Trichoderma Koningiopsis (Hypocreaceae) has the Smallest Mitogenome of the Genus *Trichoderma*

María Lorena Castrillo (✉ mlc_827@hotmail.com)

Universidad Nacional de Misiones Facultad de Ciencias Exactas Químicas y Naturales

<https://orcid.org/0000-0002-4999-1672>

Gustavo Angel Bich

Universidad Nacional de Misiones Facultad de Ciencias Exactas Químicas y Naturales

Pedro Dario Zapata

Universidad Nacional de Misiones Facultad de Ciencias Exactas Químicas y Naturales

Mario Carlos Nazareno Saparrat

National University of La Plata: Universidad Nacional de la Plata

Laura Lidia Villalba

Universidad Nacional de Misiones Facultad de Ciencias Exactas Químicas y Naturales

Research

Keywords: *Trichoderma*, Biocontrol agent, Mitochondrial, Genome, NGS, Hypocreales, Phylogenetic relationships.

Posted Date: May 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-547419/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

The complete mitochondrial genome sequence of the mycoparasitic fungus *Trichoderma koningiopsis* was determined using the Illumina next-generation sequencing technology. We used data from our recent Illumina NGS-based project of *T. koningiopsis* genome sequencing to study its mitochondrial genome. The mitogenome was assembled, annotated, and compared with other mitogenomes. *Trichoderma koningiopsis* strain POS7 mitogenome is a circular molecule of 27,560 bp long with a GC content of 27.70%. It harbours the whole complement of the 14 conserved mitochondrial protein-coding genes (PCG) such as *atp6*, *atp8*, *atp9*, *cox1*, *cox2*, *cox3*, *cob*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*, also found in other Hypocreales. The mitogenome also contains two ribosomal RNA genes and 26 transfer RNA genes (tRNAs), 5 of them with more than one copy. Other genes also present in the assembled mitochondrial genome are a small rRNA subunit and a large rRNA subunit containing ribosomal protein S3 gene. A phylogenetic analysis was done using the 14 PCGs genes of *T. koningiopsis* strain POS7 mitogenome to compare them with those from other fungi of the Subphyla Pezizomycotina and Saccharomycotina. *T. koningiopsis* strain POS7 was clustered together with other representatives of *Trichoderma* lineage, within the Hypocreales group, which is also supported by previous phylogenetic studies based on nuclear markers. The characterization of mitochondrial genome of *T. koningiopsis* contributes to the understanding of phylogeny and evolution of Hypocreales.

Introduction

Mitochondria (mt) use electron transport coupled with oxidative phosphorylation to generate ATP and these organelles also participate in several other cellular important functions [1]. Mitochondrial DNA or mitochondrial genome, also known as mitogenome, is made up of genes that encodes a small number of proteins whose mRNAs are translated by a distinctive mitochondrial protein-synthesizing system (Burger et al. 2003; Kwak 2020). Evolutionarily mitogenome exhibit remarkable variation in conformation, size, actual gene content, arrangement and expression (Gray et al. 1999; Li et al. 2019). Within the fungi mitogenome can exist as either linear or circular molecules (Hausner 2003; Medina et al. 2020).

Many fungi show similar morphological features, which are broadly dependent upon culture conditions, and so pleomorphic, which usually difficult their identification using only traditional morphological characters. Therefore, a fungal natural classification must be including phylogenetic tools. However, these taxonomic analyses of fungi using phylogenetic inferences need to be combined with several molecular markers, such as those from the mitogenome, which can be a good complement (Chen et al. 2019).

Mt contain their own genome, and they may provide adequate information for phylogenetic studies based on their comparisons with ones from other reference taxa (Ghikas et al. 2006; Kanzi et al. 2016). However, fungal mitogenomes have been less studied than their animal or plant counterparts, limiting a complete understanding of their genetic characteristics and evolutionary histories (Aguileta et al. 2014).

Recently, development of high-throughput sequencing technologies and related bioinformatics tools have allowed considerably greater numbers of nucleotides to be characterized and the genome-scale data to be more easily assembled. Compared to the whole-genome data, mitogenomes are more readily sequenced with broader taxon sampling and a reasonable cost (Song et al. 2020). The number of complete filamentous fungal mitogenome sequences has significantly increased facilitating evolutionary and systematic studies (Mardanov

et al. 2014). Fungal mitogenomes typically contain 15 standard protein-coding genes, two rRNA genes and a variable number of tRNA genes. These protein-coding genes are *atp6*, *atp8*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, and *rps3* (Lang 2018), and some of them may be absent from certain fungal mitogenomes (Zhang and Zhang 2019).

The independent inheritance from nuclear genomes, a high copy number, and several available molecular markers has made the mitogenomes widely used as a tool for studying evolution, phylogenetics, population genetics, and comparative or evolutionary genomics for some fungi (Van de Sande 2012; Chen et al. 2019; Li et al. 2020; Wang et al. 2020).

Fungi belonging to the *Trichoderma* genus (Hypocreaceae, Hypocreales, Sordariomycetes, Pezizomycotina, Ascomycota) are ubiquitous inhabitants in different habitats, being dominate in a broad spectrum of soils and/or decaying organic matter. Species of this genus are economically important, in part because of their mycoparasitic ability, but also as they can stimulate plant resistance, and plant growth and development which increase crop production. Among these uses, *Trichoderma koningiopsis* is reported as a fungal species that can be used in biocontrol and other biotechnological uses (Castrillo et al. 2015; Samuels et al. 2006; Amerio et al. 2020).

