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Characterization of the complete mitochondrial genome of *Conus tribblei* Walls, 1977

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Abstract

The genus *Conus sensu lato* consists of 500–700 species. However, the mitochondrial genomes of only few species have been fully sequenced and reported so far. In this study, the complete mitochondrial genome of *Conus tribblei*, a member of the poorly known subgenus *Splinoconus* is sequenced with the mean coverage of 604x. The mitochondrial genome is 15,570 bp long and consists of genes encoding for 13 respiratory chain proteins, 22 tRNA, and 2 rRNA. The gene organization is highly conserved among the *Conus* species. The longest intergenic region between tRNA-Phe and cytochrome c oxidase subunit III (*cox*3), which in *C. tribblei* is 169 bp long and contains a 112-bp long segment of inverted repeat, may represent the putative control region. The control regions of *Conus* species exhibited variability in the length and position of the inverted repeats. Therefore, this region may have the potential to be used as a genetic marker for species discrimination.

Keywords

Mitogenome; control region; conoidea

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Declaration of interest
The specimen used in this study was obtained in conjunction with a collection trip supported in part by ICBG grant #1U01TW008163. This study was supported by a grant to AOL from the Emerging Interdisciplinary Research Program of the University of the Philippines System through the Philippine Genome Center. The data analysis was carried out using the high performance computing systems at the Philippine Genome Center. The mitochondrial genome sequence of *C. tribblei* is deposited in the GenBank database under the accession number KT199301. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Introduction

The genus *Conus* is a large genus, with ~500–700 species. To date, the complete mitochondrial genome sequences have been reported for only few *Conus* species (Bandyopadhyay et al. 2008; Cunha et al. 2009; Brauer et al. 2012). Although the previous studies indicate a high degree of conservation of organization and sequence of the mitochondrial genomes, interesting differences were noted, such as the novel putative control region (CR) in *Conus consors*, apparently absent in the mitochondrial DNA of other *Conus* species (Brauer et al. 2012). In this study, the complete mitochondrial genome of *Conus tribblei* as the first representative of subgenus *Splinoconus* was sequenced and annotated.

Methods

DNA was extracted from the foot tissue of *C. tribblei* specimen collected in the Philippines using DNeasy blood and tissue kit (Qiagen, USA). Three libraries with different insert sizes (170, 500 and 800 bp) were constructed, and each library was sequenced on one lane of Illumina HiSeq2000. The 100-bp long paired-end (PE) reads were filtered by FASTX (Pearson et al. 1997) and error corrected by SOAPec (Luo et al. 2012). The reads were mapped to the mitochondrial genomes of *Conus textile*, *Conus borgesi*, and *C. consors* using Bowtie2 (Langmead and Salzberg 2012). The aligned reads (184,061 PE and 30,037 single-end) were used for *de novo* assembly using ABySS-1.3.7 (Simpson et al. 2009). Open reading frames were identified using ORF finder (Stothard 2000), and tRNAscan (Lowe and Eddy 1997) was used for the identification of tRNAs. Annotation of 12S and 16S rRNA was performed by alignment of the *C. tribblei* mitochondrial genome with the reference genomes using MUMmer3 (Kurtz et al. 2004).

Results

The complete mitochondrial genome of *C. tribblei*, 15,570-bp long, was assembled with the mean coverage of 604x using *k*-mer 57. Thirteen genes encoding proteins of the respiratory chain, 22 tRNA genes and two rRNA genes were identified. The gene organization in the mitochondrial genome of *C. tribblei* and other *Conus* species are highly conserved (Fig. 1). The mitochondrial genome contained two large intergenic spacers: one between *cox*1 and *cox*2 (138 bp), and another between tRNA-Phe and *cox*3 (169 bp). The region between tRNA-Phe and *cox*3 in *C. tribblei* is longer than this region in *C. textile* (126 bp) and *C. borgesi* (127 bp) but shorter than *C. consors* (698 bp). This region in *C. tribblei* consists of a segment of 112 bp with 100% match inverted repeat sequence of 55 bp. This inverted repeat in *C. tribblei* is longer than the inverted repeat in *C. textile* (41 bp), *C. borgesi* (39bp) and the first inverted repeat in *C. consors* (21 bp); but it's shorter than the second inverted repeat in *C. consors* (455bp). This region may represent the putative control region (CR) (Brauer et al. 2012) suggested to play a role in the initiation of replication and transcription (Boore 1999). Our results show that the length of the inverted repeat in CR is variable among *Conus* species. Further survey of this region in other species of *Conus* may reveal the suitability of using this region as a genetic marker for species discrimination.

