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Complete Mitochondrial Genome of the Soft-shell clam *Mya arenaria*

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Abstract

We have sequenced and characterized the complete mitochondrial genome of the soft-shell clam, *Mya arenaria*, an important organism for environmental toxicology and aquaculture. *Mya arenaria* is located in the taxonomic order Myoida, which lacks any member with a completely annotated mitogenome. The *M. arenaria* mitochondrial genome is 17 947 bp in length. Like most marine bivalves, the circular mitogenome codes entirely on the heavy strand, with no introns. As with other bivalves, the gene order of the mitochondrion is highly rearranged. The mitogenome contains 12 protein coding genes but ATP8 is missing, consistent with about half of all bivalve genera. Twenty three tRNAs were identified. Phylogenetic analysis shows that *M. arenaria* is related most closely with the bivalves *Sinonovacula constricta*, and *Moerella iridescens*, of the infraclass Euheterodonta (unassigned). This, along with the close grouping of the phylogenetic trees, confirms a close tie between Myoida and Euheterodonta (unassigned).

Keywords

Mitogenome; Bivalvia; Mollusca; Control region; Phylogeny

Gill tissue DNA of a single soft-shell clam from New Hampshire (USA) was extracted with Trizol, processed using TruSeq kit (Illumina), sequenced on a HiSeq2000 (Illumina) at the Vanderbilt Laboratory for Advanced Technologies. Assembly was performed (CLCBio NGS Workbench v.6.0) at the Hubbard Center for Genome Studies at the UNH (N50=2999). The remaining portion was cloned into pGEM-T vector (Promega, Madison, WI, USA), according to manufacturer's specifications, and transfected using electroporation (Micropulser, Bio-Rad) and sequenced by the core facility at Mount Desert Island Biological Laboratory (Salisbury Cove, ME, USA), in a capillary DNA sequencer (Life Technologies). Annotation included: NCBI ORF Finder, DOGMA [1], tRNAScan-SE [2], MITOS (<http://>

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Declaration of interest

The authors have no conflicts of interest to report.

mitos.bioinf.uni-leipzig.de) and ARWEN1.2 (using high stringency settings) [3], MFold v 3.5 using default conditions [4], EMBOSS (<http://emboss.bioinformatics.nl/cgi-bin/emboss/palindrome>), bl2seq and/or with Clustal Omega algorithm [5]. Amino acid coding sequences were subjected to Bayesian Model testing (CLCBio NGS Workbench v.7.0 (Denmark)), alignment (Bioinformatics Research Centre, http://users-birc.au.dk/biopv/php/fabox/alignment_trimmer.php), concatenation, partitioning (MrBayes 3.2) and conversion to NEXUS format (Mesquite v. 3.0) [6]. The proportion of invariant sites was allowed to float. The substitution model selected by model testing (GTR+G+T) was applied in Bayesian Inference analyses (MrBayes v3.1 [7]). With random starting trees, MCMC chains were run for 10⁶ generations and 25% was discarded as burn-in and rerun with random starting trees.

We present the first complete mitogenome from the order Myoida. The soft shell clam, *M. arenaria*, is an economically important, benthic, marine bivalve prevalent in the intertidal shoreline [8–10]. *M. arenaria* has a contemporary distribution that includes Pacific, Atlantic, the North Sea and European waters [11]. *M. arenaria* is a filter-feeder, sensitive to environmental disruption [12]. Mollusks display a striking degree of nucleotide divergence [13], particularly in the ribosomal RNAs [14], so increasing the representation of bivalve sequences in the database has become important [15].

We have generated a completely annotated, published, mitogenome for the soft-shell clam, *Mya arenaria* (Accession # NC_024738.1). The circular *M. arenaria* mitogenome is 17 947 nt, the G + C content 34.3%, with the longest intergenic sequence at 437 nt. It contains twelve protein coding genes (*cox1, nad4* [oneintron], *nad1, cob, atp6, nad5, cox3, nad6, cox2, nad4L, nad2, nad3*), with no conservation of gene order with other mollusks except two consecutive genes matching *Hiattella arctica*. No ATPase 8 gene (*atp8*) was found, though it has been argued that all bivalves contain *atp8*. [16] The longest noncoding region of *Mya arenaria* is a ~845 nt region. This noncoding region is a putative control region [17], based on AT skew (dns) and predicted secondary structure (dns). We detected an LrRNA and SrRNA, but no overlapping genes were found. The majority of the initiation codons were standard codons, with 50% ATA and 42% ATG and a single ATT. *M. arenaria* mtDNA encodes a complete set of tRNAs, along with duplicates for leucine (tRNA^{Leu}), methionine (tRNA^{Met}) and serine (tRNA^{Ser}) (dns). Unlike some other Metazoa [18], both tRNA^{Ser} have intact DHU arms (dns). A trimmed, partitioned Bayesian phylogeny of mitogenome coding sequence shows that *Mya arenaria* shares a common ancestor with *Moerella iridescentis* and *Sinonovacula constricta*.

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Figure 1.

Bayesian phylogeny derived using consensus partitioned amino acid sequences of all protein-coding sequences in the mitogenome (4051 amino acids) from 12 bivalve taxa and one outgroup (*Loligo forbesii*). The alignment was analyzed using Mr. Bayes with GTR exchange rates + gamma distribution + topology variation. The outgroup was removed for clarity. Bar = mean distance (number of amino acid substitutions per site). GenBank Accession Numbers: *Paphia textile* (JF969277.1), *Meretrix lyrata* (KC832317.1), *Coelomactra antiquata* (JN692486.1), *Moerella iridescens* (NC_018371.1), *Sinonovacula constricta* (EU880278.1), *Hiatella arctica* (DQ632742.1), *Quadrula quadrula* (FJ809751.1).