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Complete mitochondrial genome of *Pleurocordyceps sinensis* (Hypocreales, Ascomycota), a species with uncertain family-level taxonomic assignment

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RESEARCH ARTICLE

Abstract

The complete mitochondrial (mt) genome of the ex-type strain of *Pleurocordyceps sinensis*, a fungus originally isolated from *Ophiocordyceps sinensis*, was sequenced, and assembled as a single circular DNA of 31,841 bp. The mt genome encoded 15 conserved proteins (*rps3*, *cox1*, *cox2*, *cox3*, *cob*, *atp6*, *atp8*, *atp9*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*), 2 rRNA (*rnl* and *rns*), and 25 tRNA, as well as 10 additional non-conserved open reading frames (ncORFs). Comparative analyses showed that mt genomes within the order Hypocreales encoded the same number and synteny of conserved protein coding genes despite an obvious size variation among this group of fungi. Phylogenetic analyses using 14 conserved protein sequences revealed that this fungus may not belong to the current designated family *Ophiocordycipitaceae* but is more closely related to the species of *Clavicipitaceae*. The mt genome presented herein would give valuable information on reconstructing the evolutionary history of clavicipitaceous fungi and also aid in resolving the family-level taxonomic assignment of *Polycephalomyces* s. l. species.

Keywords: *Cordyceps*; hyperparatisism; *Polycephalomyces sinensis*; taxonomy

Introduction

Pleurocordyceps sinensis (Q.T. Chen *et al.*) Y.J. Yao *et al.* was first isolated from the sclerotium of *Ophiocordyceps sinensis* collected from Kangding, Sichuan, China in June 1980, and was described as a new species named *Paecilomyces sinensis* Q.T. Chen *et al.* (Chen *et al.*, 1984). The species gained broad scientific attention since 1980s in China. Large numbers of pharmacological studies have been carried out using the only authentic strain termed CN80-2. The species was reported to have various pharmacological activities such as anti-implantation (Lin *et al.*, 1988), anti-inflammatory (Li *et al.*, 1983), anti-oxidant (Liu *et al.*, 1987, 1989, 1991), anti-tumor (Huang *et al.*, 1988; Wu *et al.*, 1986), fertility regulation (Li and Lin, 1991), immune modulation (Ge *et al.*, 1989; Lin *et al.*, 1987; Zhang *et al.*, 1998; Zheng *et al.*, 1983), and treating conditions of coronary arteriosclerotic heart disease (You *et al.*, 1986) and immunological liver injury (Cheng *et al.*, 2005; Zeng *et al.*, 2000). Because of the similarities in the pharmacological effects and chemical components, *P. sinensis* has once been recognized as the possible anamorph of *O. sinensis*. This viewpoint was widely accepted and cited when summarizing the anamorph of *O. sinensis* (Fang, 1991; Liang, 1991; Liu, 1990). The idea of using *Paecilomyces sinensis* as a substitute of

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O. sinensis was thus proposed (e.g., Cheng *et al*., 2005; Li *et al*., 1983; Zeng *et al*., 2000). However, several independent researches based on molecular evidences rejected the anamorph-teleomorph relationship between *P. sinensis* and *O. sinensis* (Chen *et al*., 2001; Jiang and Yao, 2002, 2003; Li *et al*., 2000; Zhao *et al*., 1999). Wang *et al*. (2012) placed the species in *Polycephalomyces* based on morphological and molecular analyses and found *Polycephalomyces* species formed a new clade of clavicipitaceous fungi and stated that this new clade is distinct from the known families of Hypocreales.

The genus *Polycephalomyces* was identified by Kobayasi (1941) with *P. formosus* as the type. Only three species, that is, *P. paludosus*, *P. cylindrosporus*, and *P. tomentosus*, were described in the last century (Mains, 1948; Samson *et al*., 1981; Seifert, 1986). Until recently, after the recombination of *Paecilomyces sinensis* (Wang *et al*., 2012) and several species of *Cordyceps* s. l. into the genus (Kepler *et al*., 2013), more and more new species have been discovered and described, especially from China and Southeast Asia (Crous *et al*., 2017; Wang *et al*., 2015a, 2015b; Xiao *et al*., 2018; Yang *et al*., 2020). A total of 24 species names are currently recorded by the Index Fungorum (4 April 2022, http://www.indexfungorum.org/Names/Names.asp), among which 4 were segregated to *Perennicordyceps* (Matočec *et al*., 2014). Wang *et al*. (2021) recently proposed a new genus *Pleurocordyceps* for one of the subclades within the "*Polycephalomyces* clade" (*Polycephalomyces* sensu lato). Ten species were included in the new genus including *P. sinensis*. In multigene phylogenetic analyses, species of *Polycephalomyces* s. l. usually formed a distinct clade sister to *Ophiocordycipitaceae*, although this sister relationship did not receive much statistical confidence (Kepler *et al*., 2013; Wang *et al*., 2021). In other words, the family-level taxonomic position of *Polycephalomyces* s. l. was not fully resolved; species in this group were tentatively placed in *Ophiocordycipitaceae* in most researches (e.g., Kepler *et al*., 2013; Xiao *et al*., 2018), which was accepted by the Index Fungorum. *Polycephalomyces* s. l. may represent a new family that is different from the three existing families of clavicipitoid fungi (Wang *et al*., 2021), that is, *Cordycipitaceae*, *Clavicipitaceae*, and *Ophiocordycipitaceae.* However, more evidence from morphology and molecular phylogenetics is required to support this hypothesis.

Mitochondria play various essential roles in eukaryotic cells, including respiratory metabolism, energy production, calcium homeostasis, and are also involved in cell death and aging (Basse, 2010). Mitochondrial (mt) genomes usually have a rapid rate of evolution compared with nuclear genomes, and thus are considered as powerful tools in evolutionary biology (Berbee and Taylor, 2001; Chris *et al*., 1994). Previous studies revealed that the gene contents and synteny of mt genomes of hypocrealean species were largely conserved, but in the meantime, the genome sizes expanded greatly in certain species such as *O. sinensis* (Li *et al*., 2015). Complete mt genomes have been reported for a number of species of the three clavicipitaceous fungal families, that is, *Cordycipitaceae* (Fan *et al*., 2019; Kouvelis *et al*., 2004; Sung, 2015; Zhang *et al*., 2021), *Ophiocordycipitaceae* (Abuduaini *et al*., 2021; Li *et al*., 2015; Zhang *et al*., 2016; Zhang and Zhang, 2020), and *Clavicipitaceae* (Sun *et al*., 2021; Winter *et al*., 2018), while they have not been reported for *Polycephalomyces* s. l. species so far.

