Phylogenetic Analysis of the Complete Mitochondrial Genome of *Madurella mycetomatis* Confirms Its Taxonomic Position within the Order Sordariales

Wendy W. J. van de Sande*

Department of Medical Microbiology and Infectious Diseases, Erasmus MC, Rotterdam, The Netherlands

Abstract

**Background:** *Madurella mycetomatis* is the most common cause of human eumycetoma. The genus *Madurella* has been characterized by overall sterility on mycological media. Due to this sterility and the absence of other reliable morphological and ultrastructural characters, the taxonomic classification of *Madurella* has long been a challenge. Mitochondria are of monophyletic origin and mitochondrial genomes have been proven to be useful in phylogenetic analyses.

**Results:** The first complete mitochondrial DNA genome of a mycetoma-causative agent was sequenced using 454 sequencing. The mitochondrial genome of *M. mycetomatis* is a circular DNA molecule with a size of 45,590 bp, encoding for the small and the large subunit rRNAs, 27 tRNAs, 11 genes encoding subunits of respiratory chain complexes, 2 ATP synthase subunits, 5 hypothetical proteins, 6 intronic proteins including the ribosomal protein rps3. In phylogenetic analyses using amino acid sequences of the proteins involved in respiratory chain complexes and the 2 ATP synthases it appeared that *M. mycetomatis* clustered together with members of the order Sordariales and that it was most closely related to *Chaetomium thermophilum*. Analyses of the gene order showed that within the order Sordariales a similar gene order is found. Furthermore also the tRNA order seemed mostly conserved.

**Conclusion:** Phylogenetic analyses of fungal mitochondrial genomes confirmed that *M. mycetomatis* belongs to the order of Sordariales and that it was most closely related to *Chaetomium thermophilum*, with which it also shared a comparable gene and tRNA order.

Introduction

*Madurella mycetomatis* is the most common causative agent of human mycetoma, a chronic inflammatory disease, which remains localized and involves subcutaneous tissues, fascia and bones [1]. The disease is characterised by tumefaction, discharging sinuses and the presence of fungal grains [1]. The generic criteria for *Madurella* are primarily based on tissue morphology and overall sterility on mycological media, as well as an invasive potential in human and animal hosts [2]. Since no sexual stage of *M. mycetomatis* has been discovered, the taxonomic classification of *Madurella* has long been a challenge. Especially, since there are no asexual conidia produced nor other morphological and ultrastructural characters which could be of aid in the taxonomic classification. With the development of molecular typing tools, such as sequencing of the nuclear sequences encoding for the internal transcribed spacer (ITS), the beta-tubulin gene and the ribosomal binding protein it became possible to establish the taxonomic place of *Madurella* among the ascomycetes [3,4]. Based on these nuclear sequence data, it appeared that the genus *Madurella*, consisting of five species *M. mycetomatis*, *M. grisea*, *M. pseudomyctomatis*, *M. fahalii* and *M. tropicana*, could be taxonomically differentiated into two different orders, namely the orders Sordariales and Pleosporales [3,4]. The generic type species *M. mycetomatis* belonged to the order of Sordariales together with *M. pseudomyctomatis*, *M. fahalii* and *M. tropicana*, and the genus *Madurella* appeared to be closely related to the genus *Chaetomium* [3,4].

Next to nuclear sequences it is also possible to use mitochondrial sequences for phylogenetic analyses. Mitochondria are considered descendants of an endosymbiotic *ß*-proteobacterium that was engulfed by a eukaryotic or archaeabacteria-like cell more than one billion years ago [5]. The current mitochondrial data points to a single origin of mitochondria with no transfer of mitochondria between different eukaryotes [6]. The mitochondrial DNA present in all mitochondria examined to date is believed to be a remnant of the original endosymbiont’s DNA, with the number of genes contained greatly reduced [5]. In filamentous fungi, the mitochondria are uniparental inherited and their genomes evolve faster than the corresponding nuclear DNA of the fungus [7,8,9]. Fungal mitochondrial genomes encode 5 to 100 genes, with a typical fungal mitochondrial core genome containing 14 conserved protein-coding genes, 2 tRNA genes and a variable number of...
Mitochondrial Genome of Madurella mycetomatis