Trichoderma whole genome analyses have been reported previously (Halliwell and Griffin 1973; Antal et al. 2002; Chambergro et al. 2002; Martinez et al. 2008; Kubicek et al. 2011; Studholme et al. 2013; Steindorff et al. 2014; Xie et al. 2014; Baroncelli et al. 2015, 2016; Shi-Kunne et al. 2015; Castrillo et al. 2017; Proctor et al. 2018). However, in spite of the extensive studies on biocontrol and nuclear genes of *Trichoderma* genus, information about its mitogenomes remains largely unknown. To date, even if *Trichoderma* genus has more than one hundred known species, only five mitogenomes are available, including *Trichoderma asperellum*, *Trichoderma atroviride*, *Trichoderma gamsii*, *Trichoderma hamatum*, and *Trichoderma reesei* (Kwak 2020). The characteristics of mitogenomes belonging to different representatives of *Trichoderma* genus are needed to elucidate the understanding of the evolution and potential technological use of specific isolates of this genus. The objectives of this work were to determine the complete mitogenome sequence of the *T. koningiopsis* POS7 strain and to compare its mitogenome organization with that of other known mitogenome from Subphylum Pezizomycotina.

Materials And Methods

Strain

T. koningiopsis strain POS7 (Hypocreaceae) was isolated from soil samples (Posadas, Misiones, Argentina) (Castrillo et al. 2017). *T. koningiopsis* POS7 was sequenced, NCBI ID: 337941, Bioproject PRJNA356137, Biosample SAMN06106985, WGS MRBD 00000000, ITS reference sequence KT030879, and was deposited at the Universidad Nacional de Misiones under accession number LBM116.

Mitochondrial genome assembly

In this study, the mitogenome of *T. koningiopsis* POS7 was determined from HiSeq Illumina reads data from whole genome sequencing of *T. koningiopsis* POS7 were used (Castrillo et al. 2017). All reads (7,773,936 reads) were used to map and assemble by comparing with mitochondrial DNA of other species of *Trichoderma* using

the software Geneious 9.1.8 (Kearse et al. 2012). The reported mitogenomes of *T. gamsii* and *T. asperellum* (NC_030218 and NC_037075, respectively) were used as reference to map reads and assemble the mitogenome of *T. koningiopsis*. A total of 88,833 reads were retrieved from the complete genome sequencing project to assemble the mitogenome sequence. The contigs were manually curated into the final single genomic sequence. A single mitochondrial contig was extracted from the assembly for further analyses.

Mitochondrial genome annotation

Initial open reading frames were identified using ORF finder (<https://www.ncbi.nlm.nih.gov/orffinder/>) using the mold mitochondrial genetic code and Geneious 9.1.8 program. The protein-coding genes, rRNA, and tRNA genes of *T. koningiopsis* were predicted and curated manually based on comparisons with previously published *Trichoderma* mitogenome.

This initial annotation was helped using MFannot online tool (https://megasun.bch.umontreal.ca/cgi-bin/dev_mfa/mfannotInterface.pl) based on Genetic Code 4 (Molds). The tRNAs genes were also identified using tRNAscan-SE 2.0 (Chan and Lowe 2019). Putative functional assignments of genes were made based on sequence similarity to characterize fungal mitochondrial genes using BLASTN searches against NCBI databases (Altschul et al. 1990). Then manual examination was performed to rectify possible annotation errors. After confirming the presence of all the conserved mt genes in the assembled contig, the displayed overlaps at both ends were used to circularize the mitogenome.

Codon usage was determined using the online Sequence Manipulation Suite software (Stothard 2000), and using the genetic code 4 for mold mitochondrial. The physical map of *T. koningiopsis* POS7 mitogenome was generated using the same software Geneious 9.1.8.

Phylogenetic analyses

Phylogenetic analyses were performed using the 14 conserved mitochondrial genes of a database constructed with selected taxa from the Subphylum Pezizomycotina (Cardona et al. 2018). In addition, three species of the Subphylum Saccharomycotina, as *Candida albicans*, *Candida glabrata*, and *Wickerhamomyces canadensis* were used as outgroups.

We retrieved the complete mitogenome of 18 species available in GenBank (Table 1). The sequences of 14 conserved protein genes namely *atp6*, *atp8*, *atp9*, *cox1*, *cox2*, *cox3*, *cob*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6* from each species were concatenated and used for phylogenetic analyses.

Sequence alignment was performed using the Clustal W program of the MEGA 6.06 package (Tamura et al. 2013) with parameters set to default. Phylogenetic reconstruction were inferred using the Maximum Likelihood (ML) and Neighbor Joining (NJ) methods using the Mega 6.06 package (Tamura et al. 2013) with parameters set to default. Bootstrap values were determined using 1000 replicates.

Table 1
Species included in the phylogenetic analysis.

Genbank Accession number	Species (Order, Class)	Class	Order	mitogenome size (bp)	Reference
NC_023268	<i>Acremonium chrysogenum</i>	Sordariomycetes	Hypocreales	27,266	Jelen et al. 2016
NC_022835	<i>Metacordyceps chlamydosporia</i>	Sordariomycetes	Hypocreales	25,615	Jelen et al. 2016
NC_018046	<i>Candida albicans</i>	Saccharomycetes	Saccharomycetales	33,928	Jelen et al. 2016
NC_017930	<i>Fusarium oxysporum</i>	Sordariomycetes	Hypocreales	34,477	Jelen et al. 2016
NC_008248	<i>Verticillium dahlia</i>	Sordariomycetes	Glomerellales	27,184	Jelen et al. 2016
KR704425	<i>Verticillium nonalfalfae</i>	Sordariomycetes	Glomerellales	26,139	Jelen et al. 2016
NC_008068	<i>Metarhizium anisopliae</i>	Sordariomycetes	Hypocreales	24,673	Jelen et al. 2016
NC_004514	<i>Lecanicillium muscarium</i>	Sordariomycetes	Hypocreales	24,499	Jelen et al. 2016
NC_001329	<i>Podospora anserine</i>	Dothideomycetes	Mycosphaerellales	100,314	Jelen et al. 2016
KC683708	<i>Neurospora crassa</i>	Sordariomycetes	Sordariales	64,840	Jelen et al. 2016
D31785	<i>Wickerhamomyces canadensis</i>	Saccharomycetes	Saccharomycetales	27,694	Ghikas et al. 2006
AJ511533	<i>Candida glabrata</i>	Saccharomycetes	Saccharomycetales	20,063	Ghikas et al. 2006
MH211586	<i>Beauveria bassiana</i>	Sordariomycetes	Hypocreales	31,258	Genbank
NC_037075	<i>T. asperellum</i>	Sordariomycetes	Hypocreales	29,999	Kwak 2020
MN125601	<i>T. atroviride</i>	Sordariomycetes	Hypocreales	32,758	Kwak 2020
NC_030218	<i>T. gamsii</i>	Sordariomycetes	Hypocreales	29,303	Kwak 2020
MF287973	<i>T. hamatum</i>	Sordariomycetes	Hypocreales	32,763	Kwak 2020
NC_003388	<i>T. reesei</i>	Sordariomycetes	Hypocreales	42,130	Kwak 2020