In this study, the complete mt genome of the type strain (CN 80-2) of the species *Pleurocordyceps sinensis* was sequenced, described, and compared with other hypocrealean species. Phylogenetic analyses using 14 conserved protein sequences were also performed to study the phylogenetic relationship between this species and other clavicipitaceous fungal groups.

Materials and Methods

Fungal isolation and cultivation

The ex-type strain (CN80-2) of *Pleurocordyceps sinensis* used in this study was isolated from a sclerotium of *O. sinensis* collected from Kangding, Sichuan, China in June 1980 (Chen *et al*., 1984). Stock strain was maintained at 4°C on potato dextrose gar (PDA) slants. Seed cultures were grown in 250-mL Erlenmeyer flasks, containing 50 mL liquid potato-dextrose medium, by shaking at 100 rpm at 25°C for 10 days. Mycelia were harvested and washed with distilled water using vacuum filtration to remove extracellular polysaccharides, frozen with liquid nitrogen, and vacuum freeze-dried using a freeze dryer. Dried mycelia were then sent to the genome sequencing company.

DNA extraction and genome sequencing

Genomic DNA was extracted by the sequencing company using TIANamp Yeast DNA Kit (TIANGEN Biotech Co., Ltd., Beijing, China) according to the manufacturer's instruction. The amount and quality of total DNA were visualized by 1% agarose gel electrophoresis and quantified with a Qubit2.0® Fluorometer (Life Technologies, New York, USA). A 20 K library was prepared from sheared genomic DNA (containing both mt and nuclear sequences) using a 20-Kb template library preparation workflow. Twelve single molecule real time (SMRT) sequencing cells were sequenced on PacBio RS II sequencing platform (Pacific Biosciences, Menlo Park, CA) with P6 polymerase and C4 sequencing chemistry at Tianjin Biochip Corporation (Tianjin, China).

Mitochondrial genome assembly and annotation

Mt genome of the strain CN80-2 was assembled and annotated following a procedure described in Li *et al*. (2015). The adapter sequences, reads with length <50 bp, or average quality <0.75 (defined as low quality) were filtered before assembling. The mt sequences were extracted from filtered reads matching each read against the fungal mt genome database (https://www.ncbi.nlm.nih.gov/genome/browse#!/organelles/), pre-assembled and corrected using BLASR (Chaisson and Tesler, 2012). Corrected reads were retained and then re-assembled with the Celera Assembler program (Myers *et al*., 2000). The assembly was further refined with Quiver (Chin *et al*., 2013). A circular double-stranded DNA was finally obtained and proceeded to an online annotation tool MFannot using the Mold, Protozoan, and Coelenterate Mitochondrial Code (Beck and Lang, 2010). The annotated mt genome was submitted to GenBank under the accession number OK017430. The annotated mt genetic map was generated by Circos software (Krzywinski *et al*., 2009) and modified with Adobe Illustrator® CS5 (Version 15.0.0, Adobe®, San Jose, CA).

Phylogenetic analyses and comparative genomics

All the 82 complete mt genomes available from GenBank (accessed on 28 March, 2021) within the order Hypocreales were downloaded and used for phylogenetic and/or comparative genomic analysis. Among which *Ophiocordyceps camponoti-floridani* EC05 (CM022976) was used only for genome comparison but not included in phylogenetic analyses, as it seems to be incorrectly assembled; an unclassified mt genome (NC_049089) was also excluded due to the large numbers of possible sequencing errors or possible assembly mistakes. A number of mt genomes were found to be incorrectly or incompletely annotated. For example, the *rps3* gene was not predicted in a number of species. Those genomes were re-annotated following the same procedure used in this study with missing genes replenished and wrongly predicted genes manually corrected. A phylogenetic tree was constructed using 14 conserved protein-coding genes (*cox1*, *cox2*, *cox3*, *cob*, *atp6*, *atp8*, *atp9*, *nad1*, *nad2*, *nad3*, *nad4*, *nad5*, *nad4L*, and *nad6*). Protein sequences were aligned with BioEdit version 7.0.9.0 (Hall, 1999) and refined manually. Maximum likelihood (ML) phylogenetic analyses were performed with RAxML v.7.2.659 (Stamatakis, 2006) using the LG substitution matrix and default parameters. Bootstrap values were calculated with 1000 re-sampling iterations using an approximate likelihood ratio test. Three mt genomes from two species of the order *Glomerellales*, that is, *Colletotrichum lindemuthianum* (NC_023540) and *Verticillium dahliae* (NC_008248 and CM019738), were used as outgroups (Supplementary Table S1).

The gene contents and synteny of mt genomes within the order Hypocreales and related outgroup species were compared and analyzed.

Results

Genome sequencing and assembly

A total of 23,757 reads (171,039,810 bp) were identified as mt among 601,168 reads (5,005,308,071 bp) of the raw sequencing output for the whole genome of *Pleurocordyceps sinensis*. The lengths of the putative mt reads ranged from 276 bp to 45,245 bp with an average length of 7200 bp, reaching a coverage depth of 5371× over the mt genome of the species. The mt reads were passed through the program BLASR and assembled with Celera Assembler program and Quiver, resulting in a circular DNA of 31,841 bp (Figure 1).

Conserved protein genes and nonconserved open reading frames

The mt genome of *P. sinensis* had a low GC content of 25.46% and encoded 15 protein genes conserved within the order Hypocreales, including seven subunits of the electron transport complex I (*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*), cytochrome b (*cob*), three subunits of complex IV (*cox1*, *cox2* and *cox3*), three F0 subunits of the ATP-synthase complex (*atp6*, *atp8*, and *atp9*), and the *rps3* gene, which encodes 40S ribosomal protein S3 (Table 1, Figure 1). In addition to those genes, 10 non-conserved open reading frames (ncORFs) (7194 bp totally in length) were also predicted, among which two (ncORF3 and ncORF9) were found to encode homing endonucleases (HEs) with motif patterns GIY-YIG and LAGLIDADG, respectively (Table 1).

