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Differential Expression of *Hirsutella sinensis* Genes and Intraspecific Genetic Variation among *H. sinensis* Strains

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ABSTRACT

Genetic heterogeneity has been documented among the 17 genotypes of *Ophiocordyceps sinensis*. However, intraspecific genetic variations in *Hirsutella sinensis* (Genotype #1 of *O. sinensis*) and differential expressions of *H. sinensis* genes have rarely been recognized at the genome and transcriptome levels.

Objective: To explore expressions of the *H. sinensis* genes and intraspecific genetic variations at the genome-transcriptome levels.

Methods: To cross-analyze GenBank sequences of the assembled genome/mitogenome and transcriptome assemblies from *H. sinensis* strains and natural *Cordyceps sinensis*, and unassembled shotgun genome sequences and multiple PCR-amplified gene sequences from >300 *H. sinensis* strains.

Results: Many assembled and unassembled genome sequences were genetically variable and differentially occurred in the genome assemblies of various *H. sinensis* strains. Low sequence similarities of some gene transcripts were also found between the transcriptomic sequences of *H. sinensis* strain L0106 and natural *C. sinensis*. Many genes, including mating-type genes, were differentially transcribed in *H. sinensis* and natural *C. sinensis*. Other genes, including ribosomal 5.8S gene, were transcriptional silencing in *H. sinensis* and natural *C. sinensis*. Multiple genome and transcriptome repeats of numerous genes were identified, some of which contain scattered nonsense, missense, or frame shift mutant alleles.

Discussion: Differential expressions of *H. sinensis* genes and apparent intraspecific genetic variations exist among the *H. sinensis* strains. Inconsistent occurrence of the mating-type genes in *H. sinensis* and their paradoxical transcriptions challenge the hypotheses of homothallism and pseudohomothallism for *H. sinensis* and suggest heterothallism. Such genetic and transcriptional variations among *H. sinensis* strains significantly impact on the proteomic, chemical, therapeutic and safety profiles of natural *C. sinensis* and various mycelial fermentation products that are manufactured using arbitrarily selected strains, warning careful verification of *H. sinensis* strains prior to academic and industrial/commercial uses.

Key Words: Natural *Cordyceps sinensis*; *Hirsutella sinensis* (Genotype #1 of *Ophiocordyceps sinensis*); Differential transcription; Mating-type genes, Intraspecific variations; Genome; Mitogenome; Transcriptome

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INTRODUCTION

Natural Cordyceps sinensis is a precious therapeutic agent in traditional Chinese medicine with a rich history of clinical use for health maintenance, disease amelioration, post-disease recovery, and antiaging therapy [1-3]. The Chinese Pharmacopoeia defines natural *C. sinensis* as an insect-fungi complex, containing the *Ophiocordyceps* sinensis fruiting body and a dead larva from the Hepialidae family [4-6]. Studies over the past 2 decades have demonstrated its heterokaryotic multicellular structure and dramatic genetic heterogeneity with at least 17 genotypes of *O. sinensis* and >90 fungal species spanning >37 genera with using various molecular approaches [7-36]. Many publications on natural *C. sinensis* and *O. sinensis* have primarily focused on *Hirsutella sinensis* (Genotype #1 and the postulated anamorph of *O. sinensis*) through traditional mycology technology based on fungal morphology and growth characteristics. However, the observation of *Hirsutella*-like and *H. sinensis*-like morphologies has generated uncertainty among mycologists in taxonomic determinations of multiple mutant genotypes of *O. sinensis* and fungi in the families Clavicipitaceae and Ophiocordycipitaceae and in the genera *Harposporium* and *Polycephalomyces* [6,12-13,16,30,32,37]. The sequences of AT-biased genotypes of *O. sinensis* reside not in the genome of the GC-biased *H. sinensis* but in the genomes of independent *O. sinensis* fungi, indicating that the *O. sinensis* genotypes belong to independent *O. sinensis* fungi [5-11,14,17-19,26,28,35-36,38].

Molecular marker polymorphism assays and multigene analyses have demonstrated apparent polymorphic alterations in multiple *H. sinensis* strains isolated from *C. sinensis* specimens collected from the same or geographically different production areas [16,39-45]. However, knowledge of intraspecific genetic variations in Genotype #1 *H. sinensis* at the genome and transcriptome levels remains limited.

In ascomycetous fungi, the mating system is usually controlled by the mating-type (MAT) loci [46]. Bushley et al [24]. and Hu et al [26]. detected 4 mating-type genes of *MAT1-1* and *MAT1-2* idiomorphs in the genomes of the *H. sinensis* strains CS68-2-1229 and Co18 and hypothesized homothallism/pseudohomothallism for *H. sinensis*. Zhang et al [39]. and Zhang and Zhang [47] found the differential existence of MAT1-1-1 and/or MAT1-2-1 genes in various *H. sinensis* strains, hypothesizing facultative hybridization for *H. sinensis* for differential occurrence of (pseudo-) homothallism and heterothallism based on different genetic materials in various *H. sinensis* strains. However, these hypotheses were solely based on genetic evidence without considering expressions of these *H. sinensis* mating-type genes.

We explored in this study the differential transcription of *H. sinensis* genes and intraspecific genetic variations in *H. sinensis* through comprehensive cross-analysis of the assembled and unassembled shotgun genome and mitogenome sequences from *H. sinensis* strains, PCR-amplified sequences from multiple *H. sinensis* strains, transcriptome assemblies from natural *C. sinensis* and *H. sinensis* strain L0106. The results of this study reveal differential transcription of *O. sinensis* genes (including the ribosomal 5.8S and mating-type genes) in response to natural and unnatural conditions and significant intraspecific genetic variations in the genome and transcriptome sequences of *H. sinensis*.

METHODS

Gene, Genome, and Mitogenome Sequences of *H. Sinensis* Strains

Four sets of the assembled shotgun genome sequences, ANOV00000000, LKHE00000000, LWBQ00000000, and JAAVMX000000000 of *H. sinensis* strains Co18, 1229, ZJB12195, and IOZ07, respectively, and 2 complete mitogenome sequences (KP835313 of strain 1229 and KY622006 of natural *C. sinensis*) are available in GenBank [26,28-29,35-36,48]. (**Table S1**) lists 312 *H. sinensis* strains that were used to obtain assembled shotgun genome and mitogenome sequences, PCR-amplified gene sequences, unassembled shotgun genome sequences, transcriptome shotgun assemblies and a group of mRNA sequences [4,13,16,24,26,28-29,33,35-36,39,41,49-60].

Sequencing and Assembling Methods for Shotgun Genome and Mitogenomes Sequences

Genomic DNA from strain Co18 was sequenced with the Roche 454 GS FLX system (Illumina HiSeq: 454), and the shotgun sequences were assembled using SOAPdenovo v.1.05 and Newbler v.2.3 under accession #ANOV01000001-ANOV01025873 [26]. Genomic DNA from strain 1229 was sequenced with Illumina HiSeq sequencing technology, and the shotgun sequences were assembled using ABySS v.1.2.3 under accession #LKHE01000001-LKHE01003687 [28]. Genomic DNA from strain ZJB12195 was sequenced with Illumina sequencing technology (Hiseq 2000 Sequencing System), and the shotgun sequences were assembled under accession #LWBQ01000001-LWBQ01000618 using SOAPdenovo v.2.0 [35]. Genomic DNA from strain IOZ07 was sequenced with PacBio Sequel sequencing technology, and the shotgun sequences were assembled under accession #JAAVMX010000001-JAAVMX010000023 using Canu v.1.7 [36].

The mitochondrial sequences of *H. sinensis* strain 1229 were extracted from the filtered reads containing both nuclear and mitochondrial genomes. The corrected reads were fully assembled with the Celera Assembler program, refined with Quiver, and verified by PCR amplification. The mitogenome sequence KP835313 for strain 1229 is accessible in GenBank [29].

Natural *C. sinensis* specimens were purchased in Guoluo of Qinghai Province, China. Total DNA was extracted from the *C. sinensis* stroma, and randomly sheared to fragments with an average size of 20 kb for sequencing on a PacBio RS II sequencing platform. The mitochondrial genome was assembled through Hierarchical Genome Assembly Process (HGAP) workflow, including preassembly, error correction, Celera assembly and polishing with Quiver [61]. The mitogenome sequence KY622006 for natural *C. sinensis* is accessible in GenBank [57].

The PCR-Amplified Sequences of OSRC14, OSRC19, OSRC27, And OSRC32 Marker Genes

The OSRC14 marker gene sequences are under the GenBank accession numbers: KM197544, JQ277381-JQ277382, JQ277386, JQ277389-JQ277392, JQ325373, JQ325377, JQ325381-JQ325382, JQ325386, JQ325390, JQ325397-JQ325398, JQ325402, JQ325408-JQ325409, JQ325422, JQ325429,

JQ325431, JQ325438, JQ325442-JQ325443, JQ325451, JQ325455, JQ325458, JQ325461-JQ325462, JQ325464, and JQ325472-JQ325487 for 47 *H. sinensis* strains (**Table S1**) [39,41-42].

The OSRC19 marker gene sequences of *H. sinensis* are under the GenBank accession numbers: JM973741 and JQ277405-JQ277408 for 5 *H. sinensis* strains (**Table S1**) [39,42]).

The OSRC27 marker gene sequences of *H. sinensis* are under the GenBank accession numbers: JQ277433-JQ277436, JQ325605, JQ325609, JQ325613-JQ325614, JQ325618, JQ325634, JQ325640-JQ325641, JQ325654, JQ325661, JQ325671, JQ325675, JQ325683, JQ325687, JQ325690, JQ325693-JQ325694, JQ325696, and JQ325704-JQ325718 for 37 *H. sinensis* strains (**Table S1**) [39,41-42].

The OSRC32 marker gene sequences of *H. sinensis* are under the GenBank accession numbers: JM973601, JQ277445, JQ277447-JQ277448, JQ325721, JQ325725-JQ325726, JQ325729-JQ325730, JQ325734, JQ325750, JQ325756-JQ325757, JQ325760, JQ325770, JQ325777, JQ325780, JQ325784, JQ325787, JQ325791-JQ325792, JQ325799, JQ325803, JQ325806, JQ325809-JQ325810, JQ325812, JQ325820-JQ325822, JQ325824-JQ325825, JQ325829-JQ325831, and JQ325833 for 36 *H. sinensis* strains (**Table S1**) [39,41-42].

PCR-amplified Sequences of Other *H. sinensis* genes

Multiple PCR-amplified *H. sinensis* sequences in GenBank also include in this cross-analysis: 116 sequences of MAT1-1-1 gene, 183 sequences of MAT1-2-1 gene, 125 sequences of serine protease gene (csp1), 45 sequences of beta-tubulin 1 gene (β-tub1), 51 sequences of translation elongation factor 1-alpha gene (tef1α), 41 sequences of the largest subunit of RNA polymerase II (rpb1), 9 sequences of the second largest subunit of RNA polymerase II (rpb2), as well as partial 18S gene (nrSSU EF468971) and 28S gene (nrLSU EF468827) sequences of strain EFCC7287 [4,16,24,26,39,41,47,49-52,54-60].