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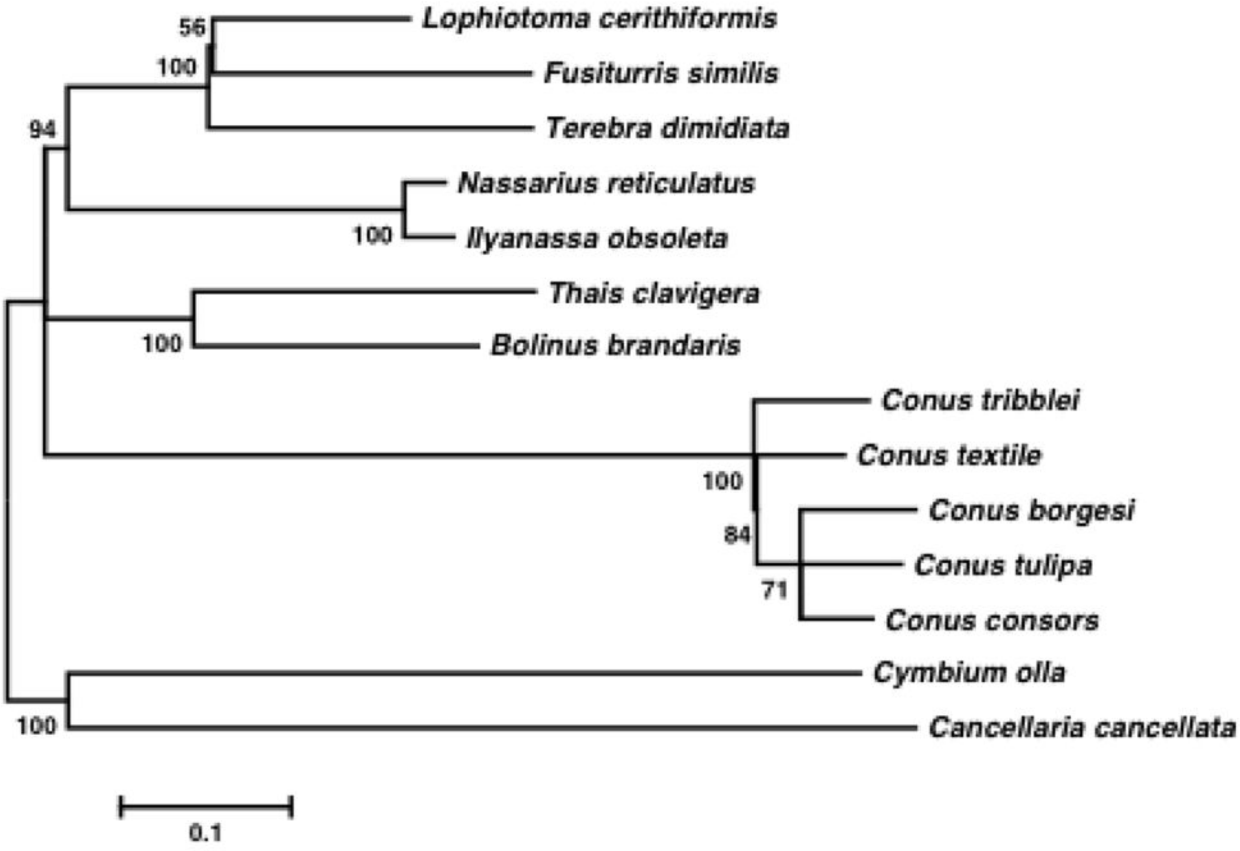

Fig. 1.

Phylogenetic relationship within Neogastropods. The evolutionary history was inferred using the Maximum likelihood method based on the General Reversible Mitochondrial+Freq model from a single concatenated data set of deduced amino acid sequences of 13 mitochondrial protein-coding genes. A discrete Gamma distribution was used to model evolutionary rate differences among cites. The rate variation model allowed for some sites to be evolutionary invariable. The bootstrap values are indicated on the nodes. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011). *Conus tulipa* (KR006970), *Conus borgesi* (EU827198), *Conus consors* (KF887950), *Conus textile* (DQ862058), *Terebra dimidiata* (EU827196), *Fusiturris similis* (EU827197), *Lophiotoma cerithiformis* (DQ284754), *Cancellaria cancellata* (EU827195), *Cymbium olla* (EU827199), *Bolinus brandaris* (EU827194), *Thais clavigera* (DQ159954), *Ilyanassa obsoleta* (DQ238598) and *Nassarius reticulatus* (EU827201).