All conserved protein coding genes and ncORFs were found on the positive strand and oriented clockwise except for ncORF1 and ncORF2, which were on the negative strand and anticlockwise oriented. It was found that the *nad2*/*nad3* genes were joined and *nad4L*/*nad5* genes were fused, that is, the initial codon of the *nad3* gene (ATG) followed the terminal codon of the *nad2* gene (TAA), and the terminal codon of *nad4L* (TAA) uses the same nucleotide A with the initial codon (ATG) of *nad5* (Figure 1, Table 1). Other protein-coding genes and ncORFs were separated by either long or short intergenic regions (Figure 1).

The 15 protein-coding genes and 10 predicted ncORFs employed the standard fungal mt start codon ATG, except the *cox1* and ncORF10, which were initiated by

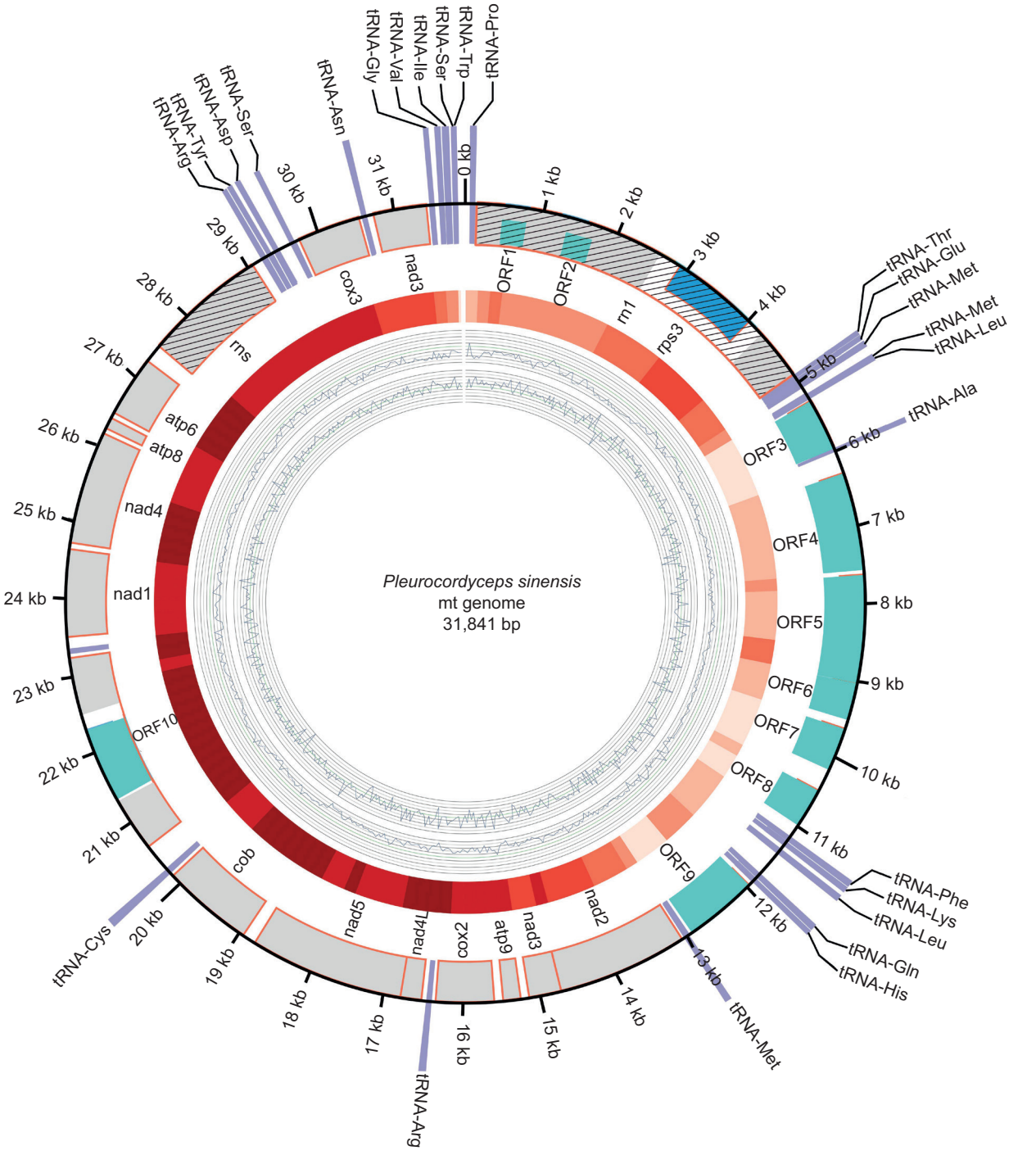

Figure 1. Genetic map of the mitochondrial genome of *Pleurocordyceps sinensis*. Shading blocks with orange frame indicate exons of predicted coding genes, with gene names labeled on the inner side; *rnl* and *rns* genes were marked with slashes; the *rps3* was in blue and nested within an intron of the *rnl* gene; two introns that located in *rnl* and *cox1*, respectively, were shown in blank. Thin flint lines protruding outside of the outer circle indicate tRNAs. Ten predicted ncORFs were shown as light blue blocks. The red circle represented sequence coverage with the highest at 5,200× and the lowest at 4,300× (average at 4,750×). The outer and inner diagram of curves represented GC content and GC skew, respectively.

Table 1. Mitochondrial genome annotation of *Pleurocordyceps sinensis*.