Shotgun Transcriptome Assemblies of *H. sinensis* Strain L0106 and Natural *C. sinensis* and mRNA Sequences of Strain L0106

Two transcriptome assemblies are accessible in GenBank. A specimen of natural *C. sinensis* (unknown maturational status) was collected in Kangding County, Sichuan Province, China. Total RNA from this specimen was sequenced using 454 technologies. The sequences longer than 50 bp. from the 454 reads were assembled into unique sequences (containing contigs and singletons) using the GS De Novo Assembler software v 2.6 or Newbler 2.6 (454 Life Sciences Corporation, USA). The shotgun sequences were assembled under GenBank accession #GAGW01000001-GAGW01016676 using Newbler v.2.3 and 2.6 [53].

The transcriptome assembly GCQL00000000 was derived from fermented mycelia of strain L0106. The mycelia were collected for total RNA extraction from cultures grown for 3, 6, and 9 days. Total RNA (20 mg per sample) was subjected to mRNA purification and total mRNA was used to construct a cDNA library and sequenced using Illumina HiSeq sequencing technology. The shotgun nucleotide sequences were assembled under GenBank accession #GCQL01000001-GCQL01020586 using SOAPdenovo v.2.0 [33]. Additional 41 mRNA sequences (KP090933-KP090973) from strain L0106 were also sequenced with Illumina sequencing technology.

Sequence Alignment Analysis

All genome, mitogenome, and transcriptome sequences and other PCR-amplified DNA sequences were analyzed using the MegaBlast or discontinuous MegaBlast programs provided by GenBank (https://blast.ncbi.nlm.nih.gov/).

RESULTS

The ITS1-5.8S-ITS2 Sequences in the Genomes of *H. sinensis* Strains

A single copy of ITS1-5.8S-ITS2 sequences was identified in genomes ANOV00000000, LKHE00000000, and LWBQ00000000 of strains Co18, 1229, and ZJB12195, respectively, using the 2nd generation of sequencing technology but multiple copies of ITS1-5.8S-ITS2 sequences in genome JAAVMX000000000 of strain IOZ07 with using the 3rd generation of sequencing technology (**Table 1**) [26,28,35-36]. These *H. sinensis* ITS sequenc-

Table 1: Comparisons of the ribosomal ITS1-5.8S-ITS2 sequences of *H. sinensis* strains.

H. sinensis strain	ITS1-5.8S-ITS2 sequence		% Similarity to GC-biased AB067721 (59→549) of strain GYOKUJU
	Accession #	Range & direction	
1229	LKHE01000582	2,132→2,622	100% (491/491)
Co18	ANOV01021709	896→1,386	99.8% (490/491)
ZJB12195	LWBQ01000008	991,797→992,287	99.4% (488/491)

IOZ07	JAAVMX010000002	18,688,917→18,689,407	100% (491/491)
		18,702,095→18,702,586	97.4% (485/498)
	JAAVMX010000008	13,823→14,313	100% (491/491)
		1,199→1,687	99.2% (488/492)
	JAAVMX010000017	9,147←9,637	100% (491/491)
		21,791←22,281	100% (491/491)
		34,435←34,925	100% (491/491)
		47,079←47,569	100% (491/491)
	JAAVMX010000018	13,381→13,871	100% (491/491)
		26,076→26,566	100% (491/491)
		38,771→39,261	100% (491/491)
		51,467→51,958	99.0% (488/493)
	JAAVMX010000019	700→1,186	97.0% (479/494)
		19,404→19,894	100% (491/491)
		32,048→32,538	100% (491/491)
		6,233→6,733	95.5% (476/498)
		44,729→45,251	91.8% (480/523)

es are GC-biased and 80.1%-89.9% similar to the sequences of AT-biased Genotypes #4-6, #15-17 of *O. sinensis*.

(**Figure 1**) shows illustratively the locations of the *H. sinensis* ITS1-5.8S-ITS2 (59→549 of AB067721; 896→1,386 of ANOV01021709; 2,132→2,622 of LKHE01000582; 991,797→992,287 of LWBQ01000008; multiple segments of JAAVMX000000000) and several partial 18S and 28S gene sequences of strains GYOKUJU, Co18, 1229, ZJB12195, IOZ07, EFCC7287, and YN07-8. LKHE01000582 (dark blue), LWBQ01000008, ANOV01021709/ANOV01022831/ANOV01024581, and JAAVMX010000002/JAAVMX010000008/JAAVMX010000017-JAAVMX010000019 (light blue) are the assembled genome sequences from *H. sinensis* strains 1229, ZJB12195, Co18, and IOZ07, respectively [26,28,35-36]. AB067721 (red) is the PCR-amplified ITS1-5.8S-ITS2 sequence of strain GYOKUJU [13]. EF468971 and EF468827 (green) are the PCR-amplified partial 18S and 28S genes sequences, respectively, from strain EFCC7287 [4]. JM973574-JM973579 (brown) are unassembled shotgun partial 28S gene sequences of strain YN07-8 [39].

The 5.8S Gene Sequence of *H. sinensis* Strains

The ribosomal 5.8S gene sequence (218→373 of AB067721 of strain GYOKUJU) is 100% homologous to most of the 5.8S genome sequences of strains Co18, 1229, ZJB12195 and IOZ07 [13,26,28,35-36]. However, several copies of the 5.8S gene of strain IOZ07 contain some mutant alleles (**Figure S1**): 18,702,254→18,702,409 (97.5%) of JAAVMX010000002, 858→1,011 (95.6%) of JAAVMX010000018, and 6,396→6,550 and 44,889→45,046 (95.6% and 98.7%) of JAAVMX010000019. The mismatched alleles may cause translational interruptions of the once-functional 5.8S gene due to nonsense, frame shift, or missense allelic mismatches. All the 5.8S gene sequences of GC-biased *H. sinensis* (Genotype #1 of *O. sinensis*) are 79.8%-89.6% similar to the 5.8S gene sequences of the AT-biased Genotypes #4-6, #15-17 of *O. sinensis* [6,9,26,28,35-36]. No additional genome sequences of strains Co18, 1229, ZJB12195, and IOZ07 show >89.9% similarities with any of the AT-biased 5.8S gene sequences, comfirming that the sequences of the AT-biased genotypes of *O. sinensis* belong to the genomes of independent fungi [6,8–11]. The transcriptome assembly

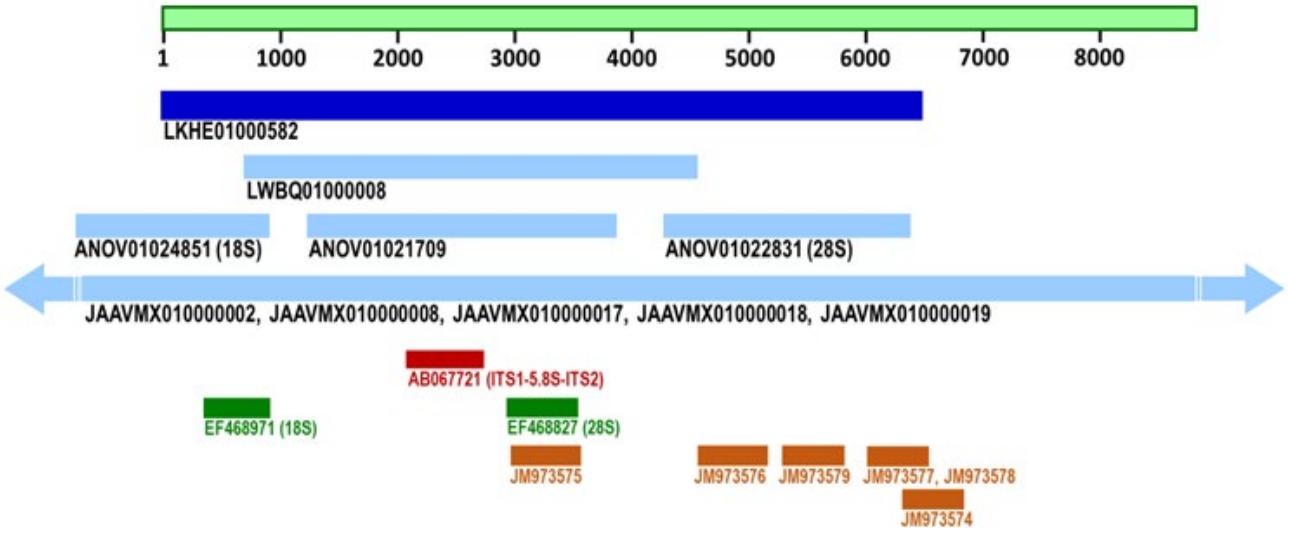


Figure 1: Illustration of the locations of the genome segments of nuclear ribosomal DNA relative to the genome assembly sequence LKHE01000582

GAGW00000000 of natural *C. sinensis* contains no 5.8S gene sequence [53].

The 18S Gene Sequence of LKHE01000582 of *H. sinensis* Strain 1229

The ribosomal 18S gene segment 1→2,132 of LKHE01000582 of strain 1229 is 99.9%-100% homologous to the non-overlapped sequences ANOV01024851 (427→1,198) and ANOV01021709 (1→927) of strain Co18 and LWBQ01000008 (990,360→991,828) of strain ZJB12195 and 100% homologous to multiple segments of the genome assembly JAAVMX000000000 of strain IOZ07: 18,686,785→18,688,948 of JAAVMX010000002; 990,360→991,828 of JAAVMX010000008; 9,606→11,768, 22,250→24,412, 34,894→37,056, 47,538→49,700 of JAAVMX010000017; 11,250→13,412, 23,945→26,107, 36,640→38,802 of JAAVMX010000018; and 4,095→6,233, 17,273→19,435, 29,917→32,079 of JAAVMX010000019 [26,28,35–36]. However, the 18S gene sequence of LKHE01000582 is 97.1%-98.9% similar to 5 other 18S gene segments: 18,699,944→18,702,095 of JAAVMX010000002, 1→1,199 of JAAVMX010000008, 1→730 of JAAVMX010000018, and 4,095→6,265 and 42,577→44,760 of JAAVMX010000019, with scattered transition, transversion and insertion/deletion mutant alleles, which may lead to nonsense, frame shift, or missense mutations of the 18S gene.

The PCR amplified partial 18S gene sequence (EF468971) of *H. sinensis* strain EFCC7287 is 99.4%-99.8% homologous to 18S sequences JX968024-JX968028 obtained from various *H. sinensis* strains using the same pair of primers. EF468971 is >98.6% homologous to the assembled genome sequences ANOV01024581, LKHE01000582, LWBQ01000008, JAAVMX010000002, JAAVMX010000008, and JAAVMX010000017-JAAVMX010000019 of strains Co18, 1229, ZJB12195, and IOZ07, respectively [4,26,28,35–36].

