

**"DUI in Bivalves. Transcriptomics, mitogenomics and supranumerary ORFs:  
Functional analysis"  
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Contrary to classical strict maternal inheritance of mitochondrial DNA represented by most of animal species (including humans), mussels of the genus *Mytilus* inherit their mitochondria according to doubly uniparental inheritance model (DUI). In DUI bivalves mitochondrial DNA (mtDNA) is passed in “mother-daughter” and “father-son” manner, where females transmit their mitochondrial DNA (F mtDNA) to both daughters and sons, while males transmit only one of their mtDNA (M mtDNA) exclusively to sons. As a result, males exhibit heteroplasmy with the sequence divergence reaching up to 50 % (~24 % in genus *Mytilus*).

During the last decade, a new F-specific open reading frame (ORF) of considerable 375 base pairs length and presumable functional importance was discovered in mussels mtDNA. Similar open reading frame has been identified in several species from *Mytilus edulis* complex, as well as in the somewhat evolutionary more distant *M. californianus* and *M. coruscus*. This suggests that it is kept in this evolutionary line since at least 13 million years ago. Moreover, it is likely that the homologous ORF also exists in mitogenome of *Musculista senhousia*, a mussel from the Crenellinae subfamily also presenting DUI. These data suggest that there are previously unknown mitochondrial genes that can have key biological functions, such as regulation of mtDNA transmission or sex determination. Presence of additional open reading frames suggests that mitochondrial genomes may perform significantly more functions than expected.

The first part of the thesis shows a novel approach to the research on doubly uniparental inheritance of mitochondria based on data from high-throughput next generation RNA sequencing (NGS RNA-seq). Due to close relationship to the DUI possessing species *Venerupis philippinarium* (common family Veneridae), Chilean clam from genus *Eurhomalea* was selected as a candidate for transcriptomic analysis. Bioinformatic analysis of sequencing data revealed the presence of only a single set of mitochondrial genes (including *atp8*), proving the lack of DUI phenomenon in *E. rufa*.

The second part of the thesis focuses on an additional open reading frame (*forf*) localized in the control region (CR) of the *Mytilus edulis* mitochondrial DNA. This DNA

fragment contains all the necessary structural motifs expected from a protein coding gene: start, stop codon and conserved at species level nucleic acid sequence. Furthermore, the group of homologous open reading frames (in closely related bivalves with DUI) is perceived as candidate for a key element regulating doubly uniparental inheritance of mitochondria. Obtained results based on high-throughput next generation DNA sequencing (NGS DNA- seq), western blot and mass spectroscopy seem to support the hypothesis that this protein is indeed expressed, nevertheless there is still some room left for speculations. Detection of the *forf* was limited to the mantle tissues of male specimens in the initial period of gametes maturation.

The third part deals with the problem of “missing” ATPase 8 subunit gene in mitochondrial genome of *Mytilus edulis* mussels. Publication of the first bivalve mitogenome (i.e. *M. edulis*) in 1992, created a paradigm that some of the bivalves may not possess *atp8* gene coded by mitochondrial DNA. Results obtained *via* western blot technique and BN/SDS-PAGE two-dimensional electrophoresis combined with ATP synthase complex enzymatic activity detection, confirmed the presence and expression of ATPase 8 subunit in both female (F) and male (M) mitochondrial *Mytilus edulis* genomes.