Genes	Strands	Positions	Lengths (bp)	Introns	Start/stop codons	Anticodons
tRNA-Pro [P]	+/CW	53–125	73			TGG
rnl	+/CW	155–4876	4722	IA (1643), 2583–4225		
ncORF1	–/CW	818–519	300		ATG/TAA	
ncORF2	–/CW	1581–1252	330		ATG/TAA	
rps3	+/CW	2816–4132	1317		ATG/TAA	
tRNA-Thr [T]	+/CW	4793–4863	71			TGT
tRNA-Glu [E]	+/CW	4869–4941	73			TTC
tRNA-Met [M1]	+/CW	4942–5012	71			CAT
tRNA-Met [M2]	+/CW	5019–5091	73			CAT
tRNA-Leu [L]	+/CW	5177–5258	82			TAA
ncORF3 (GIY-YIG)	+/CW	5295–5942	648		ATG/TAA	
tRNA-Ala [A]	+/CW	5933–6005	73			CGC
ncORF4	+/CW	6307–7539	1233		ATG/TAA	
ncORF5	+/CW	7629–8987	1359		ATG/TAA	
ncORF6	+/CW	9018–9470	453		ATG/TAA	
ncORF7	+/CW	9619–10,149	531		ATG/TAA	
ncORF8	+/CW	10,473–10,958	486		ATG/TAG	
tRNA-Phe [F]	+/CW	11,156–11,228	73			GAA
tRNA-Lys [K]	+/CW	11,230–11,302	73			TTT
tRNA-Leu [L2]	+/CW	11,354–11,435	82			TAG
tRNA-Gln [E2]	+/CW	11,764–11,836	73			TTG
tRNA-His [H]	+/CW	11,841–11,914	74			GTG
ncORF9 (LAGLIDADG)	+/CW	11,968–12,888	921		ATG/TAA	
tRNA-Met [M3]	+/CW	12,947–13,019	73			CAT
nad2	+/CW	13,061–14,737	1677		ATG/TAA	
nad3	+/CW	14,738–15,151	414		ATG/TAA	
atp9	+/CW	15,260–15,484	225		ATG/TAA	
cox2	+/CW	15,598–16,344	747		ATG/TAA	
tRNA-Arg [R1]	+/CW	16,391–16,461	71			ACG
nad4L	+/CW	16,526–16,795	270		ATG/TAA	
nad5	+/CW	16,795–18,792	1998		ATG/TAA	
cob	+/CW	18,951–20,120	1170		ATG/TAA	
tRNA-Cys [C]	+/CW	20,176–20,247	72			GCA
cox1	+/CW	20,597–23,225	2629	IB (1036), 21,336–22,372	ATA/TAA	
ncORF10	+/CW	21,335–22,285	933		ATA/TAA	
tRNA-Arg [R2]	+/CW	23,276–23,346	71			TCT
nad1	+/CW	23,495–24,616	1122		ATG/TAA	
nad4	+/CW	24,699–26,156	1458		ATG/TAA	
atp8	+/CW	26,228–26,374	147		ATG/TAA	
atp6	+/CW	26,450–27,235	786		ATG/TAA	
rns	+/CW	27,513–29,036	1524			
tRNA-Tyr [Y]	+/CW	29,189–29,272	84			GTA
tRNA-Asp [D]	+/CW	29,284–29,357	74			GTC
tRNA-Ser [S1]	+/CW	29,370–29,452	83			GCT
tRNA-Asn [N]	+/CW	29,619–29,690	72			GTT
cox3	+/CW	29,733–30,542	810		ATG/TAA	
tRNA-Gly [G]	+/CW	30,578–30,648	71			TCC

(continues)

Table 1. Continued

Genes	Strands	Positions	Lengths (bp)	Introns	Start/stop codons	Anticodons
nad6	+/CW	30,732–31,418	687		ATG/TAA	
tRNA-Val [V]	+/CW	31,452–31,524	73			TAC
tRNA-Ile [I]	+/CW	31,580–31,651	72			GAT
tRNA-Ser [S2]	+/CW	31,656–31,742	87			TGA
tRNA-Trp [T]	+/CW	31,755–31,826	72			TCA

Note: +, genes encoded on positive strain; –, genes encoded on negative strain; CW, genes were clockwise oriented.

ATA. In addition, 24 of those genes used TAA as the stop codon except the ncORF8, which used TAG (Table 1).

Noncoding RNAs

In addition to the 15 protein-coding genes, a large and a small ribosomal RNA (*rnl* and *rns*, respectively) and 25 tRNA genes were also identified (Table 1). The tRNA genes ranged in size from 71 to 87 bp and could correspond to 20 amino acids. A majority of amino acids were coded by only one tRNA gene; however, Serine (Ser), Arginine (Arg), Methionine (Met), and Leucine (Leu) had 2, 2, 3, and 2 tRNA genes, respectively (Table 1). All noncoding RNAs (tRNA, rRNA) were found on the positive strand and oriented clockwise.

Intronic and intergenic regions

Exons of protein-coding genes, rRNA and tRNA genes, had a total length of 20,873 bp accounting for 65.55% of the mt genome. Ten ncORFs (7,194 bp) accounted for 22.59% of the mt genome. Only two introns (group I) were predicted, including one further classified into subgroup IA (1,643 bp) in *rnl* and one classified into subgroup IB in *cox1* (1,036 bp), respectively, making up 8.5% of the entire mt genome. The intergenic sequences had a total length of 1,070 bp covering 3.4% of the genome.

Gene component and synteny

Although different numbers of ncORFs (hypothetical proteins) would be predicted for hypocrealean fungi, the content and synteny of 15 protein-coding genes remained largely conserved, except in a few cases. For instance, location of the *cox2* gene shifted in three species of *Acremonium chrysogenum*, *A. fuci*, and *Clonostachys rose* comparing to other Hypocreales; an additional copy of *rps3* and *atp9* gene was found in *Beauveria malawiensis* and *Fusarium solani* IISc-1; an extra copy of *nad1* and *nad4* was found from *F. oxysporum* UASWS AC1 (KR952337); the location of the two genes were found to be reversed in *F. oxysporum* f. sp. *matthiolae* (CM019668); and the mt genome of *F. oxysporum* f. sp. *fragariae* GL1381 (CM029251) was found to lose *cox3* and *nad6* genes and possess an extra reversed copy of genes of *cob*, *cox1*, *nad1*, *nad4*, *atp8*, and *atp6*; an even extreme case was found in *Sarocladium implicatum* in which three genes (*cob*, *cox3*, and *nad6*) were lost and the *nad4* gene shifted its location from the *nad1-atp8* junction to a position between *rps3* and *nad2* (Supplementary Table S1).