The 18S gene segment (1→2,132) of LKHE01000582 of *H. sinensis* strain 1229 is 99.0%-100% homologous to multiple transcriptome sequences of natural *C. sinensis*: GAGW01005077/GAGW01005078/GAGW01005953/GAGW01012978/GAGW01013875/GAGW01016121/GAGW01013937 [28,53]. The longest transcript GAGW01005077 overlaps with other 6 transcripts.

The 18S gene sequence EF468971 of strain EFCC7287 is 98.6%-99.8% homologous to the overlapped or partially overlapped transcriptome sequences: GAGW01005077/GAGW01005078/GAGW01013937 of natural *C. sinensis*, indicating that the transcripts might be derived from independent fungi that co-colonized in natural *C. sinensis* [4,53].

The 28S Gene Sequence of LKHE01000582 of *H. sinensis* Strain 1229

The ribosomal 28S gene segment 2,623→6,454 of LKHE01000582 of strain 1229 is 82.2%-100% similar to multiple 28S genome segments: ANOV01021709 and ANOV01022831; LWBQ01000008; and JAAVMX010000002/JAAVMX010000008/JAAVMX010000017-JAAVMX010000019 of strains Co18, 1229, and IOZ07, respectively [26,28,35-36], containing numerous, scattered insertion/deletion mutations and some transition and transversion mutant alleles, some of which may cause nonsense, frame shift, or missense mutations of the 28S genes (**Table 2**).

Table 2: Comparisons of the ribosomal 28S sequences of *H. sinensis* strains

Strain	28S gene sequence		% Similarity vs. LKHE01000582 (2,623→6,454) of strain 1229	
	Accession #	Range & direction		
Co18	ANOV01021709	1,387→2,626	100% (1,240/1,240)	Not overlapped
	ANOV01022831	1→2,057	95.6% (2,018/2,110)	
ZJB12195	LWBQ01000008	995,230→996,749	98.7% (1,507/1,527)	
		992,288→994,347	96.9% (1,996/2,060)	
		93,757→94,224	82.2% (447/544)	
IOZ07	JAAVMX010000002	18,689,408→18,693,238	99.8% (3,828/3,832)	
		18,702,587→18,706,423	98.9% (3,807/3,851)	
	JAAVMX010000008	14,314→18,144	99.8% (3,828/3,832)	
		1,688→5,516	99.5% (3,817/3,838)	
	JAAVMX010000017	5,316←9,146	99.8% (3,828/3,832)	
		17,960←21,790	99.8% (3,828/3,832)	
		30,604←34,434	99.8% (3,828/3,832)	
		43,248←47,078	99.8% (3,828/3,832)	
		55,943←58,385	99.8% (2,440/2,445)	
	JAAVMX010000018	13,872→17,702	99.8% (3,828/3,832)	
		26,567→30,397	99.8% (3,828/3,832)	
		39,262→43,092	99.8% (3,828/3,832)	
		1,188→5,007	99.3% (3,808/3,836)	
		51,959→55,162	98.4% (3,168/3,218)	
	JAAVMX010000019	19,895→23,725	99.8% (3,828/3,832)	
		32,539→36,371	99.8% (3,827/3,835)	
		6,734→10,576	98.8% (3,810/3,858)	
		45,253→49,110	98.1% (3,804/3,877)	

Cross-analysis revealed that the 28S genome sequence ANOV01022831 of *H. sinensis* strain Co18 is 92.9%-95.7% similar to the genome segments of LKHE01000582/LKHE01002349, JAAVMX010000002/JAAVMX010000008/JAAVMX010000017-JAAVMX010000019, and LWBQ01000008/LWBQ01000038 of strains 1229, IOZ07, and ZJB12195 with multiple insertions/deletions and transition and transversion point mutations [26,28,35-36].

The PCR-amplified partial 28S RNA gene sequence EF468827 of *H. sinensis* strain EFCC7287 is 99.5%-99.8% homologous to other 28S gene sequences JX968029-JX968033 of various *H. sinensis* strains and the genome sequences ANOV01021709, LKHE01000582, LWBQ01000008, and JAAVMX010000002/JAAVMX010000008/JAAVMX010000017-JAAVMX010000019 of strains Co18, 1229, ZJB12195, and IOZ07, respectively [4,26,28,35-36].

Of the 254 unassembled shotgun *H. sinensis* sequences (JM973567-JM973820) of *H. sinensis* strain YN07-8, JM973574-JM973579 are partial 28S gene sequences at various locations (Figure 1) [39]. JM973577 and JM973578 are overlapped partially overlapped with JM973574.

JM973575 of strain YN07-8 is 99.5%-99.8% homologous to the overlapped sequence EF468827 of *H. sinensis* strain EFCC7287 and genome sequence ANOV01021709 of train Co18 [4,26,39]. JM973574 and JM973576-JM973579 locate downstream of LWBQ01000008 and do not align with any part of the genome sequence LWBQ00000000 (**Figure 1**) [35,39]. JM973574 and JM973577-JM973578 of strain YN07-8 are 98.7%-100% homologous to the genome segments of ANOV01022831, LKHE01000582, and JAAVMX010000002/JAAVMX010000008/JAAVMX010000017-JAAVMX010000019 of strain Co18, 1229, and IOZ07, respectively [26,28,36,39]. JM973576 and JM973579 of strain YN07-8 are 99.3%-99.7% homologous to segments of JAAVMX010000002/JAAVMX010000008/JAAVMX010000017-JAAVMX010000019 and LKHE01000582 of strains IOZ07 and 1229 but only 95.3% and 86.2% similar to ANOV01022831 of strain Co18 with scattered insertion/deletion, transition, and transversion mutant alleles (**Figure S2**).

The 28S gene segment (2,623→6,454) of LKHE01000582 of *H. sinensis* strain 1229 is 97.6%-100% homologous to at least 23 segments of the transcriptome assembly GAGW00000000 of natural *C. sinensis*, but <97% similar to at least 11 other GAGW00000000 segments with scattered transition, transversion, and insertion/deletion, mutant alleles (**Table S2**) [28,53]. Some of these transcriptome sequences are overlapped or partially overlapped, likely indicating divergent genome sources of multiple fungi co-colonized in natural *C. sinensis*.

The overlapped 28S gene sequences EF468827 and JM973575 of strains EFCC7287 and YN07-8 are 99.4%-100% homologous to the transcriptome sequences GAGW01000468/GAGW01000467/GAGW01005959/GAGW01013228/GAGW01014446 of natural *C. sinensis*, some of which are overlapped [4,39,53].

The partial 28S gene sequences JM973574, JM973576-JM973579 of strain YN07-8 are 99.4%-100% homologous to transcriptome sequence GAGW01000465 but 78.5%-100% similar to other 6-10 overlapped transcriptome sequences of natural *C. sinensis* [39,53].

Cross-analysis revealed that the transcriptome sequence 1→720 of GAGW01000465 of natural *C. sinensis* is 99.4%-100% homologous to the genome sequences of LKHE01000582 (4,282→5,001), ANOV01022831 (5→724); LWBQ01000008 (overlapped 994,000→994,347 and 996,402→996,749) of strains 1229, Co18, and ZJB12195, and numerous segment sequences of strain IOZ07: 18,691,067→18,691,786 and 18,704,249→18,704,967 of JAAVMX010000002; 3,349→4,068; and 15,973→16,692 of JAAVMX010000008; 6,768←7,487; 19,412←20,131; 32,056←32,775; 44,700←45,419; and 57,395←58,113 of JAAVMX010000017; 2,836→3,555; 15,531→16,250; 28,226→28,945; 40,921→41,640; and 53,621→54,339 of JAAVMX010000018; and 8,410→9,128; 21,554→22,273; 34,198→34,917; and 46,925→47,644 of JAAVMX010000019 [26,28,36,53].

Second part (720→1,682) of the transcriptome sequence GAGW01000465 is 99.3%-99.5% homologous to the genome sequences of LKHE01000582 (5,341→6,306) of strain 1229 and numerous segment sequences of strain IOZ07: 18,692,126→18,693,091 of JAAVMX010000002; 4,406→5,369 and 17,032→17,997 of JAAVMX010000008; 5,463←6,428; 18,107←19,072; 30,751←31,716; 43,395←44,360; and 56,090←57,055 of JAAVMX010000017; 3,895→4,860; 16,590→17,555; 29,285→30,250; 41,980→42,945; and 54,678→55,162 of JAAVMX010000018; and 9,465→10,430; 22,613→23,578; 35,259→36,223; and 47,988→48,952 of JAAVMX010000019 [26,28,36,53]. Slightly lower similarities (97.8%-98.1%) were also found between the transcriptome segment 720→1,682 of GAGW01000465 and genome sequences: 18,705,306→18,706,280 of JAAVMX010000002, 54,678→55,162 of JAAVMX010000018, and 47,988→48,952 of JAAVMX010000019 with scattered insertion/deletion point mutant alleles and a few transition and transversion point mutations [36,53]. This segment of GAGW01000465 is only 93.5% similar to the genome sequence ANOV01022831 (5→724) of strain Co18 with multiple mismatched alleles [26,53].

Mating-Type Genes of *H. sinensis*

Table 3 lists 235 *H. sinensis* strains that contain either or both MAT1-1-1 and MAT1-2-1 genes listed in GenBank [24,26,28,35-36,40,62]. Twenty two of the strains contain only MAT1-1-1 gene but no MAT1-2-1 gene; 63 contain only MAT1-2-1 gene but no MAT1-1-1 gene; and 150 contain both MAT1-1-1 and MAT1-2-1 genes.

Table 3: *H. sinensis* strains contain either or both MAT1-1-1 and MAT1-2-1 genes listed in GenBank.