Materials and Methods

Isolate

*M. mycetomatis* strain mm55, isolated from the lesion of a 22-year-old patient seen in the Mycetoma Research Centre, University of Khartoum, Sudan, was used in this study. Written informed consent was obtained from this patient and ethical clearance was obtained from Soha University Hospital Ethical Committee. This strain was isolated by direct culture of the black grains obtained by a deep biopsy and identified by morphology, PCR-RFLP and sequencing of the ITS region [16]. This strain is used in the only mouse model of eumycetoma in use today and considered the type strain in phylogenetic and antifungal susceptibility testing as well [17,18,19,20]. The strain was maintained on Sabouraud Dextrose Agar (Difco Laboratories, Paris, France) at 37°C. Passage to fresh medium was done on a monthly basis.

DNA Extraction

Three-week-old *Madurella* cultures were scraped from Sabouraud agar plates, frozen in liquid nitrogen and ground with a mortar and pestle. DNA was extracted from the resulting pulp with the Promega Wizard Kit (Promega). To the grind mycelia, 300 μl lysis solution was added and mixed by pipetting gently. From this step onwards, the yeast protocol from the Promega Wizard Kit was used according to the manufacturer's instructions.

Sequencing and Assembling of the Mitochondrial Genome

The genome of *M. mycetomatis* was sequenced using Roche GS junior titanium 454 sequencing according to the manufactures instructions. In short DNA was fragmented by nebulisation to an average fragment length of 600–900 bp after which the fragments were amplified and coupled to capture beads using the emPCR amplification kit Lib-L for the GS Junior Titatium Series (roche). In total 5×10⁶ coupled beads were deposited on the GS junior titanium picotiterplates (Roche) and sequenced. To assemble the mitochondrial genome the GS de novo assembler of Roche was used. The two ends of the assembled sequence were amplified with primers mmmitofw (5′-TCATGGCTTAGATGGTTG-3′) and mmmitorf (5′-GAGCTATAGTGGCTCCTAGT-3′) and resequenced by sanger sequencing to confirm the circular nature of the mitochondrial genome.

Annotation of the Mitochondrial Genome

Open reading frames (ORFs) were searched with CLC sequence viewer version 6.5.1 (CLC bio, Aarhus, Denmark) and annotated manually using the published mitochondrial genomes of *Podospora anserina*, *Sordaria macrospora* and *Neurospora crassa*. For hypothetical proteins a cut off of 100 amino acids was used. Codon usage was determined by using the Sequence Manipulation Suite version 2 (www.bioinformatics.org/sms2/codon_usage.html). tRNAs were identified by using tRNAscan-SE 1.21 [21,22], ARAGORN v1.2 [23], ARWEN [24] and RNAmaseal [25] software programs. A tRNA was determined to be a true tRNA if it was found with at least 2 out of 4 software programs.

Phylogenetic Analysis

To compare the *M. mycetomatis* mitochondrial genome with the genome of other fungal mitochondrial genomes, the amino-acid sequences of the protein-encoding genes *atp6, atp8, atp9, cob, cox1, cox2, cox3, nad1, nad2, nad3, nad4, nad4L, nad5 and nad6* were aligned by clustalW using the Mega 5.05 software package [26]. The sequences of the selected proteins were extracted from the fungal mitochondrial genomes deposited in the GenBank database. The aligned amino-acid sequences were used to construct a maximum likelihood tree with 1000 bootstrap replicates based on the cpREV model using Mega 5.05 [26].

Genbank Accession Number

The mtDNA sequence of *M. mycetomatis* strain mm55 was deposited in GenBank under accession number JQ013502.