Genbank Accession number	Species (Order, Class)	Class	Order	mitogenome size (bp)	Reference
MT816499	<i>T. koningiopsis</i>	Sordariomycetes	Hypocreales	27,560	This work.

Results And Discussion

General features of *T. koningiopsis* POS7 mitochondrial genome

The rapid development of next generation sequencing technologies has resulted in increasing numbers of available mitogenomes (Brankovics et al. 2017; Li et al. 2019). Fungal mitogenomes are among the most variable ones compared to others from several eukaryotic groups both in size and organization (Hausner 2003; Formey et al. 2012; Li et al. 2019).

So far, 415 fungal mitochondrion NCBI Reference Sequences (RefSeq) are available in NCBI Database (<https://www.ncbi.nlm.nih.gov/nuccore>, May 2021). The overall average size of fungal mitogenomes sequenced is 50,512 bp (Jelen et al. 2016), though it can vary greatly, ranging from 12,055 bp in *Rozella allomyces* to 235,849 bp in *Rhizoctonia solani* or 272,238 bp in *Morchella importuna* (James et al. 2013; Losada et al. 2014; Li et al. 2020).

In this study, the mitogenome of the mycoparasitic fungus *T. koningiopsis* POS7 had a total size of 27,560 bp. The mtDNA sequence mean coverage in the assembly was 326X. This newly sequenced mitogenome was deposited in the GenBank database with the accession number MT816499. The size of *T. koningiopsis* POS7 mitogenome was the smallest genome among the 6 mitogenomes available in the *Trichoderma* genus (Table 1).

The GC content in organisms is considered to be influenced by mutational bias, selection, and biased recombination related to DNA repair, which could provide useful information regarding the evolution of different species (Li et al. 2019). The GC content of the *T. koningiopsis* POS7 mitogenome was 27.7%. This is in concordance with the other known *Trichoderma* mitogenomes evidencing no particular skew, ranging from 27.2% in *T. reesei* to 28.3% in *T. gamsii*. These findings indicate that *Trichoderma* species may ultimately be subject to similar selection pressures; nevertheless, deeper studies with additional genomic regions are needed to confirm this.

Forty-three genes were detected into *T. koningiopsis* POS7 mitogenome, all of them being located on the sense strand. The latter is in accordance with most Ascomycota mitogenomes where all genes are transcribed from the same DNA strand (Ghikas et al. 2006). However, in some Ascomycota some genes are located on the antisense strand as in *V. nonalfalfae* (Haridas and Gantt 2010; Costa et al. 2012; Jelen et al. 2016).

Protein-Coding Genes and Codon Usage

The *T. koningiopsis* POS7 mitogenome had the conserved set of 14 mitochondrial protein-coding genes: the ATP synthase subunits (*atp6*, *atp8*, and *atp9*), the cytochrome oxidase subunits (*cox1*, *cox2*, and *cox3*), the

reduced nicotinamide adenine dinucleotide ubiquinone oxidoreductase subunits (*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*), and the apocytochrome b (*cob*).

Codon usage analyses indicated that the most frequently used codons in *T. koningiopsis* POS7 mitogenome were ATT (for isoleucine; Ile), TTT (for phenylalanine; Phe), and ATA (for isoleucine; Ile). The high frequency of AT content of these codons is in agreement to the high AT content of the six *Trichoderma* mitogenomes (from 71.1–72.8%).

Genes were also analyzed with respect to their start and stop codons. Among the predicted genes, the “AUG” initiation codon was mostly prevalent, with only the gene *rps3* using the alternative “AUU” initiation codon (Deng et al. 2016). In all cases the stop codon was “UAA”, followed by two alternative stop codons exhibiting similar frequency (Table 2).

Table 2
Gene features of the *T. koningiopsis* POS7 mitochondrial genome.

Gene	Start position	Stop position	Length	Strand	Start Codon	Stop Codon
<i>cox1</i>	68	1,660	1,593	+	AUG	UAA
<i>cox2</i>	21,428	22,177	750	+	AUG	UAA
<i>cox3</i>	8,900	9,983	1,084	+	AUG	UAA
<i>cob</i>	25,811	26,980	1,170	+	AUG	UAA
<i>nad1</i>	2,238	3,395	1,158	+	AUG	UAA
<i>nad2</i>	18,761	20,428	1,668	+	AUG	UAA
<i>nad3</i>	20,429	20,842	414	+	AUG	UAA
<i>nad4</i>	3,537	4,994	1,458	+	AUG	UAA
<i>nad4L</i>	22,485	22,754	270	+	AUG	UAA
<i>nad5</i>	22,854	24,920	2,067	+	AUG	UAA
<i>nad6</i>	10,228	10,980	753	+	AUG	UAA
<i>atp6</i>	5,764	6,546	783	+	AUG	UAA
<i>atp8</i>	5,434	5,580	147	+	AUG	UAA
<i>atp9</i>	21,038	21,262	225	+	AUG	UAA
<i>rps3</i>	14,393	15,793	1,401	+	AUU	UAA

Ribosomal Protein, Transfer RNA, and Ribosomal RNA Genes

In *T. koningiopsis* POS7 mitogenome the mitochondrial ribosomal protein S3 (*rps3*) gene was found with a sequence length of 1,401 bp. This gene was also found in the mitogenomes of *T. atroviride* (1,389 bp), *T. hamatum* (1,350 bp), and *T. reesei* (1,425 bp).