Containing only MAT1-1-1 gene (N=12)	Containing only MAT1-2-1 gene (N=30	Containing both MAT1-1-1 & MAT1-2-1 genes (N=55)	
CS09-143	CS09-225	1229	SC04
CS09-229	CS26-277	Co18	SC05
CS68-2-1228	CS34-291	CS09-111	SC06
GS03	CS36-1294	CS09-121	SC07
IOZ07	CS37-295	CS18-266	SC08
SC08	CS70-1211	CS2	SC09_65
SC09_97	CS71-1220	CS25-273	SC09_200

XZ05_6	ID10_1	CS560-961	TB01
XZ06_260	NP10_1	CS561-964	TB02
XZ07_H2	QH02	CS6-251	TB03
XZ09_95	QH05	CS68-2-1229	TB04
YN09_61	QH09_111	CS68-5-1216	TB05
	QH09_157	CS70-1208	TB06
	QH-YS-199	CS71-1218	TB07
	SC09_47	CS71-1219	TB08
	SC09_57	CS76-1284	XZ05_2
	SC09_77	CS91-1291	XZ05_8
	SC-3	GS01	XZ12_16
	SC-5	GS02	YN01
	XZ06_124	GS04	YN02
	XZ-LZ06_1	GS05	YN03
	XZ-LZ06_108	QH01	YN09_3
	XZ-LZ07_H1	QH03	YN09_22
	XZ-NQ_154	QH04	YN09_51
	XZ-NQ_180	QH06	YN09_64
	XZ-SN_44	QH07	
	YN09_6	QH08	
	YN-1	SC01	
	YN-4	SC02	
	ZJB12195	SC03	

Bushley et al [24]. detected 3 mating-type genes of *MAT1-1* idiomorph in the genome sequence KC437356 of strain CS68-2-1229: MAT1-1-1 (6,530→7,748), MAT1-1-2 (4,683→6,183), and MAT1-1-3 (3,730→4,432). These genes are 99.9%-100% homologous to the genome assemblies: LKHE01001116 (3,691←4,909; 5,374←6,874; 7,125←7,827), JAAVMX010000001 (6,698,911→6,700,129; 6,696,939→6,698,439; 6,695,986→6,696,688), and ANOV01017390 (302←1,519) and ANOV01017391 (276←1,776; 2,027←2,729) of strains 1229, IOZ07, and Co18, respectively, but absent from the genome assembly LWBQ0000000 of strain ZJB12195 [24,26,28,35-36]. The MAT1-1-1 sequence of KC437356 is 98.4%-100% homologous to 34 MAT1-1-1 gene sequences of *H. sinensis* in GenBank, but no other MAT1-1-2 or MAT1-1-3 gene sequence of *H. sinensis* listed in GenBank.

The MAT1-1-1, MAT1-1-2, and MAT1-1-3 sequences of KC437356 of strain CS68-2-1229 are absent from the transcriptome assembly GCQL00000000 of *H. sinensis* strain L0106 [24,33]. The MAT1-1-1 sequence is 94.2%-94.6% similar to the transcriptome segment (297←1,129) GAGW01008880 of natural *C. sinensis* with a 48-nt. deletion between nucleotides 358 and 359 of GAGW01008880 [53].

The MAT1-2-1 sequence JQ325153 of *H. sinensis* strain GS09_121 is 99.7%-99.9% homologous to the sequences of the genome assemblies LWBQ01000021 (238,864←239,736), LKHE01001605 (13,851←14,723), and ANOV01000063 (9,319→10,191) of strains Co18, 1229 and ZJB12195, respectively, but absent from the genome assembly JAAVMX000000000 of strain IOZ07 [24,26,28,35-36,42]. JQ325153 is 97.3%-100% homologous to 85 other MAT1-2-1 sequences of *H. sinensis* strains (Table 3).

JQ325153 is 99.6% homologous to segment 388←671 of the transcriptome assembly GCQL01020543 of strain L0106 but 90.3% similar to segment 672←1,153 of GCQL01020543 with a 52-nt. deletion [24,33]. However, the sequence of the MAT1-2-1 gene was absent from the transcriptome assembly GAGW00000000 of natural *C. sinensis* [53].

Translation Elongation Factor 1α (tef1α) Gene

Sequence hologogies of 98.8%-100% were found among the translation elongation factor 1α (tef1α) genes of 52 *H. sinensis* strains [4,42,50,52,54-58]. The tef1α gene was found less sensitive in fungal species identification, because the sequence of *H. sinensis* tef1α gene is 96.2%-96.9% similar to the sequences of *O. robertsii* (EF468766; KC561979), *O. karstii* (KU854945; KU854946), *O. lanpingensis* (KC417462; KC417463), and other *Hirsutella* sp. (KY415601).

The tef1α sequence EF468767 of strain EFCC7287 is 99.5%-99.7% homologous to 4 overlapped or partially overlapped transcriptome sequences of natural *C. sinensis*: GAGW01000517 (332←1,005), GAGW01003987 (1←263), GAGW01014172 (250←479), and GAGW01013074 (1→212) [4,53]. It is absent from the transcriptome assembly GCQL00000000 of the *H. sinensis* strain L0106 [4,33], indicating that tef1α gene was in a silent status in *H. sinensis*. EF468767 and its longest transcriptome sequence GAGW01000517 are 99.4%-99.8% homologous to a single gene copy (JAAVMX010000011, ANOV01000106, LKHE01001641, and LWBQ01000064) of each of the genome assemblies of strains IOZ07, Co18, 1229, and ZJB12195, respectively [4,26,28,35-36,53]. Some of the overlapped tef1α transcripts may likely be derived from various fungi co-colonized in natural *C. sinensis*.

The Largest and Second Largest Subunits of RNA Polymerase II (rpb1 and rpb2) Genes

Sequence hologogies of 98.8%-100% were found among the largest subunit of RNA polymerase II (rpb1) genes of 41 *H. sinensis* strains [4,42,52,54-56]. EF468874 (rpb1) of *H. sinensis* strain EFCC7287 is 100% homologous to a single gene copy (ANOV010001113, LKHE01001285, LWBQ01000001, and JAAVMX010000003) of each of the genome sequences of strains Co18, 1229, ZJB12195, and IOZ07, respectively [4,26,28,35-36]. EF468874 is 99.0%-100% to the transcriptome sequences GCQL01000113 of *H. sinensis* strain L0106 and GAGW01009638 of natural *C. sinensis* [4,33,53].

Sequence hologogies of 99.4%-100% were found among the second largest subunit of RNA polymerase II (rpb2) genes of 9 *H. sinensis* strains [4,42,52,54-56]. EF468924 (rpb2) of *H. sinensis* strain EFCC7287 is 99.4% homologous to the genome sequences ANOV010007657, LKHE01001069, LWBQ01000010, and JAAVMX010000012 of strains Co18, 1229, ZJB12195, and IOZ07, respectively, but 94.3% similar to another ZJB12195 sequence LWBQ01000044 with a 39-nt. insertion [4,26,28,35-36]. EF468924 is 97.6%-100% homologous to the transcriptome sequences GCQL01011291 of *H. sinensis* strain L0106 and 3 non-overlapped transcripts (GAGW01012703/GAGW01015334/GAGW01001851) of natural *C. sinensis* [4,33,53].

Serine Protease (csp1) Gene

Sequence hologogies of 96.5%-100% were found among the serine protease (csp1) genes of 125 *H. sinensis* strains [15,39,42,54]. JQ325256 (csp1) of strain GS09-225 is 99.0%-100% homologous to the genome sequences ANOV0100009487, LKHE01000343, LWBQ01000085, JAAVMX010000012 and JAAVMX010000021 of strains Co18, 1229, ZJB12195, and IOZ07, respectively [4,26,28,35-36]. JQ325256 is 65.9%-70.0% similar to the overlapped transcriptome sequences GCQL01005668 and GCQL01005996 of *H. sinensis* strain L0106 but absent from the transcriptome assembly GAGW00000000 of natural *C. sinensis* [4,33,53], indicating transcriptional silencing of csp1 gene in strain L0106 and natural *C. sinensis*.

Beta-Tubulin 1 (β-tub1) Gene

Sequence hologogies of 96.9%-100% were found among the beta-tubulin 1 (β-tub1) genes of 45 *H. sinensis* strains [15,39,42,54]. JX968019 (β-tub1) of strain QH09-201 is 99.4%-99.8% homologous to the genome assemblies ANOV01001731, LKHE01000036, LWBQ01000017/LWBQ01000184, and JAAVMX010000003 of strains Co18, 1229, ZJB12195, and IOZ07, respectively [26,28,35-36]. JX968019 is 100% homologous to 3 segments (1→312; 311→613; 611→838) of the transcriptome assembly GAGW01002303 of natural *C. sinensis* and 99.0% to a short transcript (1←197) of GCQL01016424 (241 bp) of strain L0106 [33,42,53].

Mitogenome Sequences of *H. sinensis* and Natural *C. sinensis*

Complete mitogenome sequence KP835313 of *H. sinensis* strain 1229 is 99.9% (157,454/157,584) homologous to KY622006 of natural *C. sinensis* with 119 gaps (up to 8-base) of insertions/deletions, but 11 pairs of the short mitogenome segments of KP835313 and KY622006 share 84.9%-98.3% similarities with multiple transition, transversion, and insertion/deletion point mutations [33,48].

Blasting KP835313 of *H. sinensis* strain 1229 against the assembled genome sequences in GenBank database revealed best hits on 217 subject sequences of strain Co18, 1229, ZJB12195 and IOZ07, 138 of which are highly homologous (97.1%-100%) to KP835313, but 79 are <97% similar to KP835313 with scattered transition, transversion and insertion/deletion mutant alleles [26,28-29,35-36].

Of the 254 unassembled shotgun genome sequences of strain YN07-8, sequences JM973570-JM973573, and JM973767 share 99.4%-99.9% homologies with the mitogenomic sequence KP835313 [29,39].

Blasting the mitogenome sequence KP835313 against the assembled transcriptome sequences hits on 1,455 *O. sinensis* transcriptome sequences, many of which are overlapped [29,33,53]. KP835313 is 96.8%-100% homologous to 250 best-hit transcriptome sequences, 198 (ranging 496-4820 nt. in length) of which are segments of the transcriptome GAGW00000000 of natural *C. sinensis* and the rest 52 (ranging 533-3227 nt. in length) are segments of the transcriptome GCQL00000000 of strain L0106.

Blasting the segment 5,261→5,451 of KP835313 hits on 85 overlapped GAGW00000000 transcripts of natural *C. sinensis* that cover >90% of the segment length of KP835313, or 101 transcript repeats that cover >80% of the segment length of KP835313 [33,53]. These GAGW00000000 transcripts share 94.4%-100% similarities with KP835313 sequences with scattered insertion/deletion, transition and transversion mutant alleles in some of the repeats, indicating the GAGW00000000 transcript repeats might likely be derived from multiple fungi co-colonized in natural *C. sinensis*.

Another mitogenome segment 1→506 of KP835313 shares 99.4% homology with mitochondrial RNA ligase gene transcript GAGW01012749 of natural *C. sinensis* [33,53]. GAGW01012749 is 97%-100% homologous to 64 overlapped GAGW00000000 transcripts that cover ≥ 90% of the length of GAGW01012749, or to 122 transcript repeats that cover ≥ 80% of the length of GAGW01012749 with scattered insertion/deletion and transition and transversion mutant alleles.

Both segments (1→506 and 5,261→5,451) of KP835313 did not align to any part of the transcriptome assembly GCQL00000000 of strain L0106 [29,33].

Genome Sequence ANOV01021101 of *H. sinensis* Strain Co18

Segment 1→2,009 of ANOV01021101 of strain Co18 is 97.1% similar to segments 22,379→24,387 of LKHE01000642 and 435,899→437,907 of JAAVMX010000012 of strains 1229 and IOZ07, respectively (**Figure S3**), but absent from the genome assembly LWBQ00000000 of strain ZJB12195 [26,28,35-36]. Cross-analysis showed that LKHE01000642 is 97.1%-100% homologous to segments of JAAVMX010000012, LWBQ01000085, ANOV01002198/ANOV01021101/ANOV01021102 of strains IOZ07, ZJB12195, and Co18, respectively.