Results and Discussion

Genome Organization

The mitochondrial genome of *M. mycetomatis* is a typical circular DNA molecule with a length of 45,590 bp. This mitochondrial genome size is small in comparison with the published mitochondrial genomes belonging to the order of the Sordariales. These genomes range from 64,840 nt (*Neurospora crassa* as stated by the Broad Institute) to 127,206 nt (*Cladophotium thermophilum*) [14,27,28,29]. This difference in genome size is due to the variation in intergenic regions and the presence of hypothetical proteins. In overall, the mitochondrial genome of *M. mycetomatis* is highly compact, with 80% of the genome encoding for structural genes. The genome encodes for the small and the large subunit RNA genes, have a slightly higher G+C content, namely 28.8%. The regions of the encoding RNA genes, have a slightly higher G+C content, namely 28.8%. This is in agreement with values found for other fungal mitochondria [10].

Protein Coding Genes

The *M. mycetomatis* mitochondrial genome has the following genes encoding proteins involved in respiratory chain complexes: ATP synthase subunits 6 and 8 (*atp6* and *atp8*), but not for subunit 9, apocytochrome b (*cob*), the cytochrome c oxidase subunits 1, 2 and 3 (*cox1, cox2* and *cox3*) and NADH dehydrogense subunits 1, 2, 3, 4, L, 5, and 6 (*nad1, nad2, nad3, nad4L, nad5* and *nad6*) (Table 1). Most of these proteins are highly conserved within fungal mitochondrial genomes [13,27,30], only for the *nad* genes and the *atp9* some variation is noted. No nad genes are present in most of the yeasts and in some fungi *atp9* is located in the nuclear genome or
on a different, independent circular molecule, rather than in the mitochondrial genome [12,27,32]. Next to the proteins involved in respiratory chain complexes, the mitochondrial DNA encodes for 5 hypothetical proteins and 6 intronic proteins including ribosomal protein S3 (rps3). Of the 5 hypothetical proteins only hypothetical proteins 1 and 3 do not show any homology with other known genes. For the other hypothetical genes some homology is found at the protein level. Hypothetical protein 2 shows homology with an unnamed protein product with accession number CAA38821, found in the mitochondrion of *Podospora anserina* (e-value: 3e-08, max identity 38%). Hypothetical protein 4 shows homology with YP_003127070, an GIY-YIG endonuclease found in an intronic sequence after exon3 of cox1 gene [27,33]. Hypothetical protein 5 does not show a LAGLIDADG domain in its sequence and therefore probably does not function as a LAGLIDADG endonuclease. Hypothetical proteins 4 and 5 are probably remnants of endonucleases but do not function as endonucleases any more, the precise function, if any, remains unknown.

**Introns**

In the coding genes of the mitochondrial genome of *M. mycetomatis*, a total of 8 introns are found. All introns are group I introns (table 1). Two introns are found in both the large...
| Gene | Start position | Stop position | Length (nt) | Length (aa) | Start-Codon | Stop-codon | Intron Start | Intron Stop | Intron type | Intron Start | Intron Stop | Intron type | Intron Start | Intron Stop | Intron type | Intron Start | Intron Stop | Intron type | Intron Start | Intron Stop | Intron type |
|------|----------------|---------------|-------------|-------------|-------------|------------|--------------|-------------|-------------|--------------|-------------|-------------|--------------|-------------|-------------|-------------|--------------|-------------|-------------|--------------|-------------|-------------|
| trnL  | 5823           | 10438         | 4616        | 6145        | 6271        | IA         | 8269         | 9860        | IA          | 8269         | 9860        | IA          | rps3         | 8493        | 9740        | 1248        | 415          | ATG         | TAA         |
| trnT  | 10459          | 10529         | 71          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnE  | 10655          | 10735         | 81          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnW  | 10655          | 10726         | 72          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnM  | 11299          | 11371         | 73          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnP  | 5574           | 5648          | 75          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| rnl   | 5823           | 10438         | 4616        | 6145        | 6271        | IA         | 8269         | 9860        | IA          | 8269         | 9860        | IA          | rps3         | 8493        | 9740        | 1248        | 415          | ATG         | TAA         |
| trnT  | 10459          | 10529         | 71          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnE  | 10655          | 10735         | 81          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnW  | 10655          | 10726         | 72          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnM  | 11299          | 11371         | 73          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnM  | 11386          | 11458         | 73          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnL  | 11554          | 11638         | 85          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnL  | 11697          | 11769         | 73          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnF  | 11773          | 11845         | 73          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnF  | 11773          | 11845         | 73          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnQ  | 12029          | 12101         | 73          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnH  | 12305          | 12377         | 73          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| trnM  | 12482          | 12553         | 72          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |
| nad2  | 12708          | 14390         | 1683        | 560         | ATG         | TAA        |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |              |
| nad3  | 14319          | 16094         | 1704        | 137         | ATG         | TAA        |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |              |
| cox2  | 16436          | 18502         | 2067        | 250         | ATG         | TAG        |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |              |
| trnL  | 18894          | 18967         | 74          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |              |
| trnR  | 19515          | 19585         | 71          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |              |
| trnR  | 19957          | 20029         | 73          |             |             |             |              |              |             |              |              |             |             |              |              |              |              |              |              |              |              |              |
Table 1. Cont.

