

Exploring the mtDNA *rnl* and *nad4* genes in *Ophiostoma*  
species for novel introns and homing endonucleases

by

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## Abstract

Fungal mitochondria are variable in size due to the presence of potential mobile elements such as group I and group II introns and homing endonuclease genes (HEGs). In this work the mitochondrial large ribosomal subunit gene (*mt-rnl*) of *Ophiostoma ulmi* and related species have been screened for the presence of introns and intron encoded proteins. Five introns have been noted in different regions of the *rnl* gene of *O. ulmi* and related species. Based on this *rnl* survey and *rnl* data from Genbank, an *rnl* intron landscape for ascomycetous and basidiomycetous fungi was generated by using bioinformatic based analysis. A total number of 23 possible intron insertion sites were found in the *rnl* gene of ascomycetous and basidiomycetous fungi. The results also indicate that regions of the *rnl* gene are more prone to intron invasion than others. The second project dealt with the evolution of mitochondrial ribosomal protein S5 (*rps3*) gene within the filamentous ascomycetes fungi. Within members of this group of fungi the *rps3* gene typically is a component of the mL2449 group I intron but there are free-standing forms of *rps3*. The study examined if these free standing forms evolved only once due to an as of yet unknown recombination event or if the *rps3* gene was transferred from the mL2449 intron to a new mtDNA locus several times during the evolution of the filamentous ascomycetes fungi. The third project was to sequence and characterize the intron and HEG found in the mitochondrial NADH dehydrogenase 4 (*nad4*) gene of an undescribed species of *Pesotum*. A 1.4 kb group IC2 intron has been identified in the *nad4* gene of *Pesotum* strain WIN (M)1630. Overall the three studies demonstrate the invasive nature of introns and their associated ORFs and the potential of these introns to influence gene structure and size variation among the fungal mtDNAs.

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## List of Abbreviations

**BLAST:** Basic Local Alignment Search Tool

**bp:** base pair

**DED:** Dutch Elm Disease

**EtBr:** Ethidium Bromide

**HEase:** Homing Endonuclease

**HEG:** Homing Endonuclease Gene

**IEP:** Intron-Encoded Protein

**LHE:** LAGLIDADG Homing Endonuclease

**LSU :** Large Subunit

**LSU rRNA:** Large Subunit ribosomal RNA

**mRNA:** messenger RNA

**mt:** mitochondrial

**mtDNA:** mitochondrial DNA

**NADH:** *Nicotinamide Adenine Dinucleotide* plus Hydrogen flavin adenine dinucleotide

**nad4:** NADH dehydrogenase subunit 4

**NCBI:** National Center for Biotechnology Information

**nt:** nucleotide

**ORF:** Open Reading Frame

**PCR:** Polymerase Chain Reaction

***rnl:*** mitochondrial large subunit ribosomal RNA gene

***rns:*** mitochondrial small subunit ribosomal RNA gene

***rps3:*** ribosomal protein S3 gene

**Rps3:** ribosomal protein S3

**rRNA:** ribosomal RNA

**RT:** Reverse Transcriptase

**RT-PCR:** Reverse Transcription Polymerase Chain Reaction

**SOC:** Super Optimal broth with Catabolite repression

**SSU:** Small Subunit

**SSU rRNA:** Small Subunit ribosomal RNA

**tRNA:** transfer RNA