Segment 368→2,009 of ANOV01021101 is 99.9% homologous to segment 80→1,721 of the transcriptome sequence GCQL01010475 of strain L0106 [26,33]. Segment 24→638 of ANOV01021102 of strain Co18 is 100% homologous to overlapped transcripts GCQL01014530 (1←649) and GCQL01010475 (1,873→2,487) of strain L0106 [26,33]. ANOV01021101 and ANOV01021102 did not align to any part of the transcriptome assembly GAGW00000000 of natural *C. sinensis* [26,53]. These results indicate transcriptional silencing of the genes of all fungi co-colonized in natural *C. sinensis* and transcriptional activation of the genes in strain L0106.

Genome Sequence LKHE01000676 of *H. sinensis* Strain 1229

The sequence LKHE01000676 of strain 1229 is 97.0%-100% homologous to the multiple genome sequences ANOV01000288/ANOV01000289/ANOV01000811/ANOV01000812/ANOV01001676/ANOV01007159/ANOV01009876/ANOV01009877/ANOV01022491, JAAVMX010000004, and LWBQ01000084, but shares low similarities with many other segment sequences of the genome assemblies ANOV00000000, JAAVMX000000000, and LWBQ00000000 of strains Co18, IOZ07, and ZJB12195, respectively (**Table S3**). For instance,

segment 827→1,351 of LKHE01000676 is 82.0%-91.9% similar to segments of ANOV01006005, JAAVMX010000001/JAAVMX010000004, LWBQ01000135, and many other segments of strains Co18, IOZ07, and ZJB12195, respectively [26,28,35-36]. **Figure 2** show sequence comparisons with scattered transition and transversion mutations and multiple insertion/deletion mutant alelles.

Cross-analysis revealed that the lengthy segments 4,337→9,693 and 11,500→18,158 of ANOV01000289 of strain Co18 are 91.0%-96.5% similar to segments 6,005←12,651 and 15,974←21,281 of LKHE01000676; 5,342←11,988 and 15,311←20,620 of JAAVMX010000004 of strains 1229 and IOZ07, respective-

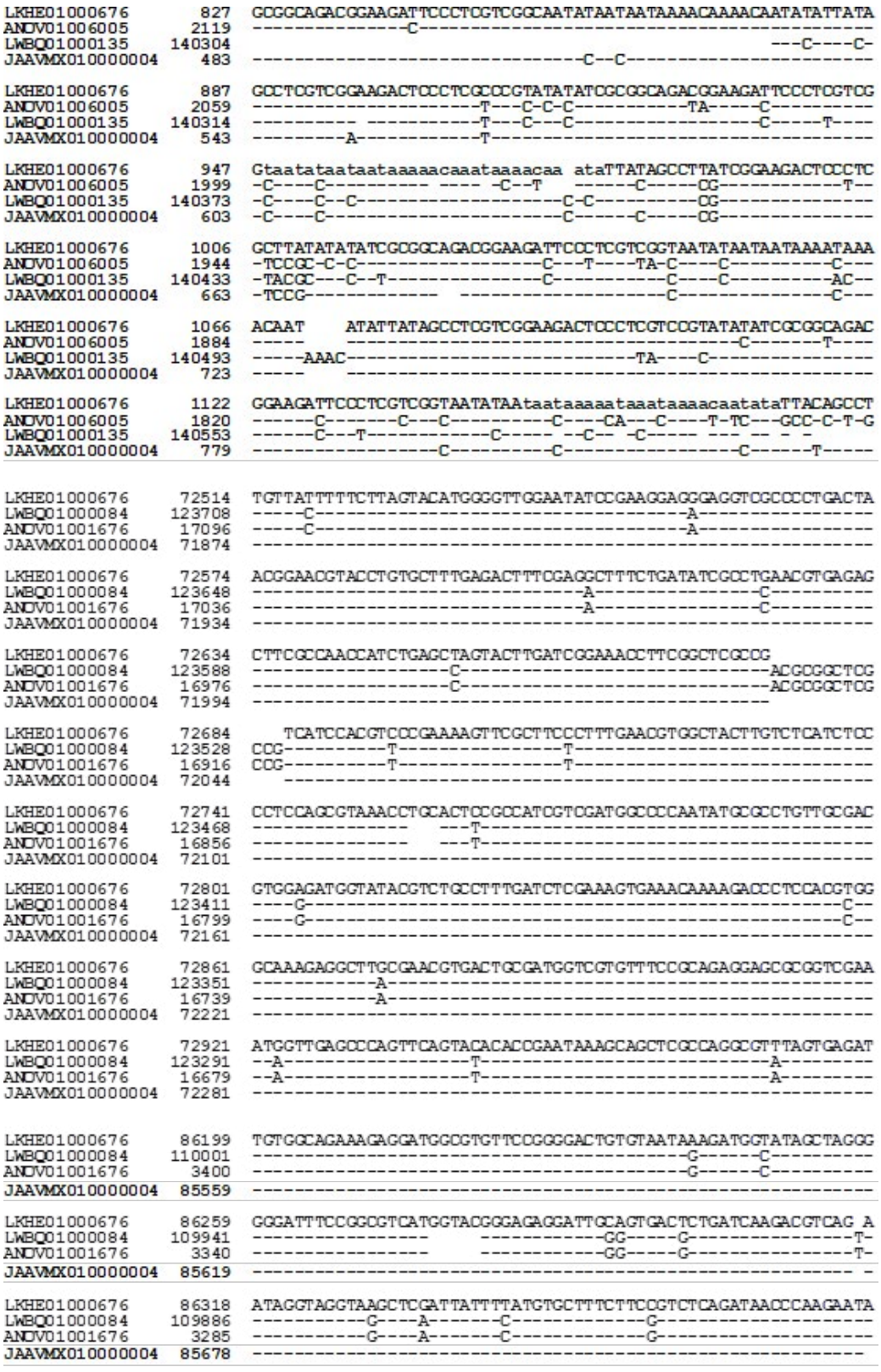


Figure 2: Alignment of the genome assembly segments LKHE01000676 with other genomic sequences

ly, with scattered transition, transversion, insertion/deletion mutant alleles [26,28,36]. Within these lengthy segments of ANOV01000289, 2 shorter segments 5,485→6,702 and 16,257→17,663 are 99.2%-99.9% homologous to the segments of LWBQ01000084 of strain ZJB12195 [26,35].

Segment 4,490→4,881 of ANOV01006005 of strain Co18 is 97.7% homologous to segment 4,011,040→4,011,426 of JAAVMX010000001 and segment 52,870→53,256 of LKHE01002757 of strains IOZ07 and 1229, respectively [26,28,35-36]. This segment of ANOV01006005 is only 91.8% similar to segment 350,593→351,007 of LWBQ01000080 of strain ZJB12195 with scattered transition, transversion, insertion/deletion mutations.

Table 4 shows the alignments with similarities <97% between the genome segments of LKHE01000676 of strain 1229 and the transcriptome sequences of GAGW00000000 and GCQL00000000 of natural *C. sinensis* and strain L0106 with scattered transition, transversion, and insertion/deletion mutations [28,33,53]. Several segments of LKHE01000676 were transcribed in natural *C. sinensis* but not in strain L0106, or vice versa, indicating alternative on-or-off of gene transcription in natural *C. sinensis* and strain L0106, or differential post-transcriptional modifications in response to the natural and unnatural conditions.

Table 4: Comparison of the genome segment sequences of LKHE01000676 with transcriptome sequences

Query segment of LKHE01000676	Subject transcriptome sequence segment		% Similarity	Note
	Accession #	Range & direction		
1,847→2,330	GAGW01005792	1→479	90.7% (439/484)	
3,287→3,816	GCQL01001639	5→513	94.9% (504/531)	
5,386→5,827	GAGW01002271	1→435	97.0% (423/436)	
6,664→7,561	GCQL01010633	1→896	96.1% (864/899)	
	GAGW01002270	1←396	94.2% (376/399)	
12,283→12,651	GAGW01014001	1→370	94.4% (353/374)	
	GCQL01011474	1→285	93.8% (271/289)	
14,111→15,149	GCQL01011474	283→1,326	96.8% (1,014/1,048)	
17,291→18,242	GCQL01000318	986→1,326	96.5% (329/341)	
	GAGW01001243	859→1,407	96.4% (530/550)	
0000		518→858	96.2% (328/341)	
19,006→20,474	GAGW01001246	1←1,414	95.0% (1,399/1,472)	Overlapped with GAGW01001246
	GAGW01001244	1←260	94.2% (245/260)	
	GAGW01001245	1←919	83.9% (818/975)	
	GCQL01004794	1←453	89.8% (407/453)	
	GCQL01013071	8→597	87.2% (532/611)	
20,592→22,337	GCQL01006612	359→636	95.7% (266/278)	
		641→1,272	95.6% (605/633)	
		8→359	93.3% (334/358)	
	GAGW01003631	41→318	95.7% (266/278)	
22,376→22,660	GCQL01019951	1←285	96.5% (275/285)	
28,034→28,569	GCQL01010894	1→525	93.3% (500/536)	
33,387→34,679	GAGW01000758	294→1,057	93.1% (760/816)	
	GAGW01012024	2←589	96.6 % (568/588)	Overlapped
	GAGW01012122	1→580	91.7% (532/580)	
44,841→46,018	GCQL01005661	1→1,144	93.7% (1,117/1,192)	
50,206→51,036	GCQL01003788	10→790	93.6% (778/831)	
68,282→69,541	GAGW01006524	1,751←2,956	94.1% (1,187/1,261)	
	GCQL01019941	1←281	82.4% (277/336)	
92,442→93,636	GAGW01003452	1→250	94.4% (237/251)	
	GAGW01003450	1←621	89.5% (561/627)	
95,026→95,351	GAGW01000444	63→320	96.9% (250/258)	Overlapped
	GAGW01000445	1←256	96.9% (248/256)	
	GAGW01000442	1←247	96.8% (239/247)	
	GAGW01000616	1←263	95.1% (250/263)	
107,232→110,427	GCQL01005615	1→2,902	95.8% (2,842/2,967)	
	GAGW01009911	75→869	92.2% (733/795)	

121,758→123,734	GCQL01008379	1,124←2,896	89.2% (1,764/1,977)
	GAGW01006329	1,457→3,231	89.2% (1,763/1,977)
124,838→125,131	GCQL01008379	1←288	93.6% (277/296)

Genome Sequence LWBQ01000028 of *H. sinensis* Strain ZJB12195

Segments 80,533→92,360; 104,744→115,873; 118,393→131,981; 134,541→148,041; 162,533→174,118; 185,839→196,118; 226,274→235,333; 235,360→294,143; 249,222→264,107; 276,078→290,663 (and more) of LWBQ01000028 of strain ZJB12195 are 99%-100% homologous to many segments of ANOV01000226/ANOV01006525, LKHE01002847, and JAAVMX010000008 of strains Co18, 1229, and IOZ07, respectively [28,33,53]. Several other segments of LWBQ01000028, however, share 85.7%-100% similarities with the genome sequences of strains Co18, 1229, and IOZ07, as shown in (**Figure S4 and Table 5**) [26,28,35-36].