| Gene | Start position | Stop position | Length (nt) | Length (aa) | Start-Codon | Stop-codon |
|------|----------------|---------------|-------------|-------------|-------------|------------|
| nad4L | 20252          | 20521         | 270         | 89          | ATG         | TAA        |
| nad5  | 20521          | 24611         | 4091        | 643         | ATG         | TAA        |
| cob   | 26392          | 28848         | 2457        | 395         | ATG         | TAA        |
| x1    | 28913          | 29464         | 552         | 183         | ATG         | TAG        |
| trnC  | 29762          | 29832         | 71          |             |             |            |
| cox1  | 29979          | 34122         |            | 566         | ATG         | TAG        |
| x2    | 34251          | 34715         | 465         | 154         | ATG         | TAA        |
| x3    | 34968          | 35975         | 1008        | 335         | ATG         | TAA        |
| x4    | 37164          | 38372         | 1209        | 402         | ATT         | TAA        |
| nad1  | 38765          | 39880         | 1116        | 371         | ATG         | TAA        |
| nad4  | 40576          | 42006         | 1431        | 476         | ATG         | TAA        |
| atp8  | 42159          | 42311         | 153         | 50          | ATG         | TAA        |
| atp6  | 42703          | 43491         | 789         | 262         | ATG         | TAA        |
| x5    | 44033          | 44881         | 849         | 282         | ATG         | TAA        |

Genomic organization of the mitochondrial genome of *Madurella mycetomatis*. In this table the start and stop positions and the lengths of the resulting nucleotide and amino acid sequences are shown for of all genes, tRNAs, introns and intronic proteins. For the introns the group and subgroups are also stated. The classification of these introns are based on the conservation of the core sequences and structural motifs as indicated by Michel et al. [48].

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ribosomal subunit (intron IA) and in \( \text{cox1} \) (both intron IB). Single introns are found in \( \text{cob} \) (intron ID), \( \text{cox2} \) (intron IC2), \( \text{nad3} \) (intron IC2) and \( \text{nad5} \) (intron IA) (table 1). Group I introns are considered to be mobile genetic elements which interrupt protein-coding and structural RNA genes [34]. One of the features of group I introns is that they themselves are often invaded with smaller genes that encode mobility-promoting activities that enables the DNA element to move within and between genomes, usually so-called homing endonucleases [34]. In the \( M. \text{mycetomatis} \) mitochondrial DNA we find five intronic proteins, located in the introns of \( \text{cob} \), \( \text{cox1} \) (in each intron one), \( \text{cox2} \) and \( \text{nad3} \) which encode for such homing endonucleases and one intronic protein which encode for ribosomal protein S3 (rps3). Of the four families of homing endonuclease proteins only endonucleases with the conserved amino acid sequence motifs LAGLIDADG (intron proteins 1, 2, and 4) and GIY-YIG (intron proteins 3 and 5) are found. The endonuclease assignment was supported by BLAST analysis and motif identification using PFAM. LAGLIDADG homing endonucleases are found in two forms: a single LAGLIDADG motif that dimerizes and double-motif forms derived from a gene fusion event between two monomeric forms [35]. The endonucleases found in the \( M. \text{mycetomatis} \) mitochondrial genome are all with double-motif forms.