Phylogenetic analyses

Eighty-one complete mt genomes representing 63 distinct species from the order Hypocreales were included in phylogenetic analyses. After excluding the ambiguous aligned regions, a total of 4,345 amino acid sequences of 14 conserved proteins were retained. All species of Hypocreales formed a well-supported clade (BP = 100%) in ML analysis. Within the clade, four family-level subclades were recognized with strong supports (BP = 100%), that is, *Nectriaceae*, *Bionectriaceae*, *Hypocreaceae*, and *Clavicipitaceae* (Figure 2). Species in the family *Ophiocordycipitaceae* were clustered into two subclades, one subclade that consists of four *Tolypocladium* species showed a sister group relationship with the *Clavicipitaceae* clade with low bootstrap support (BP = 75%), the other highly supported (BP = 100%) subclade comprised four *Hirsutella* species (*H. minnesotensis*, *H. rhossiliensis*, *H. thompsonii*, and *H. vermicola*) and *O. sinensis* (Figure 2). It is interesting to find that *P. sinensis* clustered with the *Clavicipitaceae* clade with 100% bootstrap support rather than grouped with either two subclades of *Ophiocordycipitaceae*.

Discussion

The complete mt genome of the ex-type strain CN 80-2 of the species *P. sinensis* described here is the first reported case for the newly proposed genus *Pleurocordyceps* (Wang *et al.*, 2021) and *Polycephalomyces* s. l. It is rather compact compared with other Hypocreales species,

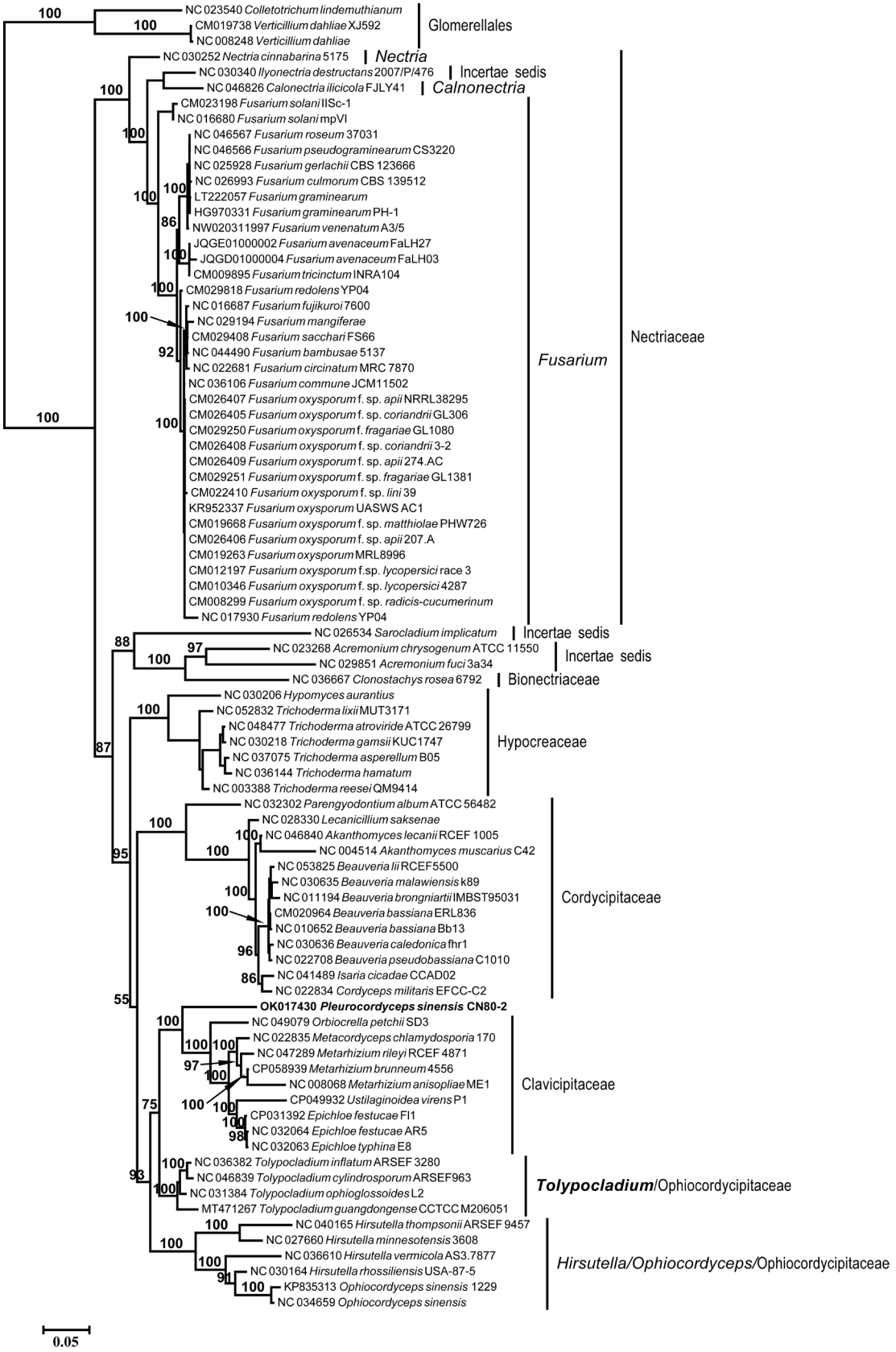

Figure 2. Phylogenetic relationships of Hypocreales inferred from 14 conserved protein sequences (*cox1, cox2, cox3, cob, atp6, atp8, atp9, nad1, nad2, nad3, nad4, nad4L, nad5, and nad6*) using the maximum likelihood method. Bootstrap values were shown above the branches. Three species in the order *Glomerellares* were used as outgroups.

especially *O. sinensis*, from which the species was isolated. The genome sizes of the two sequenced isolates of *O. sinensis* were 157,510 bp (KP835313) and 157,539 bp (NC_034659), respectively, almost five times larger than *P. sinensis*. The reason for this remarkable size variation was considered to be the presence of large numbers of repetitive regions, which mainly consisted of intronic mobile elements of HEs and reverse transcriptases (RTs) (Li *et al*., 2015). In the expanded mt genome of *O. sinensis*, 32 HEs genes (21 LAGLIDADG and 11 GIY-YIG endonuclease) and 10 RTs genes were found in group I and group II introns, respectively (Li *et al*., 2015), while only two HEs genes, that is, one GIY-YIG (ncORF3) and one LAGLIDADG endonuclease (ncORF9) were found in *P. sinensis*. After comparing mt genomes of three isolates of the same species, it was also found that larger genomes contained more introns and more intronic HEs genes in *Cordyceps militaris* (Zhang *et al*., 2015).