Table 5: Comparisons of the genome segment sequences of LWBQ01000028 with other genome sequences

Query segment of LWBQ01000028	Subject genome sequence segment		% Similarity
	Accession #	Range & direction	
79,596→82,541	ANOV01004731	885→3,775	96.3% (2,836/2,946)
	JAAVMX010000003	17,109,763←17,112,653	93.1% (2,743/2,946)
	LKHE01000694	11,826→14,722	92.0% (2,710/2,946)
	ANOV01001350	1←2,895	90.8% (2,676/2,946)
84,386→84,802	ANOV01002409	3→419	96.6% (403/417)
	LKHE01003110	106,424→106,839	94.5% (393/416)
	JAAVMX010000003	10,251,043→10,251,459	94.2% (393/417)
178,302→178,718	JAAVMX010000002	7,904,858→7,905,270	95.2% (397/417)
	LKHE01001213	2,969←3,383	94.5% (395/418)
	ANOV01013978	3,090→3,499	91.9% (384/418)
179,439→180,080	JAAVMX010000009	692,799→693,460	92.6% (613/662)
	LKHE01003155	2,038←2,699	92.6% (613/662)
180,581→180,898	LKHE01000683	145←462	96.5% (307/318)
	JAAVMX010000007	1,986,719→1,987,036	96.5% (307/318)
226,274→226,552	LKHE01002847	40,829→41,085	90.0% (252/280)
	JAAVMX010000008	1,836,903←1,837,159	
	ANOV01006525	3,281←3,537	
235,171→235,550	LKHE01002847	49,706→50,050	90.8% (345/380)
	JAAVMX010000008	1,827,939←1,828,283	
290,668→290,946	LKHE01002847	104,998→105,273	94.3% (264/280)
	JAAVMX010000008	1,772,942←1,773,217	
	ANOV01000226	5,870←6,145	
313,425→313,999	LKHE01002814	9,543→10,117	96.7% (556/575)
	JAAVMX010000006	5,835,407←5,835,791	91.2% (351/385)
	ANOV01001029	15,460→15,844	91.2% (351/385)
315,290→315,616	JAAVMX010000008	1,748,980←1,749,277	89.4% (295/330)
	LKHE01002814	11,291←11,588	89.1% (294/330)
	ANOV01007356	1,810→2,154	85.7% (299/349)
426,625→427,144	JAAVMX010000008	1,641,405←1,641,924	100% (520/520)
	ANOV01002726	17,746←18,265	99.8% (519/520)
	LKHE01002770	149,005←149,524	94.2% (490/520)
430,444→430,823	JAAVMX010000008	1,637,824←1,638,203	100% (380/380)
	LKHE01001032	1,568←1,947	100% (380/380)
	ANOV01002726	14,167←14,544	94.2% (358/380)
507,607→508,491	JAAVMX010000008	1,563,880←1,564,764	99.8% (883/885)
	LKHE01003593	25,479←26,363	92.5% (819/885)
	ANOV01000543	11,240←12,124	92.1% (815/885)

	LKHE01003291	63,024→63,516	94.1% (464/493)
593,072→593,571	JAAVMX010000002	16,452,980→16,453,478	94.1% (464/493)
	ANOV01010922	75→574	93.6 % (468/493)

(**Table S4**) shows the differentially expressed transcripts in the transcriptome assemblies of natural *C. sinensis* and *H. sinensis* strain L0106. For instance, segment 416,688→417,407 of the genome sequence LWBQ01000028 is 84.6% similar to GCQL01000179 of strain L0106 with 6 detection gaps up to 51 bp., but absent from the transcriptome assembly GAGW00000000 of natural *C. sinensis*, indicating transcriptional silencing of the gene in natural *C. sinensis* and unnatural transcriptional activation in *H. sinensis*. Low similarities (<97%) were found between some of the "paired" transcripts of natural *C. sinensis* and strain L0106. Several of these transcripts from strain L0106 are 2-3-fold in length than the transcripts from natural *C. sinensis*, such as GCQL01005624 vs. GAGW01010266, GCQL01006423 vs. GAGW01007466, GCQL01019079 vs. GAGW01008721, etc., indicating differential transcription or post-transcriptional modifications in the 2 samples.

Genome Sequence LWBQ01000037 of *H. sinensis* Strain ZJB12195

Multiple sequences of LWBQ01000037 of strain ZJB12195 are 99%-100% homologous with segments of the genome sequences ANOV00000000, LKHE00000000, and JAAVMX000000000 of strains Co18, 1229, and IOZ07, respectively [26,28,35-36]. Many short segments of the lengthy sequence LWBQ01000037 are <97% similar to other overlapped genome sequences with scatted transition, transversion and insertion/deletion mutant alleles (**Figure S5**). (**Table S5**) shows that many segment sequences of the genome LWBQ01000037 of strain ZJB12195 are 82.0%-96.2% similarities to the numerous segment sequences of LKHE00000000, JAAVMX000000000, and ANOV00000000 of strains 1229, IOZ07, and Co18 [26,28,35-36].

(**Table S6**) compares the genome segment seqeunces of LWBQ01000037 of strain ZJB12195 with fewer similarities (80.0%-97.6%) to transcriptome sequences of natural *C. sinensis* and strain L0106 [26,28,33,35-36,53]. Two genome segments (14,715→15,238 and 452,296→452,665) of LWBQ01000037 were transcribed in natural *C. sinensis* but not in strain L0106. Some transcripts of natural *C. sinensis* were 2-3-fold longer in length than those of strain L0106, corresponding to the genome segments 6,232→7,009, 39,296→39,287, and 352,166→352,524 of LWBQ01000037. However, segment 389,632→389,985 of LWBQ01000037 was transcribed >2-fold longer in strain L0106 than in natural *C. sinensis*.

The Unassembled Shotgun Genome Sequences of *H. sinensis* Strain YN07-8

Five (JM973567, JM973711, JM973713, JM973797, and JM973816) (1.97%) of the 254 unassembled shotgun genome sequences (JM973567-JM973820) of strain YN07-8 did not align to any part of *H. sinensis* genome and mitogenome sequences [26,28-29,33,35-36,39,48,53]. Among them, JM973797 and JM973816 are 100% homologous to the transcriptome sequences GAGW01010110 (41→665) and GAGW01003685 (1,636←2,243), respectively, of natural *C. sinensis*, but absent from the transcriptome GCQL00000000 of strain L0106 or genome and transcriptome sequences of other fungal strains registered in GenBank to date [33,39,53]. However, the upstream region (1→657) of GAGW01003685 is 97.7% homologous to the genome segments of JAAVMX010000003, ANOV01000070, LKHE01001512, and LWBQ01000096 of *H. sinensis* strains [26,28,33,35-36,53] IOZ07, Co18, 1229, and ZJB12195, respectively, whereas GAGW01010110 did not align to any part of the genome sequences of *H. sinensis* strains. It seems that the 5 unassembled genome sequences and the transcript GAGW01010110, as well as part of GAGW01003685, were derived from the genomes of fungi co-colonized in natural *C. sinensis* other than *H. sinensis*. The transcript GAGW01003685, however, might be incorrectly assembled with transcript segments derived from the heterogeneous fungal genomes in natural *C. sinensis*.

Six unassembled sequences (2.36%) are ribosomal 28S gene sequences (Section III.4), 5 sequences (1.97%) are mitogene sequences (Section III.10), and 163 sequences (64.2%) are 97%-100% homologous to a single copy of each of the assembled genome sequences of *H. sinensis* strains [26,28,35-36,39]. Eleven sequences (4.33%) are 98.1%-100% homologous to sequences of the genome assemblies JAAVMX000000000 and LKHE00000000 of strains IOZ07 and 1229, respectively, but unmatched to any part of ANOV00000000 or LWBQ00000000 of strains Co18 and ZJB12195. Three (1.18%) are 98.6%-99.8% homologous to a single segment of each of the genome sequences of JAAVMX000000000, ANOV00000000, and LKHE00000000, of strains IOZ07, Co18, and 1229, respectively, but JM973722 and JM973755 aligned to double, overlapped LWBQ00000000 sequences of strain ZJB12195.

Thirty unassembled sequences (11.8%) are 57.7%-96.6% similar to sequences of the genome assemblies ANOV00000000, LKHE00000000, LWBQ00000000, and JAAVMX000000000 of strains Co18, 1229, ZJB12195, and IOZ07, respectively, with scattered transition, transversion, and insertion/deletion point mutations [26,28,35-36,39]. Eight of the 30 sequences are 57.7%-95.8% similar to >100 overlapped genome sequences; whereas sequence JM973819 is 94.7%-95.2% similar to 38 overlapped genome sequences.

Thirty one unassembled sequences (12.2%) are ≥ 97% homologous to some of the assembled genome sequences but less similar (<97%) to other sequences with scattered transition, transversion, and insertion/deletion mutant alleles [26,28,35-36,39]. Six of them aligned to >100 overlapped sequences of the assembled genomes with various similarities of 79.9%-99.8%.

Transcription of the Unassembled Genome Sequences of *H. sinensis* Strain YN07-8

Sixteen (6.29%) of the 254 unassembled genome sequences

did not align to any transcriptome sequences of natural *C. sinensis* or *H. sinensis* strain L0106, indicating that these genome components were non-transcriptable or transcriptionally silent. Sixty-three sequences (24.8%) were expressed differentially in strain L0106 but not in natural *C. sinensis*; whereas 6 other sequences (2.36%) expressed in natural *C. sinensis* but not in strain L0106 and might be derived from fungi colonized in natural *C. sinensis* other than *H. sinensis*.

Among those unassembled genome sequences that are highly homologous to the assembled genome sequences of *H. sinensis* strains, 70 sequences (27.6%) were transcribed to a single transcript copy of strain L0106 and natural *C. sinensis*, whereas 46 (18.1%) transcribed in either strain L0106 or natural *C. sinensis* but not both [33,39,53]. Forty-seven (18.5%) of the unassembled sequences were transcribed with multiple overlapped transcripts in strain L0106 but 27 (10.6%) transcribed with multiple overlapped transcripts in natural *C. sinensis*. For instance, JM973748 aligned to 94 overlapped transcripts of natural *C. sinensis* and 6 overlapped transcripts of strain L0106.

The OSRC14 Marker Genes of *H. sinensis* Strains

Sequence similarities of 95.2%-100% were found among the PCR-amplified OSRC14 gene sequences of 42 *H. sinensis* strains (**Table 6**) [40]. Among them, JQ277392, JQ325408, JQ325431, JQ325442-JQ325443 and KM197544 contain numerous mismatched alleles (**Figure S6**). JQ325431 of strain XZ05-8 is 92.2%-95.2% similar to the OSRC14 sequences of 35 *H. sinensis* strains.