### Intergenic Regions

The presence of putative mitochondrial promoters are detected by comparison of the only promoter from the Sordariales, the \( \text{Neurospora crassa} \) sequence TTAG(A/T)RR(G/T)(G/C)N(A/T) [11,36,37]. Two putative promoter sequences are located within the intergenic regions and close to the 5′ end of coding genes, namely TTAGAATCTT (15885–15896) and TTAGTGGTCTA (36265–36276). Putative promoter sequence TTAGAATCTT is located 551 bp for the 5′ end of \( \text{cox2} \), while putative promoter sequence TTAGAATCTT is located 899 bp for hypothetical protein 4. Both putative promoter sequences are preceded by a 15–23 bp long AT-rich region, as is also described for other fungal species, thus strengthening the hypothesis that these sequences may indeed be mitochondrial promoters [11,37].

### Genetic Code and Codon Usage

Using the genetic mould mitochondrial code from NCBI (translation table 4), the codon usage of the \( M. \text{mycetomatis} \) mitochondrial ORFs is determined. Of the 23 ORFs, only the \( \text{cox2} \) and hypothetical protein 3 starts with the ATT initiation codon, all other genes start with the ATG initiation codon (table 1). Most of the ORF end with the in preferred TAA stop-codon, only 5 ORFs (\( \text{cox1}, \text{cox2}, \text{cox3}, \) hypothetical protein 1 and intronprotein 3) end with the TAG stop-codon [38]. As is also found for other fungi, the most frequently used amino acid in the 23 protein genes is leucine followed by isoleucine (table 2) [11]. As seen in table 2, the codon usage in \( M. \text{mycetomatis} \) mitochondrial ORFs shows a strong bias towards codons ending with a U or A since 86.8% of

### Table 2. Codon usage in protein coding genes of \( M. \text{mycetomatis} \) mitochondrial genome.

| AA | codon | % | AA | codon | % | AA | codon | % | AA | codon | % | AA | Codon | % |
|----|-------|---|----|------|---|----|------|---|----|------|---|----|-------|---|
| A  | GCG   | 0.22 | H  | CAU  | 1.31 | P  | CCG  | 0.09 | S  | UCU  | 3.11 |
| A  | GCA   | 1.46 | H  | CAC  | 0.36 | P  | CCA  | 0.64 | S  | UCC  | 0.26 |
| A  | GCU   | 2.43 | I  | AUA  | 6.07 | P  | CCU  | 2.48 | T  | ACG  | 0.15 |
| A  | GCC   | 0.26 | I  | AUA  | 4.23 | P  | CCC  | 0.10 | T  | ACA  | 2.11 |
| C  | UGU   | 0.61 | I  | AUC  | 0.56 | Q  | CAG  | 0.22 | T  | ACU  | 2.73 |
| C  | UGC   | 0.10 | K  | AAG  | 0.75 | Q  | CAA  | 1.90 | T  | ACC  | 0.16 |
| D  | GAU   | 2.92 | K  | AAA  | 5.17 | R  | AGG  | 0.07 | V  | GUG  | 0.44 |
| D  | GAC   | 0.45 | L  | UUG  | 0.85 | R  | AGA  | 2.23 | V  | GUA  | 2.78 |
| E  | GAG   | 0.82 | L  | UUA  | 9.04 | R  | CGG  | 0.06 | V  | GUU  | 2.16 |
| E  | GAA   | 3.38 | L  | CUG  | 0.26 | R  | CGA  | 0.11 | V  | GUC  | 0.17 |
| F  | UUU   | 5.41 | L  | CUA  | 1.01 | R  | CGU  | 0.42 | W  | UGG  | 0.14 |
| F  | UUC   | 1.44 | L  | CUC  | 1.46 | R  | CGC  | 0.02 | W  | UGA  | 1.11 |
| G  | GGC   | 0.50 | L  | CUC  | 0.11 | S  | AGU  | 3.31 | Y  | UAU  | 4.36 |
| G  | GGA   | 2.08 | M  | AUG  | 2.10 | S  | AGC  | 0.44 | Y  | UAC  | 0.75 |
| G  | GGU   | 3.22 | N  | AAU  | 5.68 | S  | UCG  | 0.15 | Stop UAG | 0.06 |
| G  | GGC   | 0.11 | N  | AAC  | 0.84 | S  | UCA  | 1.79 | Stop UAA | 0.23 |

