNA sequences of 13 protein-coding and two ribosomal RNA genes from *Clithon retropictus* (bold, this study; LC127067), *Neritina usnea* (KU342665), *Theodoxus fluviatilis* (KU342667), *Nerita fulgurans* (KF728888), *Nerita melanotragus* (GU810158), *Nerita tessellata* (KF728889), *Nerita versicolor* (KF728890) and *Georissa bangueyensis* (outgroup; KU342664). Asterisks denote species for which a near-complete mitogenome is available. Sequences were aligned separately for each gene using Translator X (for coding genes; Abascal et al. 2010) and MAFFT v7 (rRNA genes; Katoh & Standley 2013) with default parameters. Ambiguously aligned positions were removed using Gblocks v.0.91b (Castresana 2000) with all options for a less stringent selection. Tree reconstruction was performed in RAxML v.7.4.2 (Stamatakis 2006) using GTR + G model; nodal support estimated by 1000 thorough bootstrap replicates. Scale bar represents branch length (substitutions/site). Three limnic taxa of subfamily Neritinae (*Clithon*, *Neritina* and *Theodoxus*) were recovered as a robust clade (bootstrap probability: 98%) and sister to the also monophyletic Neritinae (*Nerita*; 99%).

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Disclosure statement

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

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