As mt genome sizes varied greatly in hypocrealean fungi, ranging from 22,376 bp of *S. implicatum* (Yao *et al*., 2016) to 272,497 bp of *Ophiocordyceps camponoti-floridani* (Will *et al*., 2020), it will be interesting to see whether the mt genome size variation was related to expansion of mobile elements of HEs and RTs and how those elements evolved. Megarioti and Kouvelis (2020) recently proposed an "aenaon" model for the evolution of HEs genes and their host introns; thus, free-standing introns and HEs genes were the ancestral form and could invade intron-free coding genes together; HEs genes and their host introns coevolved through recombination, transposition, and horizontal gene transfer. As observed in this case, the two HEs genes found in *P. sinensis* were located in intergenic regions (free-standing or sole mobile in other words) of tRNA genes (Table 1) rather than invaded into intronic regions of coding genes (intron homing). While in *O. sinensis*, HEs and RTs genes were all found to be intronic, either in group I or group II introns (Li *et al*., 2015). It may indicate that the species was earlier diverged than *O. sinensis* according to the "aenaon" model. Considering that the species was isolated from *O. sinensis* and might be a fungal parasite of the latter, and moreover, several other species in *Polycephalomyces* s. l. have often been found to associate with entomopathogenic *Cordyceps* s. l. (Kobayasi, 1941), it is reasonable to hypothesize that species of *Polycephalomyces* s. l. gained hyperparasitic ability to entomophagous fungi during the evolutionary process.

Despite the size variation of mt genome, the gene contents and synteny (gene order) are largely conserved within the order Hypocreales, generally encoding 15 known proteins and 2 rRNAs (*rnl* and *rns*) (Li *et al*., 2015). The genome size variation observed in hypocrealean fungi was probably not associated with taxonomic classification since notable variation was also observed within the same genus or even within the same species. As shown in Supplementary Table S1, mt genome sizes varied from 30,629 bp to 110,525 bp in the genus *Fusarium*, and from 34,477 bp to 52,424 bp within the species of *F. oxysporum.* This variation is largely due to the presence of various introns and the lengths of intergenic regions (Burger *et al*., 2003).

Although remarkable variation in terms of gene order, genome size, composition of intergenic regions, and presence of repeats, introns, and associated ncORFs have been observed between the major fungal phyla (Aguileta *et al*., 2014), this variance may not occur within the same fungal groups (order or below). As observed in this study, the genome size, composition of intergenic regions, and presence of repeats, introns, and associated ncORFs varied within the order Hypocreales; however, the gene content and synteny remained highly conserved even though a few exceptional cases were observed (listed in Supplementary Table S1). A part of these exceptions were probably due to the incorrect assembly. It would be interesting to know the mt genome evolutional process, that is, gene gain and loss events happened during the evolutionary history of different major fungal groups.

Most protein-coding genes and ncORFs used standard mt initial and terminal codons (ATG and TAA, respectively) in *P. sinensis*, except the ncORF8, *cox1*, and ncORF10. ncORF10 and *cox1* were initiated by ATA, and ncORF8 was terminated by TAG. It is noteworthy that *cox1* is usually found to use nonstandard start codons such as TCG, ACC, CGA, CTA, CCG, and AAA in insect mt genomes (Fenn *et al*., 2007; Wei *et al*., 2010), and ATA has been recorded to be used as the initial codon in organisms like *Pseudocohnilembus persalinus* (Gao *et al*., 2018), *Wellcomia siamensis* (Park *et al*., 2011), and *Calanus sinicus* (Wang *et al*., 2011). Although most hypocrealean species used ATG as the initial codon of *cox1* gene, exceptional cases were also reported in *Hirsutella rhossiliensis* (NC_030164) and *Calonectria ilicicola* (Gai *et al*., 2020), in which TTG were used.

The mt genome released in this study provided additional evidence that *P. sinensis* is not the anamorph of *O. sinensis* but represents another fungus, and moreover, *P. sinensis* was found to cluster with *Clavicipitaceae* rather than *Ophiocordycipitaceae* species with very strong supports (BP = 100%) in ML phylogenetic analyses (Figure 2). It is also noteworthy that the family *Ophiocordycipitaceae* was paraphyletic, which contradicts previous studies applying multi-gene phylogeny (Sung *et al*., 2007a) although the paraphyly was not well supported (Figure 2). It should be clarified whether those contradictions were due to the incongruence of phylogenies revealed by different molecular markers since mt DNA may tell different evolutionary stories than nuclear genes (Burger *et al*., 2003), or were just caused by the insufficient taxon

sampling or analytical difference. Sung *et al*. (2007b) conducted multi-gene phylogenetic analyses of clavicipitaceous fungi and compared the performance of seven loci including the nuclear ribosomal small and large subunit DNA (*nrSSU* and *nrLSU*), *ß-tubulin*, elongation factor 1*α* (*EF-1α*), the largest and second largest subunits of RNA polymerase II (*RPB1* and *RPB2*), and one mt protein-coding gene ATP Synthase subunit 6 (*mtATP6*), and found that seven genes gave incongruent topologies in higher-level relationships from each other and also from the combined dataset. It also showed that the only mt fragment (*mtATP6*) used in the study possessed localized incongruence and simultaneously provided an increased level of support for certain nodes. Phylogenetic incongruence revealed by different markers, especially those from mt and nuclear fragments, respectively, has been frequently reported and compared in different organisms (e.g. Kimball *et al*., 2021; Mikula *et al*., 2021; Zhang *et al*., 2021). It still remains unclear as to whether the nuclear genome sequences (fragments or whole genome data) or the mt genome sequences (fragments or complete data) could provide a better resolution of fungal phylogeny.

As in the case of clavicipitaceous fungi, almost all the later publications on taxonomy and phylogenetic studies accepted the backbone phylogeny created by Sung *et al*. (2007a, 2007b), and continued to use the five gene dataset (e.g. Kepler *et al*., 2013; Wang *et al*., 2021; Xiao *et al*., 2018). While the above two studies (Sung *et al*., 2007a, 2007b) failed to include species of *Polycephalomyces* s. l. Kepler *et al*. (2013) then included several species of this group and found those species represented a clade distinct from other clavicipitoid genera, and treated them as incertae sedis of Hypocreales. Further studies are needed to reconstruct a reliable phylogenetic relationship of clavicipitaceous fungi, especially the assignment of species of *Polycephalomyces* s. l. Since an increasing number of whole mt genomes have recently been sequenced and released for hypocrealean species, and even more are being proceeded, the plentiful phylogenetically informative sites from the conserved protein coding genes of the whole mt genome would provide valuable information for phylogenetic reconstruction of this important fungal group. While publically released data should be carefully treated since they could probably include assembly and annotation errors as observed in *Ophiocordyceps camponoti-floridani* EC05 (CM022976) and *Ophiocordycipitaceae* sp. (NC_049089), correct annotation and characterization are always necessary (Kortsinoglou *et al*., 2019; Megarioti and Kouvelis, 2020).