JQ325484 of strain YN09-140 is 100% homologous to the assembled genome segments 34,066→34,642 of LKHE01001606, 649,477→650,053 of JAAVMX010000011, and 6,294→6,870 of ANOV01000797 of strains 1229, IOZ07, and Co18, and 52,641←52,065 and 245,347→245,923 of LWBQ01000349 and LWBQ01000064 of strain ZJB12195, respectively [26,28,35-36,40].

Table 6: Comparisons of the OSRC14 gene sequence JQ325484 (the Query) of strain YN09-140 with other OSRC14 gene sequences (the Subject)

Accession #	*H. sinensis* strain	% Similarity vs. JQ325484 (strain YN09-140)
JQ277386	QH07-197	100% (577/577)
JQ277389	QH09-93	100% (577/577)
JQ277390	XZ07-176	100% (577/577)
JQ325373	GS09-121	100% (577/577)
JQ325377	GS09-225	100% (577/577)
JQ325402	QH09-210	100% (577/577)
JQ325422	SC09-190	100% (577/577)
JQ325429	XZ05-6	100% (577/577)
JQ325438	XZ07-133	100% (577/577)
JQ325451	XZ08-59	100% (577/577)
JQ325458	XZ09-48	100% (577/577)
JQ325462	XZ09-95	100% (577/577)
JQ325473	YN09-6	100% (577/577)
JQ325474	YN09-22	100% (577/577)
JQ325476	YN09-61	100% (577/577)
JQ325477	YN09-64	100% (577/577)
JQ325481	GS09-311	100% (577/577)
JQ325482	YN09-96	100% (577/577)
JQ325485	ID10-1	100% (577/577)
JQ325486	NP10-1	100% (577/577)
JQ325487	NP10-2	100% (577/577)
JQ325397	QH09-151	99.8% (576/577)
JQ325398	QH09-164	99.8% (576/577)
JQ325461	XZ09-80	99.8% (576/577)
JQ325472	YN09-3	99.8% (576/577)
JQ325475	YN09-51	99.7% (575/577)
JQ277391	SC09-37	98.8% (570/577)
JQ325409	SC09-47	98.8% (570/577)
JQ325455	XZ09-15	98.8% (570/577)
JQ325464	XZ09-106	98.8% (570/577)
JQ325478	YN09-72	98.8% (570/577)
JQ325479	YN09-81	98.8% (570/577)
JQ325480	YN09-85	98.8% (570/577)
JQ325483	YN09-101	98.8% (570/577)
JQ325481	YN09-89	98.6% (569/577)
JQ325408	SC09-36	96.5% (557/577)
JQ277392	XZ06-124	95.4% (561/588)
JQ325443	XZ07-H2	95.4% (561/588)
JQ325431	XZ05-8	95.2% (560/588)
JQ325442	XZ07-H1	95.2% (560/588)
KM197544	XZ12-16	95.2% (560/588)

No matches were found between OSRC14 sequences and the GCQL00000000 transcriptome assembly of strain L0106, indicating transcriptional silencing of the OSRC14 gene in *H. sinensis* [33,40]. Segments 1→67 and 364→577 of OSRC14 sequence JQ325484 of strain YN09-140 is 100% homologous to segments 451←517 and 78←291 of the GAGW01003073 transcriptome assembly of natural *C. sinensis* but another segment (125→295) of JQ325484 is 97.1% similar to segment 282←452 of GAGW01003073 with scattered transition and transversion point mutations [40,53]. Considering the integrity of PCR-amplified sequence JQ325484, the 3 segments of GAGW01003073 might have been assembled with heterogeneous shotgun transcripts derived from the genomes of independent fungi in natural *C. sinensis*. If OSRC14 gene is transcriptionally silent in *H. sinensis*, the 3 assembled GAGW01003073 transcripts might be derived from the OSRC14 genes of independent fungi in natural *C. sinensis* other than *H. sinensis*.

The OSRC19 Marker Genes of *H. sinensis* Strains

The unassembled shotgun genome segment JM973741 (the OSRC19 marker gene) of *H. sinensis* strain YN07-8 is 99.8% homologous to the PCR-amplified OSRC19 gene sequences JQ277405 and JQ277406 of strains XZ06-124 and SC09-37 but only 94.5% similar to the OSRC19 sequences JQ277407 and JQ277408 of strains QH09-93 and XZ07-176, respectively, with scattered transition and transversion point mutations (**Figure S7**) [39-41].

JM973741 of strain YN07-8 is 94.5%-98.6% homologous to segments 2,470→3,201 of ANOV01007159; 55,477←56,202

of LKHE01000676; 54837←55562 of JAAVMX010000004 of strains Co18, 1229, and IOZ07, respectively, and non-overlapped segments 140,812→141,235 and 141,367→141,660 of LWBQ01000084 of strain ZJB12195, with scattered transition and transversion, and insertion/deletion mutant alleles (**Figure 3**) [26,28,35-36,39-40]. A 131-nt (141,236→141,366) DNA segment deletion was found between the 2 aforementioned segments of LWBQ01000084.

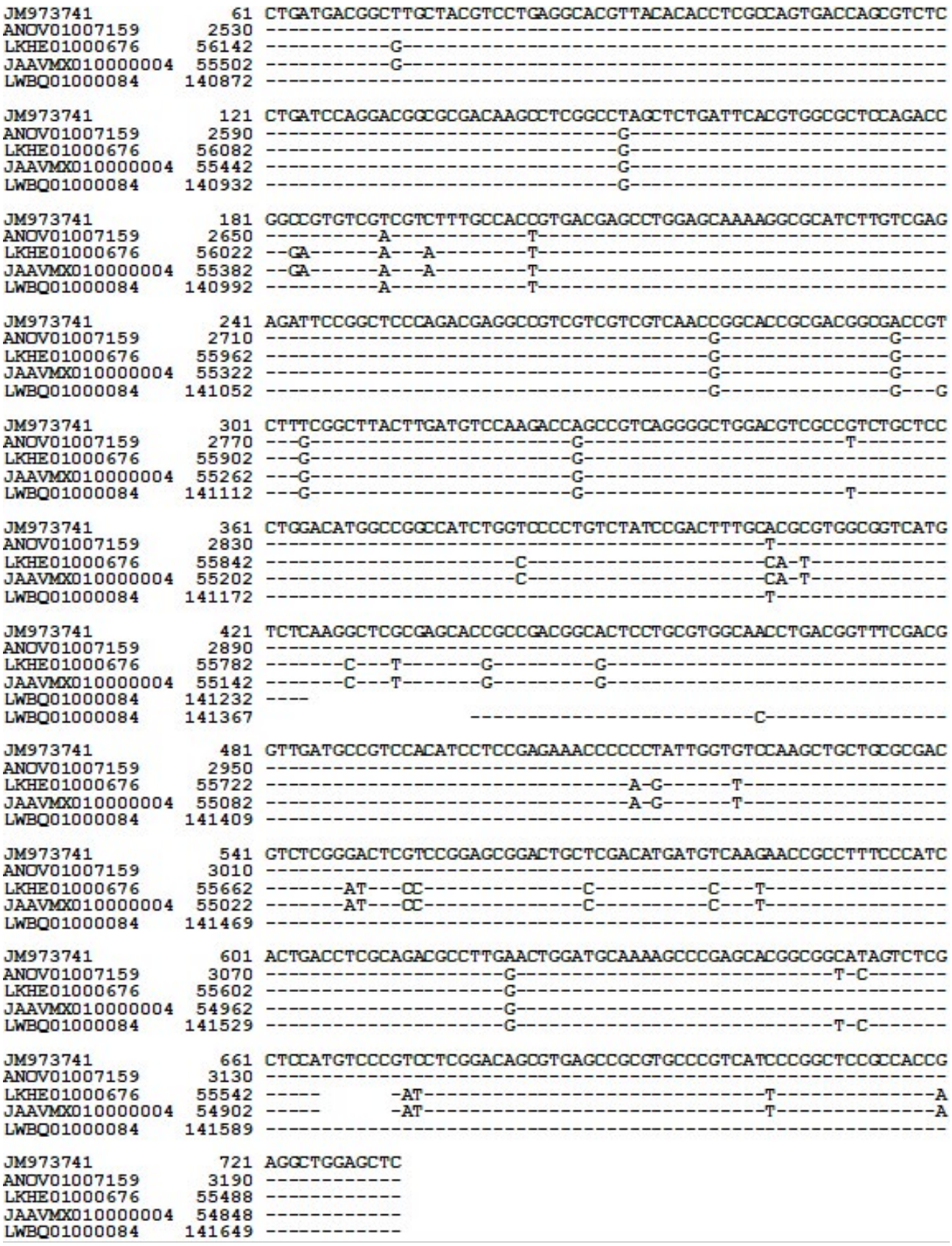


Figure 3: Alignment of the unassembled shotgun genome segment JM973741 with assembled genome sequences of *H. sinensis* strains

JM973741 did not align to any sequences of the transcriptome assemblies GAGW00000000 of natural *C. sinensis* and GCQL00000000 of strain L0106 and may represent a non-transcribed intron sequence or a silent gene.

Cross-analysis revealed that the lengthy segment 827→117,239 of LKHE01000676 of strain 1229 is 99.8% homologous to segment 191→116,599 of JAAVMX010000004 of strain IOZ07, and 98.4%-98.9% similar to much shorter segments of LWBQ01000084 (104,348←125,987) of strain ZJB12195 and ANOV01007159 (1←7,193) of strain Co18 [26,28,35-36]. Segment 827→1,348 of LKHE01000676 is 74.2%-91.9% similar to segments 140,304→140,677 of LWBQ01000135, 1,608←2,119 of ANOV01006005, 191→718 and 483→909 of JAAVMX010000004, and 4,008,152←4,008,663 of JAAVMX010000001 of strains ZJB12195, Co18, and IOZ07, respectively. Segment 191→909 of JAAVMX010000004 is 97% similar to segment 342→1068 of LKHE01000676 of strain 1229 but 82% similar to segments 1,252←2,124 of ANOV01006005 and 140,304→141,471 of LWBQ01000135 of strains Co18 and ZJB12195, respectively, with scattered transition, transversion, and inversion/deletion point mutations.

The OSRC27 Marker Genes of *H. sinensis* Strains

Sequence similarities of 93.8%-100% were found among the PCR-amplified OSRC27 gene sequences of 38 *H. sinensis* strains (**Table S7** and **Figure S8**) [40,42]. JQ325705 of strain YN09-6 is 92.6%-94.2% similar to other OSRC27 sequences of 32 *H. sinensis* strains with scattered transition, transversion, and insertion/deletion point mutations.