Across the fungi, the *rps3* gene is extremely diverse in location and organization. Some versions of this gene have been incorporated into a group I intron, others appear to have gained large insertions, microsatellite expansions, or have been invaded by homing endonucleases (Sethuraman et al. 2009; Wai et al. 2019). Also, *rps3* gene that is most likely native to mitogenome, can be lost, likely due to the presences of a nuclear-encoded analog or homolog that cloud complement for a missing mitochondrial-encoded *rps3* gene (Wai et al. 2019).

A total of 26 tRNA genes were identified on *T. koningiopsis* POS7 mitogenome using tRNAscan. Among the tRNAs, all the 20 for standard amino-acids were accounted for, but for some amino-acids multiple tRNAs were found. There were found two copies of the tRNAs genes for arginine, phenylalanine, serine, and leucine, and three copies of the tRNA gene for methionine. No introns have been found in the tRNA genes detected. The number of tRNA genes reported in the five other *Trichoderma* mitogenomes ranged from 23 to 27.

tRNA genes are an important nexus between mRNAs and protein, as they are a fundamental component of the translation machinery in that they deliver amino acids to the ribosome to translate the genetic information in a mRNA (Kirchner and Ignatova 2015). Mitochondrial tRNA mutations have been demonstrated to be associated with metabolism and various diseases in humans; however, little research has been done on tRNA mutations in fungal mitogenomes related to the growth and pathogenicity of pathogenic fungi (Chen et al. 2019).

It was reported a characteristic clustering of the *trn* genes in the mtDNA to all Pezizomycotina, just with minor differences, especially the cluster of 12 *trn* genes, located between *trnI* and *nad2* genes in all Sordariomycetes remained almost unchanged both in gene content and order (Ghikas et al. 2006; Aguileta et al. 2014).

Two mitochondrial rRNA genes were identified on *T. koningiopsis* POS7 mitogenome, namely the large subunit ribosomal RNA (*rrl*) and the small subunit ribosomal RNA (*rrs*) genes. The *rrl* gene was 4,702 bp in length with an intronic sequence of 2,387 bp containing the *rps3* gene. The *rrs* gene was a complete gene sequence of 1,503 bp without any intronic spacer identified.

Intronic and intergenic regions

Mitochondrial introns are divided into two groups (I and II) based on their secondary structure and splicing mechanism (Zhang and Zhang 2019). Group I introns are considered to be mobile genetic elements which interrupt protein-coding and RNA genes in all domains of life (Edgell et al. 2011). In fungi, introns are inserted in many different mitochondrial genes, with a strong preference for protein-coding genes, most frequently *cox1* and *cob* (Paquin et al. 1997). However, despite the small genome size, two introns were detected in the *T. koningiopsis* POS7 mitogenome.

An intron with a size of 274 bp was detected in the *cox3* gene and had high identity (BLAST) (91%, $1e^{-31}$) with the intron 1 in *cox3* gene of a representative belonging to Hypocreales, specifically *Cordyceps cicadae* (NC_041489), which is an intron Group IB. In addition, another intron with a size of 1,750 bp was detected in the *LSU* gene. The latter intron had high identity (BLAST) (92%, $1e^0$) with partial 3'-intron 1-partial 5' *LSU* gene of *T. reesei* (NC_003388). So the intronic regions accounted only for the 7.34% of this mitogenome.

Related to the other species in the genus, *T. gamsii* has one intron of 1,609 bp reported in its mitogenome, *T. asperellum* has three introns with a total size of 2,065 bp; *T. atroviride* has four introns with a total size of 2,983

bp; *T. hamatum* with five introns with a total size of 2,366 bp; and, *T. reesei* has eleven introns reported with a total size of 7,741 bp (Kwak 2020).

The high evolution rate of mitogenomes makes mitochondrial introns available to differentiate strains within the same genus or even within a species (Deng et al. 2016). Related to introns, one evolutive hypothesis suggests that within any particular host lineage, there appears to be a dynamic cycle of invasion by horizontal transmission, degeneration, and eventual loss, followed by reinvasion (Goddard and Burt 1999; Mardanov et al. 2014). It was also suggested that the number of introns could be considered one of the main factors contributing to the mitogenome size variations as it was seen here where *T. reesei* has the largest mitogenomes (Hausner 2003; Deng et al. 2016; Kanzi et al. 2016; Fan et al. 2019). However, on the basis of available studies about mitochondrial gene order, Aguileta et al. (2014) did not found a significant correlation between rearrangements of the fungal mitogenomes and the proportions of introns and intronic ORF.

Other authors indicated that the length of intergenic regions is also other key contributor to mt genomic size variation in fungi (Lin et al. 2015). In the *T. koningiopsis* POS7 mitogenome, the smallest mitogenome in the genus, the intergenic regions accounted for a total of 4,548 bp (16.5% of total mitogenome), with lengths ranging from 4 to 890 bp. The largest intergenic region was between *nad5* and *cob* genes. Meanwhile, in the *T. reesei* QM 9414 mitogenome, the species with the largest mitogenome in the genus, the intergenic regions accounted for a total of 7,405 bp (17.58% of total mitogenome), similar to the latter (Kwak 2020). So in this genus, it could be suggested that the intergenic regions were not an important force modeling mt genomic size variation.

The entire genome found in the mitochondrion of *T. koningiopsis* POS7 after its curated annotations is shown in **Fig. 1**.

Phylogenetic analyses

The most common approach for phylogenetic studies of fungi with mitochondrial sequences is using a set of conserved proteins from their annotated sequences. Since mitogenomes corresponding to the evaluated species are considered to be circular molecules, the alignment start position was arbitrarily decided as the *cox1* gene (Jelen et al. 2016).