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Conflict of Interest

The authors declare that they have no competing interests.

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Table S1. Taxa used in phylogenetic analyses and mt genome comparison of different organisms within the order Hypocreales.

Organism current Name	Accession	Order	Family	Size (Kb)	GC%																
Colletotrichum lindemuthianum	NC_023540	Glomerellales	Glomerellaceae	36.957	30.88	rps3	nad2		atp9	cox2	nad4L	nad5	nad4	atp8		atp6	nad1		cob	cox1	cox3	nad6
Verticillium dahliae	NC_008248	Glomerellales	Plectosphaerellaceae	27.184	27.32	rps3	nad2	nad3	nad1	nad4	atp8	atp6	cox3	nad6		atp9	cox2		nad4L	nad5	cob	cox1
Verticillium dahliae XJ592	CM019738	Glomerellales	Plectosphaerellaceae	27.169	27.16	rps3	nad2	nad3	nad1	nad4	atp8	atp6	cox3	nad6		atp9	cox2		nad4L	nad5	cob	cox1
Clonostachys rosea 6792	NC_036667	Hypocreales	Bionectriaceae	40.921	27.90	rps3	nad2	nad3	atp9		nad4L	nad5	cob	cox1		nad1	nad4	cox2	atp8	atp6	cox3	nad6
Epichloe festucae AR5	NC_032064	Hypocreales	Clavicipitaceae	88.744	27.52	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Epichloe festucae Fl1	CP031392	Hypocreales	Clavicipitaceae	52.960	27.18	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Epichloe typhina E8	NC_032063	Hypocreales	Clavicipitaceae	84.630	26.96	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Metacordyceps chlamydosporia 170	NC_022835	Hypocreales	Clavicipitaceae	25.615	28.28	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Metarhizium anisopliae ME1	NC_008068	Hypocreales	Clavicipitaceae	24.673	28.40	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Metarhizium brunneum 4556	CP058939	Hypocreales	Clavicipitaceae	24.965	28.25	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Metarhizium rileyi RCEF 4871	NC_047289	Hypocreales	Clavicipitaceae	62.514	26.42	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Orbiocrella petchii SD3	NC_049079	Hypocreales	Clavicipitaceae	23.794	28.12	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Ustilaginoidea virens P1	CP049932	Hypocreales	Clavicipitaceae	100.571	27.37	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Akanthomyces lecanii RCEF 1005	NC_046840	Hypocreales	Cordycipitaceae	24.643	27.24	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Akanthomyces muscarius C42	NC_004514	Hypocreales	Cordycipitaceae	24.499	27.15	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Beauveria bassiana Bb13	NC_010652	Hypocreales	Cordycipitaceae	29.961	27.25	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Beauveria bassiana ERL836	CM020964	Hypocreales	Cordycipitaceae	28.820	27.18	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Beauveria brongniartii IMBST95031	NC_011194	Hypocreales	Cordycipitaceae	33.926	27.34	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Beauveria caledonica fhr1	NC_030636	Hypocreales	Cordycipitaceae	38.316	26.26	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Beauveria lii RCEF5500	NC_053825	Hypocreales	Cordycipitaceae	59.014	25.97	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Beauveria malawiensis k89	NC_030635	Hypocreales	Cordycipitaceae	44.135	26.72	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	rps3	nad1	nad4		atp8	atp6	cox3	nad6
Beauveria pseudobassiana C1010	NC_022708	Hypocreales	Cordycipitaceae	28.006	27.54	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Cordyceps militaris EFCC-C2	NC_022834	Hypocreales	Cordycipitaceae	33.277	26.79	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Isaria cicadae CCAD02	NC_041489	Hypocreales	Cordycipitaceae	56.581	26.12	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Lecanicillium saksenae	NC_028330	Hypocreales	Cordycipitaceae	25.919	26.53	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Parengyodontium album ATCC 56482	NC_032302	Hypocreales	Cordycipitaceae	28.081	25.87	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Hypomyces aurantius	NC_030206	Hypocreales	Hypocreaceae	71.638	28.31	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Trichoderma asperellum B05	NC_037075	Hypocreales	Hypocreaceae	29.999	27.78	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Trichoderma atroviride ATCC 26799	NC_048477	Hypocreales	Hypocreaceae	32.758	28.18	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Trichoderma gamsii KUC1747	NC_030218	Hypocreales	Hypocreaceae	29.303	28.25	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Trichoderma hamatum	NC_036144	Hypocreales	Hypocreaceae	32.763	27.67	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Trichoderma lixii MUT3171	NC_052832	Hypocreales	Hypocreaceae	29.791	27.42	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Trichoderma reesei QM9414	NC_003388	Hypocreales	Hypocreaceae	42.130	27.24	rps5(3)[b,c]	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6
Acremonium chrysogenum ATCC 11550	NC_023268	Hypocreales	Incertae sedis	27.266	26.54	rps3	nad2	nad3	atp9		nad4L	nad5	cob	cox1		nad1	nad4	cox2	atp8	atp6	cox3	nad6
Acremonium fuci 3a34	NC_029851	Hypocreales	Incertae sedis	24.565	28.78	rps3	nad2	nad3	atp9		nad4L	nad5	cob	cox1		nad1	nad4	cox2	atp8	atp6	cox3	nad6
Ilyonectria destructans 2007/P/476	NC_030340	Hypocreales	Incertae sedis	42.895	28.23	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1		nad1	nad4		atp8	atp6	cox3	nad6