The percentage codon used in the protein encoding regions of atp6, atp8, cob, cox1, cox2, cox3, nad1, nad2, nad3, nad4, nad4L, nad5, nad6, hypothetical protein 1, hypothetical protein 2, hypothetical protein 3, hypothetical protein 4, hypothetical protein 5, rps3, intron protein 1, intron protein 2, intron protein 3, intron protein 4 and intron protein 5 is depicted.

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### Table 3. tRNAs identified in the genome of \( M. \text{mycetomatis} \).

| AA | Anticodon | AA | Anticodon | AA | Anticodon |
|----|-----------|----|-----------|----|-----------|
| A  | UGC       | I  | GAU       | P  | UGG       | UCA* |
| C  | GCA       | K  | UUU       | Q  | UUC       | GUAA|
| D  | GUC       | L  | AAG       | R  | ACG       |     |
| E  | UUG       | L  | UAA       | R  | UCU       |     |
| F  | GAA       | L  | UAG*      | S  | UGA       |     |
| G  | UCC       | M  | CAU**     | T  | UGU       |     |
| H  | GUG       | N  | GUU       | V  | UAC       |     |

*two tRNAs with the same anticodon were found.  **three tRNAs with the same anticodon were found.

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the codons ends with these bases. The tendency for the A and U residues in the wobble position has also been observed in other fungal genomes [12,39,40,41,42]. As expected due to the high AU content of the mitochondrial genome, the preference of A and U residues is also noted in the overall codon use. The most frequently used codons consist only of Us and As and were UUA (9.04%), AUA (6.07%), AAU (5.68%), AUA (5.17%), UAU (4.36%) and AUU (4.23%) (table 2). The least frequently used codons, CGC (0.02%), CGG (0.06%), CGG (0.06%), AGG (0.07%) and CCC (0.10%), are the codons which consist mainly of Cs and Gs (table 2).

**tRNAs**

In the *M. mycetomatis* mitochondrial genome 27 tRNAs are identified which clustered roughly in three groups (figure 1). Among the tRNAs all amino-acids are accounted for, but for some amino-acids multiple tRNAs are found (table 3). There are two tRNAs with different anticodons for arginine, four tRNAs with three different anticodons for leucine, three tRNAs with the same anticodon for methionine and two tRNAs with the same anticodon for tryptophane. All tRNAs have a cloverleaf structure except the tyrosine tRNA and the leucine tRNA with anticodon AAG, they have a TV-loop and D-loop structure respectively.

**Phylogeny and Comparative Genomics**

With the exception of the group of yeast that are lacking NADH genes, all other fungal mtDNAs contain the same essential functional genes [11]. Therefore, the sequences of these 14 conserved protein encoding genes, as well as the mitochondrial organization of these genes can be used to determine the relations between different fungal species. Amino acid sequence of 14 protein coding genes in the mitochondrial genomes of *M. mycetomatis* and 20 other fungi are used for phylogenetic tree construction (figure 2). Most nodes in this tree have high bootstrap values which indicate the robustness of the tree computed. As found by others, the mitochondrial genomes of...
Figure 3. Mitochondrial gene order of 5 members of the order Sordariales. The gene order of the protein-encoding genes, atp6, atp8, atp9, cob, cox1, cox2, cox3, nad1, nad2, nad3, nad4, nad5, nad6 and nad8 are shown for S. macrospora, N. crassa, M. mycetomatis, C. thermophilum and P. anserina. For the first four species, the positions of the tRNA genes are also depicted by using their one letter amino acid code.