Mitogenomes of *T. koningiopsis* POS7 and of 18 other selected fungi were used to construct ML and NJ phylogenetic trees. Both algorithms yielded single trees with the same branch topology (for clarity only the NJ tree is presented in **Fig. 2**).

Based on the phylogenetic analysis of the 14 conserved genes used, the 19 Ascomycota species could be divided into two major clades corresponding to the subphyla Pezizomycotina and Saccharomycotina (this latter used as outgroup). Most nodes in this tree had high bootstrap values which indicate the robustness of the phylogenetic tree inferred.

The clade Pezizomycotina was divided in three subclades including fungi with combined mitochondrial gene sets corresponding to the orders Hypocreales, Glomerellales, and Sordariales. In the Hypocreales subclade all *Trichoderma* mtDNA sequences were grouped together with the highest support value (100%).

A similar pattern of phylogeny branching was obtained for fungi of Ascomycota by Ghikas et al. (2006), for selected Basidiomycota and Ascomycota by Aguileta et al. (2016), for Pezizomycotina by Cardona et al. (2018), and for Hypocreales by Wang et al. (2017). Therefore, the combination of 14 mitochondrial gene set analyzed in this work may be a useful strategy for fungal taxa identification and phylogenetic analysis.

Even it has been reported that the mitochondrial gene order in fungi varied greatly among different species, even among strains from the same species (Li et al. 2019). In the present study, the mitochondrial gene order of *T. koningiopsis* was identical to all other *Trichoderma* mitogenomes reported, so we didn't find that a gene rearrangement occurred among *Trichoderma* species including other closely related fungal genus involving mitochondrial core genes encoding proteins.

Related to the gene order comparison, ten of the eleven compared species of the order Hypocreales analyzed have complete synteny in gene order. In contrast, the gene order in the mitogenome of *A. chrysogenum* differs only in the location of the gene *cox2*, between *nad4* and *atp8* genes. Lin et al. (2015) evaluated the mitochondrial gene order of different members belonging to Hypocreales and obtained similar results.

Even the fungal mitogenome organization is apparently quite diverse; certain features appear to be conserved in the mitogenome organization of some related fungi like the overlapping of *nad4L/nad5* so that the last base of the first gene was also the first base of the second gene, a feature that is underlined as common in mitogenomes of Sordariomycetes (Kouvelis et al. 2004). It was noted that in general the *nad4L/nad5* and *nad2/nad3* genes, tend to be next to each other, and that in these two pairs of genes existed an overlap of the stop and initiation nucleotides (Aguileta et al. 2014).

Both mitochondrial and nuclear gene sequences have been employed in efforts to reconstruct phylogenetic relationships among fungi. Also, similar phylogeny branching could be obtained using ITS genes for the same *Trichoderma* species (Castrillo 2015). But in other groups of organisms the mitochondrial data alone have less resolving power than nuclear genes where in contrast phylogenies from nuclear data is generally well-supported (Springer et al. 2001; Wiens et al. 2010).

Mitochondrial sequences have been found to be useful to determine and/or confirm phylogenetic relationships, especially in cases where nuclear genes are not reliable molecular timers to clarify conflicting phylogenies (Ghikas et al. 2010; Kanzi et al. 2016). Aguileta et al. (2014) argued that although mitochondrial genes tend to be conserved due to their universal role in cellular metabolism, fungal mt gene order is relatively free to vary, and that this variation is probably largely due to recombination. It is worth mentioning that it was reported that mt intergenic regions can serve as information for phylogenetic analysis and for the development of tools for intra- and inter-specific discrimination of fungi (Ghikas et al. 2006, 2010).

Other sources of fungal mitogenome variation are presence of repeats, variable numbers of ORFs with an unknown function and variability in the mitochondrial gene order (Jelen et al. 2016). The arrangement of mitochondrial genes has been widely employed to understand the phylogenetic status of species (Ghikas et al. 2006; Li et al. 2018; Chen et al. 2019).

In many cases, it has been shown that a single gene may not always represent the history of the genome containing it, and analyses based on this single gene may lead to wrong conclusions about the relationship of a fungus (Franco et al. 2016). Thus, the sequence of the entire mitogenome may be considered a promising alternative for phylogenetic analyses as they provide a comprehensive perspective on the positioning and types of different genes, and the presence of introns, for evaluation of relationship of a fungus. Also, because all of the mitogenomes originate from common ancestors, mitochondrial gene rearrangements have been widely used to study the origin and evolution of eukaryotes (Chen et al. 2019).

Increasing the available mt genomic data will inevitably contribute to addressing the persisting phylogenetic uncertainties in the Hypocreales group. The mitochondrial genome of *T. koningiopsis* POS7 will allow further investigations into the taxonomy, phylogenetics, conservation genetics, and evolutionary biology of this important genus as well as other closely related species.

Medina et al. (2020) reported that the mt can play key roles in fungal biology since this organelle carry both conservative mt-genes and highly variable regions in its mitogenome that are in part responsible for the phenotypic plasticity of the host. Therefore, future studies analyzing isolates of *T. koningiopsis* that differ in their mycoparasitic ability might also give information about the probability of some contribution of the mitogenome to the behaviour of these fungi in biocontrol and other biotechnological uses.

Conclusion

In conclusion, we sequenced and annotated the complete mitochondrial genome of *T. koningiopsis* POS7, which is one of the mitochondrial genome sequence reported in the genus *Trichoderma*. This study enriches the mitochondrial database of Hypocreaceae fungi and fills-in a gap for the mitogenomes of the *Trichoderma* genus. The limited number and size of introns resulted in the smallest mitogenome reported for this genus. This study is the first report on the mitochondrial genome of the species *T. koningiopsis*, and it will contribute to our understanding of the population genetics and evolution of this and related species.

Declarations

Ethics approval and consent to participate

Not applicable.

Adherence to national and international regulations

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this article.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was partially supported by the project PICT 2016-3349. This project was used to the acquisition of one PC for the genome analyses.

