

Characterization of the complete mitochondrial genome of *Ficus variegata* (Littorinimorpha: Ficidae) and molecular phylogeny of Caenogastropoda

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ABSTRACT

Ficidae is a family of chiefly tropical marine gastropod mollusks with approximately 20 described species. Hitherto, there are no complete mitochondrial genome (mitogenome) of Ficidae available for the Ficoidea. Here, we determined the complete mitogenome of *Ficus variegata* Röding, 1798 representing the first species from the family Ficidae. The newly sequenced mitogenome consists of 13 protein-coding genes, two ribosomal *RNA* genes, and 22 transfer *RNA* genes. All of 13 PCGs use ATG as initiation codons and end with conventional stop codons TAA and TAG, and the genome organization is similar to those of other documented caenogastropod mitogenomes. Tonnoidea and Ficoidea were recovered as sister group in the Caenogastropoda tree.

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Ficidae is a family of medium to large marine gastropods that found worldwide, chiefly distributed in subtropical and tropical region, living in waters from the shallows to approximately 100 meters deep, it is the only family in the superfamily Ficoidea (Bouchet et al. 2017). Ficidae were previously assigned to superfamily Tonnoidea (e.g. Wenz 1941), until Riedel (1994) removed ficids from this superfamily, because neither conchological characters (including fossils) nor anatomical features reveal a common derived character, and established superfamily Ficoidea. So far, several molecular phylogenies based on partial genomes have recovered the close affinities of Tonnoidea and Ficoidea, as in they were recovered as sister groups on the phylogenetic tree (Hayashi 2005; Zou et al. 2011). The monophyletic clade formed by tonnoideans and ficoideans have been the main reason of neogastropods being polyphyletic. However, no complete mitogenome sequence from the family Ficidae is available in the NCBI database to date, hinder the understanding of the phylogenetic position of Ficidae within Caenogastropoda in the complete mitochondrial genome perspective.

In this study, the complete mitochondrial genome of *Ficus variegata* was determined by next-generation sequencing (NGS) platform. Specimens of *F. variegata* were collected from Hainan province (114°06'56"E, 31°49'25"N), China. After collection, all samples were immediately stored in 100% ethanol and deposited at Ocean University of China (voucher number: FCG20150930; contact information: Qi Li, email: qili66@ouc.edu.cn). The total genomic DNA was isolated from 1 g of foot tissue following the CTAB method modified by Winnepenninckx et al. (1993), the quality of the DNA was

visualized on 1.0% agarose gel. Total genomic DNA was sequenced on an Illumina HiSeq X sequencer using a PE150 protocol. The raw reads were filtered using Trimomatics (Bolger et al. 2014). Short-read DNA sequences were assembled using de novo assembly using Ray (Boisvert et al. 2012) with a *k*-mer of 31.

The complete mitochondrial genome of *F. variegata* (Genbank accession number MW376482) is 15,736 bp in length, consists of 37 genes, including 13 protein-coding genes, 22 *tRNA* genes, and two *rRNA* genes according to the annotation results from MITOS (Bernt et al. 2013) and ORF Finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). All of the PCGs and most of tRNAs are encoded on the heavy strand, while *trnY*, *trnM*, *trnC*, *trnW*, *trnQ*, *trnG*, *trnE*, and *trnT* are located on the light strand. The gene arrangement of *F. variegata* was in accordance with the consensus gene order shared by most caenogastropod mt genomes (Osca et al. 2015), which provide additional evidence that the gene order in caenogastropoda is highly conserved. The nucleotide composition of *F. variegata* mitogenome was clearly biased toward A/T nucleotides, the overall AT content (74.1%) is distinctly higher than the GC content (25.9%), similar to other molluscan taxa reported (Grande et al. 2008). All the protein-coding genes start their open reading frame with ATG, the most common stop codon is TAA, whereas the stop codon TAG is used in *atp8* and *cox3*.