the yeast species cluster apart from the mitochondrial genomes obtained from filamentous fungi [10]. As is seen in figure 2, M. mycetomatis clusters amongst other species of the order Sordariales with high bootstrap support. Placing M. mycetomatis in the order Sordariales is in line with previous observations based on the nuclear sequences SSU, ITS, betatubulin 2 and ribosomal binding protein 2 [3,4]. Based on an extensive phylogenetic comparison of the SSU rDNA sequence of M. mycetomatis with that of 157 other members of the Ascomycota belonging to the orders Chaetothyriales, Diaporthales, Dothideales, Eurotiales, Halosphaeriales, Hypocreales, Lecanorales, Leotiales, Microascales, Onygenales, Ophiostomatales, Pezizales, Pleosporales, Sordariales, Taphriniales and Tuberales it appeared that M. mycetomatis clustered among the members of the order Sordariales while M. grisea clustered among the members of the order Pleosporales [3]. In order to determine the phylogenetic place of M. mycetomatis within the order Sordariales, the ITS, betatubulin 2 and ribosomal binding protein 2 were also sequenced and compared to 39 members of the order Sordariales. In this latter study it appeared that M. mycetomatis was most closely related to M. tropicana, M. pseudomyctomatis and M. fahalli, but that the genus Madurella itself was most closely related to the genus Chaetomium [4]. This close relatedness to the genus Chaetomium is confirmed in this study. Based on the phylogenetic comparisons made with the mitochondrial sequence, it appears that the closest relative of M. mycetomatis is C. thermophilum.

The relatedness amongst the order Sordariales is further studied by comparing the mitochondrial organization of M. mycetomatis to the 4 complete fungal mtDNA sequences belonging to the order Sordariales. Comparable to the high similarity in amino-acid sequence and the uniform mtDNA genome organization found for dermatophytes belonging to the order Onygenales [10], the mitochondrial genome organization found for the order Sordariales is apparently also uniform (figure 3). The only exception is the mitochondrial genome organization of P. anserina, which differs from the genome organization of the other members of the order Sordariales (figure 3). This marked difference has been noted in the past, and led to the conclusion that the mitochondrial gene order in the order Sordariales was apparently quite diverse [11]. Here it is shown, that for most mitochondrial genomes in the order Sordariales this is not the case. More mitochondrial genomes are needed for the order Sordariales to determine if the gene order is indeed similar and that P. anserina is the exception, or that the gene orders are in overall more diverse within this order. When comparing the different genome organizations it appears that the genome organization of M. mycetomatis is most closely related to that of C. thermophilum (figures 3), which only differed in the presence of the gene atp9 between nad5 and cox2 in C. thermophilum and its absence in M. mycetomatis. Next to having the same gene order, the tRNA clustering in the order Sordariales is similar. Again the tRNA order of M. mycetomatis resembles that of C. thermophilum the most (figure 3). Combining the phylogenetic data, the gene order and the tRNA order it appears that the mitochondrial genome of M. mycetomatis is most closely related to the mitochondrial genome of C. thermophilum. Fungi belonging to the order Sordariales are mostly soil- and dung-inhabiting fungi [43]. N. crassa is usually found in or on burned vegetation and the soil, while het natural habitat of S. macrospora P. anserina and C. thermophilum is mainly the soil and herbivore dung [15,44,45,46]. Although DNA of M. mycetomatis has been shown to be present in soil and on thorns in the endemic area, nobody has been able to culture M. mycetomatis directly from these niches [47]. Therefore the natural habitat of M. mycetomatis still needs to be confirmed. Based on this and other studies, it is demonstrated that M. mycetomatis clusters within the order Sordariales, therefore the natural habitat of M. mycetomatis might be sought on similar substrates. To discover the natural niche of this fungus could lead to strategies in the prevention of this mutilating disease.
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