Authors' contributions

MLC and GAB designed the research and wrote the manuscript. PDZ, MCNS and LLV supervision the research.

Acknowledgments

The authors would also like to thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina). GAB is a postgraduate CONICET fellowship holder. MLC is an assistant researcher in CONICET, Argentina. PDZ and MCNS are independent researchers in CONICET, Argentina.

References

- Aguileta G, De Vienne DM, Ross ON, Hood ME, Giraud T, Petit E, et al (2014) High variability of mitochondrial gene order among fungi. *Genome Biology and Evolution*. 6:451–465. doi: 10.1093/gbe/evu028
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology*. 215:403-410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Amerio NS, Castrillo ML, Bich GA, Zapata PD, Villalba LL (2020) *Trichoderma* en la Argentina: Estado del arte. *Ecología austral*. 30:113-124. doi: 10.25260/EA.20.30.1.0.945.
- Antal Z, Manczinger L, Kredics L, Kevei F, Nagy E (2002) Complete DNA sequence and analysis of a mitochondrial plasmid in the mycoparasitic *Trichoderma harzianum* strain T95. *Plasmid*. 47:148-152. doi:10.1006/plas.2001.1559.
- Baroncelli R, Piaggieschi G, Fiorini L, Bertolini E, Zapparata A, Pé ME, et al (2015) Draft Whole-Genome Sequence of the Biocontrol Agent *Trichoderma harzianum* T6776. *Genome Announcements*. 3:e00647-15. doi:10.1128/genomeA.00647-15.
- Baroncelli R, Zapparata A, Piaggieschi G, Sarrocco S, Vannacci G (2016) Draft Whole-Genome Sequence of *Trichoderma gamsii* T6085, a Promising Biocontrol Agent of *Fusarium* Head Blight on Wheat. *Genome Announcements*. 4:e01747-15. doi.org/10.1128/genomeA.01747-15.
- Brankovics B, van Dam P, Rep M, de Hoog GS, van der Lee TA, Waalwijk C, et al (2017) Mitochondrial genomes reveal recombination in the presumed asexual *Fusarium oxysporum* species complex. *BMC Genomics*. 18:735. doi.org/10.1186/s12864-017-4116-5.

- Burger G, Gray MW, Lang BF (2003) Mitochondrial genomes: anything goes. *Trends in Genetics*. 19:709-716. doi.org/10.1016/j.tig.2003.10.012.
- Cardona NL, Franco-Sierra ND, Correa Alvarez J (2018) Complete mitogenome of the biocontroller fungus *Purpureocillium* sp.(Ascomycota, Ophiocordycipitaceae, Hypocreales). *Mitochondrial DNA Part B*. 3:1158-1160. doi.org/10.1080/23802359.2018.1522982.
- Castrillo ML (2015) Caracterización de celulasas secretadas por aislamientos de *Trichoderma*, nativos de la provincia de Misiones (Argentina) aplicables en la etapa de sacarificación. Doctoral thesis. Universidad Nacional de Misiones, Misiones, Argentina.
- Castrillo ML, Bich GA, Kramer GR, Velazquez JE, Rodriguez MD, Zapata PD, et al (2015) Utilization of synthetic and semi- synthetic culture media for endo-1,4- β - glucanases secretion by *Trichoderma koningiopsis*. *Procedia Materials Science*. 8:786-792. doi.org/10.1016/j.mspro.2015.04.136.
- Castrillo ML, Bich GÁ, Modenutti C, Turjanski A, Zapata PD, Villalba LL (2017) First whole-genome shotgun sequence of a promising cellulase secretor, *Trichoderma koningiopsis* strain POS7. *Genome Announcements*. 5:e00823-17. doi: 10.1128/genomeA.00823-17.
- Chambergro FS, Bonaccorsi ED, Ferreira AJ, Ramos ASP, Ferreira Júnior JR, Abrahao-Neto J, et al (2002) Elucidation of the metabolic fate of glucose in the filamentous fungus *Trichoderma reesei* using expressed sequence tag (EST) analysis and cDNA microarrays. *Journal of Biological Chemistry*. 277:13983-13988. doi:10.1074/jbc.M107651200.
- Chan PP, Lowe TM (2019) tRNAscan-SE: Searching for tRNA Genes in Genomic Sequences. *Methods in Molecular Biology*. 1962:1–14. doi.org/10.1007/978-1-4939-9173-0_1
- Chen C, Li Q, Fu R, Wang J, Xiong C, Fan Z, et al (2019) Characterization of the mitochondrial genome of the pathogenic fungus *Scytalidium auriculariicola* (Leotiomyces) and insights into its phylogenetics. *Scientific Reports*. 9:1-12. doi.org/10.1038/s41598-019-53941-5
- Costa GG, Cabrera OG, Tiburcio RA, Medrano FJ, Carazzolle MF, Thomazella DP, et al (2016) The mitochondrial genome of *Moniliophthora roreri*, the frosty pod rot pathogen of cacao. *Fungal Biol-UK*. 116:551-562. doi: 10.1016/j.funbio.2012.01.008.
- Deng Y, Zhang Q, Ming R, Lin L, Lin X, Lin Y, et al (2016) Analysis of the mitochondrial genome in *Hypomyces aurantius* reveals a novel two intron complex in fungi. *International Journal of Molecular Sciences*. 17:1049. doi: 10.3390/ijms17071049.
- Edgell DR, Chalamcharla VR, Belfort M (2011) Learning to live together: mutualism between self-splicing introns and their hosts. *BMC Biology*. 9:22. doi:10.1186/1741-7007-9-22
- Fan WW, Zhang S, Zhang YJ (2019) The complete mitochondrial genome of the Chan-hua fungus *Isaria cicadae*: a tale of intron evolution in Cordycipitaceae. *Environmental Microbiology*. 21:864-879. doi: 10.1111/1462-2920.14522.