Sarocladium implicatum	NC_026534	Hypocreales	Incertae sedis	22.376	26.12	rps3	nad4	nad2	nad3	atp9	cox2	nad4L	nad5		cox1	nad1		atp8	atp6		
Calonectria ilicicola FJLY41	NC_046826	Hypocreales	Nectriaceae	39.891	28.48	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium graminearum	LT222057	Hypocreales	Nectriaceae	100.131	32.38	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium culmorum CBS 139512	NC_026993	Hypocreales	Nectriaceae	103.844	31.68	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium pseudograminearum CS3220	NC_046566	Hypocreales	Nectriaceae	110.525	31.64	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium mangiferae	NC_029194	Hypocreales	Nectriaceae	30.629	31.25	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum F11	NC_017930	Hypocreales	Nectriaceae	34.477	30.98	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum f. sp. lini 39	CM022410	Hypocreales	Nectriaceae	38.745	31.02	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum f. sp. fragariae GL1381	CM029251	Hypocreales	Nectriaceae	40.945	31.32	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6		
Fusarium oxysporum f. fragariae GL1080	CM029250	Hypocreales	Nectriaceae	45.629	32.08	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum f. sp. apii 274.AC	CM026409	Hypocreales	Nectriaceae	45.699	32.13	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum f. sp. apii NRRL38295	CM026407	Hypocreales	Nectriaceae	45.699	32.13	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum f. sp. coriandrii 3-2	CM026408	Hypocreales	Nectriaceae	45.699	32.13	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum f. sp. coriandrii GL306	CM026405	Hypocreales	Nectriaceae	45.699	32.13	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium commune JCM11502	NC_036106	Hypocreales	Nectriaceae	47.526	32.42	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum f. sp. radicis-cucumerinum Forc016	CM008299	Hypocreales	Nectriaceae	47.541	32.23	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum f. sp. apii 207.A	CM026406	Hypocreales	Nectriaceae	47.671	32.30	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium tricinctum INRA104	CM009895	Hypocreales	Nectriaceae	48.506	33.05	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum MRL8996	CM019263	Hypocreales	Nectriaceae	48.644	32.00	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium avenaceum FaLH27	JQGE01000002	Hypocreales	Nectriaceae	49.396	33.06	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium avenaceum FaLH03	JQGD01000004	Hypocreales	Nectriaceae	49.402	33.09	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium redolens YP04	CM029818	Hypocreales	Nectriaceae	49.602	32.05	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum UASWS AC1	KR952337	Hypocreales	Nectriaceae	51.536	31.91	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium oxysporum f.sp. lycopersici race 3	CM012197	Hypocreales	Nectriaceae	52.353	31.46	rps3		nad2	nad3	atp9	cox2	nad4L	nad5	cob	cox1	nad1	nad4	atp8	atp6	cox3	nad6

(*continues*)

Table S1. Continued

Organism current Name	Accession	Order	Family	Size (Kb)	GC%															
Fusarium oxysporum f. sp. matthiolae PHW726	CM019668	Hypocreales	Nectriaceae	52.365	31.54	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad4	nad1	atp8	atp6	cox3	nad6
Fusarium oxysporum f. sp. lycopersici 4287	CM010346	Hypocreales	Nectriaceae	52.424	31.47	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium fujikuroi 7600	NC_016687	Hypocreales	Nectriaceae	53.753	32.61	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium solani IISc-1	CM023198	Hypocreales	Nectriaceae	59.514	28.78	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob	atp9	cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium sacchari FS66	CM029408	Hypocreales	Nectriaceae	59.755	32.35	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium solani mpVI	NC_016680	Hypocreales	Nectriaceae	62.978	28.88	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium bambusae 5137	NC_044490	Hypocreales	Nectriaceae	63.593	31.92	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium circinatum MRC 7870	NC_022681	Hypocreales	Nectriaceae	67.109	31.45	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium venenatum A3/5	NW_020311997	Hypocreales	Nectriaceae	78.612	31.71	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium roseum 37031	NC_046567	Hypocreales	Nectriaceae	93.160	31.74	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium gerlachii CBS 123666	NC_025928	Hypocreales	Nectriaceae	93.428	31.91	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Fusarium graminearum PH-1	HG970331	Hypocreales	Nectriaceae	95.638	31.83	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Nectria cinnabarina 5175	NC_030252	Hypocreales	Nectriaceae	69.895	28.71	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Hirsutella minnesotensis 3608	NC_027660	Hypocreales	Ophiocordycipitaceae	52.245	28.42	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Hirsutella rhossiliensis USA-87-5	NC_030164	Hypocreales	Ophiocordycipitaceae	62.483	28.21	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Hirsutella thompsonii ARSEF 9457	NC_040165	Hypocreales	Ophiocordycipitaceae	62.509	29.82	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Hirsutella vermicola AS3.7877	NC_036610	Hypocreales	Ophiocordycipitaceae	53.793	25.27	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Ophiocordyceps camponoti-floridani EC05a	CM022976	Hypocreales	Ophiocordycipitaceae	272.497	27.56	rps3	nad2	nad3	atp9	cox2	nad4L		cob		cox1(3)	nad1(3)	nad4(2)	atp8	atp6	cox3	nad6
Ophiocordyceps sinensis	NC_034659	Hypocreales	Ophiocordycipitaceae	157.539	30.20	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Ophiocordyceps sinensis 1229	KP835313	Hypocreales	Ophiocordycipitaceae	157.510	30.19	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Ophiocordycipitaceae sp.a	NC_049089	Hypocreales	Ophiocordycipitaceae	66.785	30.58	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Pleurocordyceps sinensis CN80-2	OK017430	Hypocreales	Ophiocordycipitaceae	31.841	25.46	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Tolypocladium cylindrosporum ARSEF963	NC_046839	Hypocreales	Ophiocordycipitaceae	34.698	26.97	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Tolypocladium inflatum ARSEF 3280	NC_036382	Hypocreales	Ophiocordycipitaceae	25.328	27.79	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Tolypocladium ophioglossoides L2	NC_031384	Hypocreales	Ophiocordycipitaceae	35.159	27.53	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6
Tolypocladium guangdongense GD15	MT471267	Hypocreales	Ophiocordycipitaceae	46.102	26.10	rps3	nad2	nad3	atp9	cox2	nad4L	nad5	cob		cox1	nad1	nad4	atp8	atp6	cox3	nad6

aTaxa used in comparative analysis while excluded from phylogenetic reconstruction due to the possible error occurred during sequencing and assembly;
bOriginally annotated as rps5 but really represent rps3;
cExceptionals from the majority were highlighted.