The molecular phylogeny of *Caenogastropoda* was reconstructed based on deduced amino acids of 13 protein-coding genes (Figure 1). A total of 52 mitogenomes that covering three main lineages of Caenogastropoda were selected, two

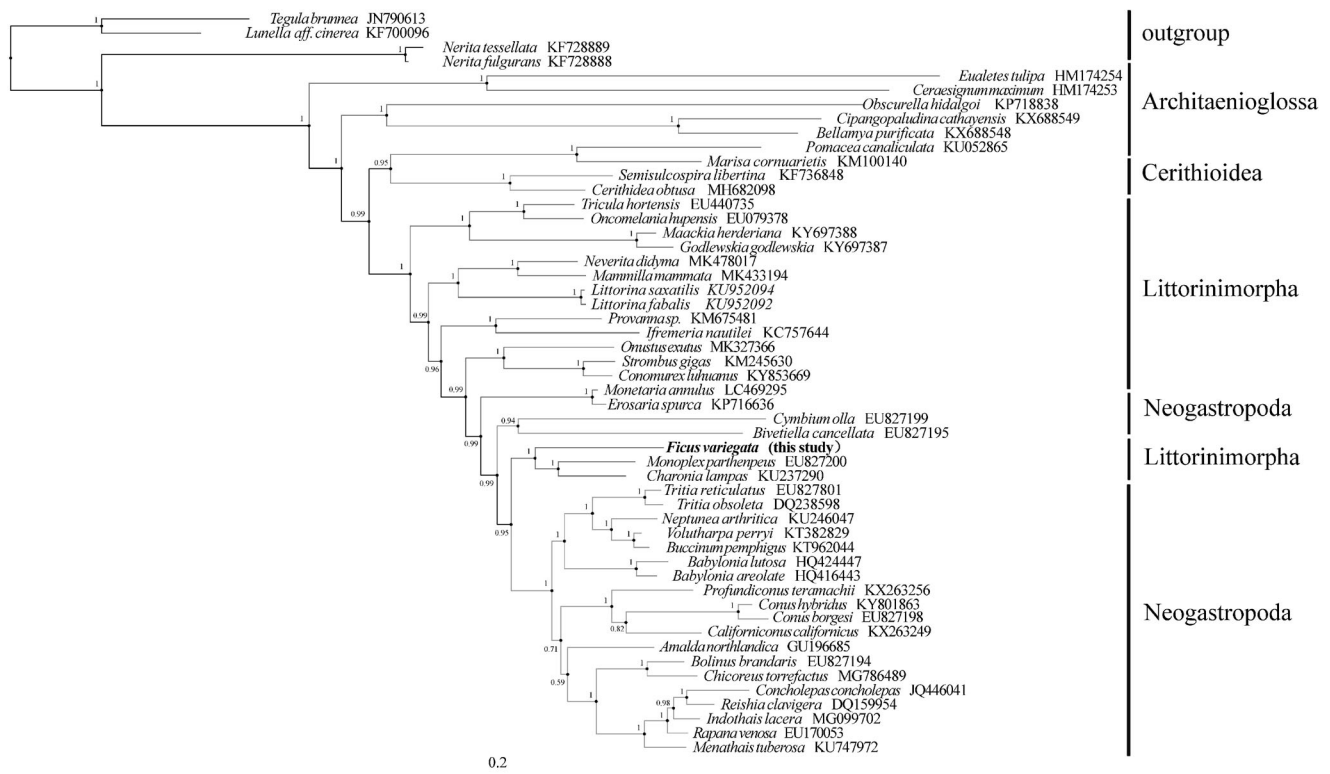


Figure 1. The phylogenetic relationships of *Caenogastropoda* based on deduced amino acids of 13 protein-coding genes using MrBayes version 3.1.2 (Ronquist et al. 2012), run four simultaneous Monte Carlo Markov chains (MCMC) for 10 million generations, sampling every 1000 generations, and discarding the first 25% generations as burn-in to prevent sampling before reaching stationarity.

vetigastropods and two neritimorphs were used as outgroup to root the phylogenetic tree. Tonnoidea and Ficoidea were recovered as sister group with substantial statistical support, which breaks the monophyly of neogastropods, further confirmed that Neogastropoda is a polyphyletic group. Aside from Tonnoidea and Ficoidea, the following Neogastropoda lineages branch off successively: Buccinoidea, Muricoidea, Olivoidea, and Conoidea.

In conclusion, the complete mitogenome of *F. variegata* reported in this study provided important information for the characterization of Ficidae mitogenome, and add additional resolution to the phylogenetic relationship of *Caenogastropoda*.

Disclosure statement

No potential conflict of interest was reported by the authors.

Specimen information

A specimen was deposited at Ocean University of China (<https://www.ouc.edu.cn/>), contact person: Qi Li, email: qili66@ouc.edu.cn under the voucher number FCG20150930.

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Data availability statement

The data that support the findings of this study are openly available in NCBI at <https://www.ncbi.nlm.nih.gov> under the accession number MW376482, or available from the corresponding author. The raw sequence data were deposited in SRA, accession number SRR13484475.

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