- Formey D, Molès M, Haouy A, Savelli B, Bouchez O, Bécard G, et al (2012) Comparative analysis of mitochondrial genomes of *Rhizophagus irregularis*–syn. *Glomus irregulare*–reveals a polymorphism induced by variability generating elements. *New Phytology*. 196:1217-1227. doi: 10.1111/j.1469-8137.2012.04283.x.
- Franco MEE, López SMY, Saparrat MCN, Balatti PA (2016) Mitochondrial and nuclear gene sequences to infer the phylogeny of Pezizomycotina (Ascomycota). In American Phytopathological Society Annual Meeting.
- Ghikas DV, Kouvelis VN, Typas MA (2006) The complete mitochondrial genome of the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae*: gene order and trn gene clusters reveal a common evolutionary course for all Sordariomycetes, while intergenic regions show variation. *Archives of Microbiology*. 185:393. doi: 10.1007/s00203-006-0104-x.
- Ghikas DV, Kouvelis VN, Typas MA (2010) Phylogenetic and biogeographic implications inferred by mitochondrial intergenic region analyses and ITS1-5.8 S-ITS2 of the entomopathogenic fungi *Beauveria bassiana* and *B. brongniartii*. *BMC Microbiology*. 10:1-15. doi.org/10.1186/1471-2180-10-174
- Goddard MR, Burt A (1999) Recurrent invasion and extinction of a selfish gene. *Proceedings of the National Academy of Sciences USA*. 96:13880-13885. doi: 10.1073/pnas.96.24.13880.
- Gray MW, Burger G, Lang BF (1999) Mitochondrial evolution. *Science*. 283:1476–1481. doi.org/10.1126/science.283.5407.1476
- Halliwell G, Griffin M (1973) The nature and mode of action of the cellulolytic component C1 of *Trichoderma koningii* on native cellulose. *Biochemical Journal*. 135:587-594. doi:10.1042/bj1350587
- Haridas S, Gannt JS (2010) The mitochondrial genome of the wood-degrading basidiomycete *Trametes cingulata*. *FEMS Microbiology Letters*. 308:29-34. doi:10.1111/j.1574-6968.2010.01979.x
- Hausner G (2003) Fungal Mitochondrial Genomes. *Fungal Genomics*. 3, 101.
- James TY, Pelin A, Bonen L, Ahrendt S, Sain D, Corradi N, et al (2013) Shared signatures of parasitism and phylogenomics unite Cryptomycota and microsporidia. *Current Biology*. 23:1548-1553. doi: 10.1016/j.cub.2013.06.057.
- Jelen V, De Jonge R, Van de Peer Y, Javornik B, Jakše J (2016) Complete mitochondrial genome of the Verticillium-wilt causing plant pathogen *Verticillium nonalfalfae*. *PLoS ONE*. 11:e0148525. doi.org/10.1371/journal.pone.0148525
- Kanzi AM, Wingfield BD, Steenkamp ET, Naidoo S, van der Merwe NA (2016) Intron derived size polymorphism in the mitochondrial genomes of closely related Chrysosporthe species. *PLoS ONE*. 11:e0156104. doi.org/10.1371/journal.pone.0156104.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*. 28:1647-1649. doi: 10.1093/bioinformatics/bts199.

- Kirchner S, Ignatova Z (2015) Emerging roles of tRNA in adaptive translation, signalling dynamics and disease. *Nature Reviews Genetics*. 16:98-112. doi.org/10.1038/nrg3861.
- Kouvelis VN, Ghikas DV, Typas MA (2004) The analysis of the complete mitochondrial genome of *Lecanicillium muscarium* (synonym *Verticillium lecanii*) suggests a minimum common gene organization in mtDNAs of Sordariomycetes: phylogenetic implications. *Fungal Genetics and Biology*. 41:930-940. doi: 10.1016/j.fgb.2004.07.003.
- Kubicek CP, Herrera-Estrella A, Seidl-Seiboth V, Martinez DA, Druzhinina IS, Thon M, et al (2011) Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*. *Genome Biology*. 12:R40. doi:10.1186/gb-2011-12-4-r40.
- Kwak Y (2020) Complete mitochondrial genome of the fungal biocontrol agent *Trichoderma atroviride*: Genomic features, comparative analysis and insight into the mitochondrial evolution in *Trichoderma*. *Frontiers in Microbiology*. doi: 10.3389/fmicb.2020.00785
- Li Q, Wang Q, Jin X, Chen Z, Xiong C, Li P, et al (2019) Characterization and comparative analysis of six complete mitochondrial genomes from ectomycorrhizal fungi of the *Lactarius* genus and phylogenetic analysis of the Agaricomycetes. *International Journal of Biological Macromolecules*. 121:249-260. doi.org/10.1016/j.ijbiomac.2018.10.029.
- Li Q, Yang L, Xiang D, Wan Y, Wu Q, Huang W, et al (2020) The complete mitochondrial genomes of two model ectomycorrhizal fungi (*Laccaria*): Features, intron dynamics and phylogenetic implications. *International Journal of Biological Macromolecules*. 145: 974-984. doi.org/10.1016/j.ijbiomac.2019.09.188.
- Lin R, Liu C, Shen B, Bai M, Ling J, Chen G, et al (2015) Analysis of the complete mitochondrial genome of *Pochonia chlamydosporia* suggests a close relationship to the invertebrate-pathogenic fungi in Hypocreales. *BMC Microbiology*. 15:1-15. doi: 10.1186/s12866-015-0341-8.
- Losada L, Pakala SB, Fedorova ND, Joardar V, Shabalina SA, Hostetler J, et al (2014) Mobile elements and mitochondrial genome expansion in the soil fungus and potato pathogen *Rhizoctonia solani* AG-3. *FEMS Microbiology Letters*. 352:165-173. doi: 10.1111/1574-6968.12387.
- Mardanov AV, Beletsky AV, Kadnikov VV, Ignatov AN, Ravin NV (2014) The 203 kbp mitochondrial genome of the phytopathogenic fungus *Sclerotinia borealis* reveals multiple invasions of introns and genomic duplications. *PLoS ONE*. 9:e107536. doi: 10.1371/journal.pone.0107536.
- Martinez D, Berka RM, Henrissat B, Saloheimo M, Arvas M, Baker SE, et al (2008) Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). *Nature Biotechnology*. 26:553-560. doi:10.1038/nbt1403.
- Medina R, Franco MEE, Bartel LC, Martinez Alcántara V, Saparrat MCN, Balatti PA (2020) Fungal Mitogenomes: Relevant features to Pplanning plant disease management. *Frontiers in Microbiology*. 11:978. doi: 10.3389/fmicb.2020.00978.

- Paquin B, Laforest MJ, Forget L, Roewer I, Wang Z, Longcore J, et al (1997) The fungal mitochondrial genome project: evolution of fungal mitochondrial genomes and their gene expression. *Current Genetics*. 31:380-395. doi.org/10.1007/s002940050220
- Proctor RH, McCormick SP, Kim HS, Cardoza RE, Stanley AM, Lindo L, et al (2018) Evolution of structural diversity of trichothecenes, a family of toxins produced by plant pathogenic and entomopathogenic fungi. *PLoS Pathogens*. 14:e1006946. doi:10.1371/journal.ppat.1006946.
- Samuels GJ, Dodd SL, Lu BS, Petrini O, Schroers HJ, Druzhinina IS (2006) The *Trichoderma koningii* aggregate species. *Studies in Mycology*. 56:67-133. doi:10.3114/sim.2006.56.03.
- Sethuraman J, Majer A, Iranpour M, Hausner G (2009) Molecular evolution of the mtDNA encoded rps3 gene among filamentous ascomycetes fungi with an emphasis on the ophiostomatoid fungi. *Journal of Molecular Evolution*. 69:372-385. doi: 10.1007/s00239-009-9291-9.
- Shi-Kunne X, Seidl MF, Faino L, Thomma BP (2015) Draft Genome Sequence of a Strain of Cosmopolitan Fungus *Trichoderma atroviride*. *Genome Announcements*. 3:e00287-15. doi:10.1128/genomeA.00287-15.
- Song N, Geng Y, Li X (2020) The mitochondrial genome of the phytopathogenic fungus *Bipolaris sorokiniana* and the utility of mitochondrial genome to infer phylogeny of Dothideomycetes. *Frontiers in Microbiology*. 11:863. doi: 10.3389/fmicb.2020.00863
- Springer MS, DeBry RW, Douady C, Amrine HM, Madsen O, de Jong WW, et al (2001) Mitochondrial versus nuclear gene sequences in deep-level mammalian phylogeny reconstruction. *Molecular Biology and Evolution*. 18:132-143. doi.org/10.1093/oxfordjournals.molbev.a003787
- Steindorff AS, Ramada MH, Coelho AS, Miller RN, Pappas GJ, Jr Ulhoa CJ, et al (2014) Identification of mycoparasitism-related genes against the phytopathogen *Sclerotinia sclerotiorum* through transcriptome and expression profile analysis in *Trichoderma harzianum*. *BMC genomics*. 15:204. doi.org/10.1186/1471-2164-15-204
- Stothard P (2000) The sequence manipulation suite: JavaScript programs for analyzing and formatting protein and DNA sequences. *Biotechniques*. 28:1102-1104. doi: 10.2144/00286ir01.
- Studholme DJ, Harris B, Le Cocq K, Winsbury R, Perera V, Ryder L, et al (2013) Investigating the beneficial traits of *Trichoderma hamatum* GD12 for sustainable agriculture-insights from genomics. *Frontiers in Plant Science*. 4:258. doi.org/10.3389/fpls.2013.00258.
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*. 30:2725–2729. doi: 10.1093/molbev/mst197.
- Van de Sande WW (2012) Phylogenetic analysis of the complete mitochondrial genome of *Madurella mycetomatis* confirms its taxonomic position within the order Sordariales. *PLoS ONE*. 7:e38654. doi.org/10.1371/journal.pone.0038654.

Wai A, Shen C, Carta A, Dansen A, Crous PW, Hausner G (2019) Intron-encoded ribosomal proteins and N-acetyltransferases within the mitochondrial genomes of fungi: here today, gone tomorrow?. *Mitochondrial DNA Part A*. 30:573-584. doi.org/10.1080/24701394.2019.1580272.

Wang X, Wang Y, Yao W, Shen J, Chen M, Gao M, et al (2020) The 256 kb mitochondrial genome of *Clavaria fumosa* is the largest among phylum Basidiomycota and is rich in introns and intronic ORFs. *IMA Fungus*. doi.org/10.1186/s43008-020-00047-7

Wang XC, Zeng ZQ, Zhuang WY (2017) The complete mitochondrial genome of the important mycoparasite *Clonostachys rosea* (Hypocreales, Ascomycota). *Mitochondrial DNA Part B*. 2:180-181. doi.org/10.1080/23802359.2017.1303344.

Wiens JJ, Kuczynski CA, Stephens PR (2010) Discordant mitochondrial and nuclear gene phylogenies in emydid turtles: implications for speciation and conservation. *Biological Journal of the Linnean Society*. 99:445-461. doi.org/10.1111/j.1095-8312.2009.01342.x

Xie BB, Qin QL, Shi M, Chen LL, Shu YL, Luo Y, et al (2014) Comparative genomics provide insights into evolution of *Trichoderma* nutrition style. *Genome Biology and Evolution*. 6:379-390. doi:10.1093/gbe/evu018.

Zhang S, Zhang YJ (2019) Proposal of a new nomenclature for introns in protein-coding genes in fungal mitogenomes. *IMA Fungus*. doi.org/10.1186/s43008-019-0015-5

Figures

Circular map of *T. koningiopsis* POS7 mitochondrion. Proteins, rRNA and tRNA genes were indicated in green. Exons were indicated in dark grey and introns were indicated in white.

Page 18/18