JQ325719 of strain NP10-2 is 100% homologous to the assembled genome sequences, ANOV01003376, LKHE01000526, LWBQ01000003, and JAAVMX010000005 of *H. sinensis* strains Co18, 1229, ZJB12195, and IOZ07, respectively [26,28,35-36,40,42]. JQ325705 of strain YN09-6 is only 93.8% similar to the OSRC27 genome sequences of the *H. sinensis* strains.

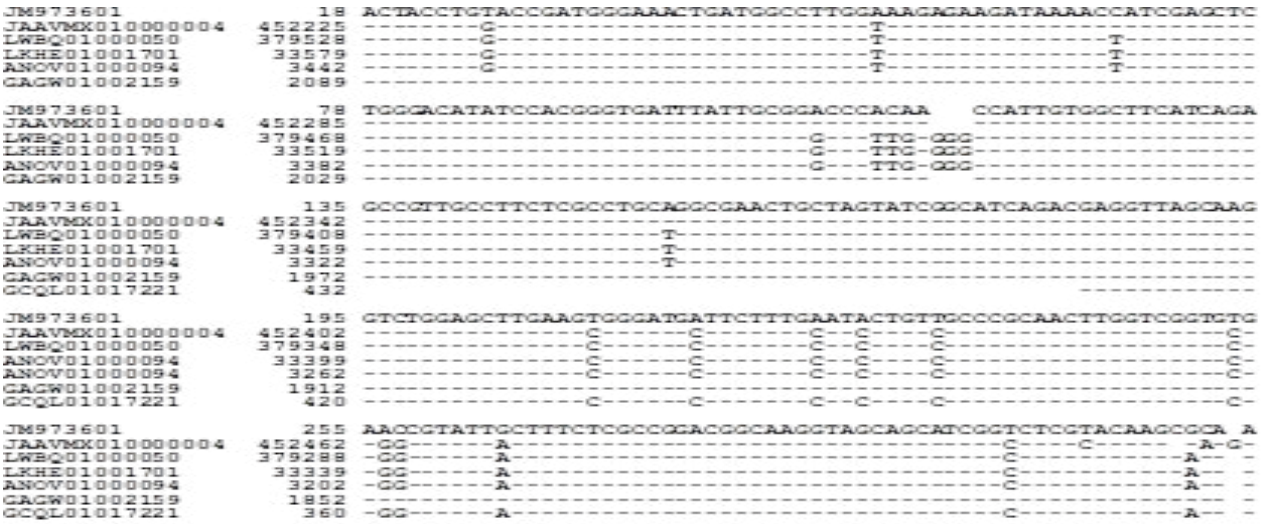
The OSRC27 gene was not transcribed in natural *C. sinensis*. Segments 1→135 and 240→574 of JQ325719 of strain NP10-2 are 99.7%-100% homologous to segments 1,651←1,985 and 1,983←2,217 of transcriptome sequence GCQL01013271 of strain L0106 with a 104-bp. segment deletion, corresponding to the genome segment 136→239 of JQ325719 [33,42]. Segments 1,983←2,217 of GCQL01013271 is completely overlapped with 20←154 of GCQL01015312 of strain L0106. JQ325705 of strain YN09-6 is 97.3%-98.5% similar to the 2 transcript segments of GCQL01013271 with scattered transition and transversion point mutations (**Figure S9**) [33,40,42].

The OSRC32 Marker Genes of *H. sinensis* Strain

The unassembled genome sequence JM973601 (the OSRC32 marker gene) of strain YN07-8 is 88.6%-100% similar to other PCR-amplified OSRC32 gene sequences of 35 *H. sinensis* strains (**Table 7** and **Figure S10**), with scattered transition and transversion mutant alleles and a DNA segment insertion/deletion [39-41]. JM973601 is 91.8-93.1% similar to the assembled genome segments 378,579←379,528 of LWBQ01000050, 32,632←33,579 of LKHE01001701, 2,495←3,442 of ANOV01000094, and 452,225→453,180 of JAAVMX010000004 of strains 1229, ZJB12195, Co18, and IOZ07, respectively, with scattered transition, transversion, and insertion/deletion point mutations (**Figure 4**) [26,28,35-36,39].

(**Table 7**)Cross-analyses revealed that ANOV01000094 of strain Co18 is 95.7%-99.6% homologous to the genome segments of LWBQ01000050 (376,024→384,921; 385,007→386,603; 386,612→392,750; 392,856→394,301; 394,358→399,049), LKHE01001701 (30,138→52,637), and JAAVMX010000004 (433,098←454,779; 454,788←455,675) of strains ZJB12195, 1229, and IOZ07, respectively [26,28,35-36]. Some of the genome sequences contain scattered transition, transversion, and insertion/deletion mutant alleles.

(**Figure 4**) also shows that JM973601 of strain YN07-8 is 94.2%-96.5% similar to the transcriptome sequences GCQL01017221 (1←432) of strain L0106 and GAGW01002159 (1,134←2,106) of natural *C. sinensis* with scattered transition, transversion, and insertion/deletion mutations [33,39,53]. The segment 1→432 of GCQL01017221 is only 94.0% similar to the segment 1,493→1,924 of GAGW01002159.



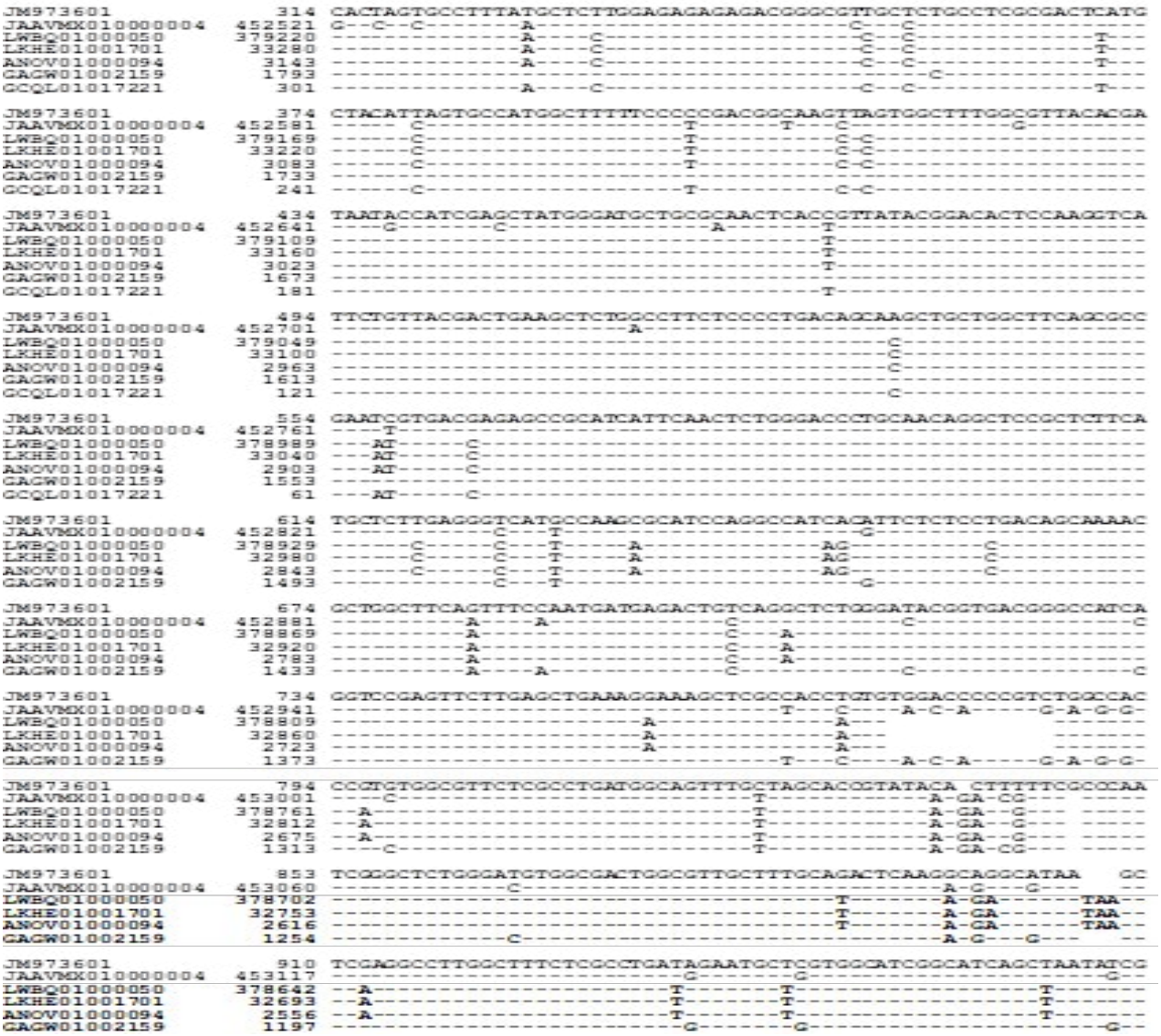


Figure 4: Alignment of the unassembled shotgun genome sequence JM973601 with the assembled genome and transcriptome OSRC32 sequences of natural *C. sinensis* and *H. sinensis* strains. sinensis

Segment 1→2,089 of GAGW01002159 of natural *C. sinensis* is 98.3% homologous to the genome sequence 452,225←454,313 of JAAVMX010000004 of strain IOZ07. However, a shorter segment 452,390←452,821 of JAAVMX010000004 is only 94.9% similar to the transcriptome sequence GCQL01017221 of strain L0106, whereas GCQL01017221 is 100% homologous to other genome sequences 2,843→3,274 of ANOV01000094, 378,929→379,360 of LWBQ01000050, and 32,980→33,411 of LKHE01001701 of strains Co18, ZJB12195, and 1229, respectively [26,28,33,35-36]. In contrast, GAGW01002159 (419→2,949) of natural *C. sinensis* is only 87.4% similar to the genome segment sequences: ANOV01000094 (1,778→4,302), LWBQ01000050 (377,864→380,388), and LKHE01001701 (31,915→34,439) of strains Co18, ZJB12195, and 1229, respectively, with scattered transition, transversion, and insertion/deletion point mutations. It is questionable whether the full or segmented transcript GAGW01002159 were derived from the genome (s) of one or more of the fungi co-colonized in natural *C. sinensis* other than *H. sinensis*, shows segment insertions/deletions in multiples of 3 to indicate open reading frames of proteins.

The OSRC11, OSRC23, and OSRC31 Marker Gene Sequences of *H. sinensis* Strains

Sequences JQ277384 (OSRC11 gene) of strain XZ06-124 and JQ277444 (OSRC31 gene) of strain XZ07-H2 are 99.5%-99.8% homologous to the assembled genome sequences ANOV01006466 and LKHE01002656 of strains Co18 and 1229 but absent from the genomes LWBQ00000000 and JAAVMX000000000 of strains ZJB12195 and IOZ07, respectively [26,28,35-36,39]. The OSRC23 gene sequence JQ277420 of strain XZ07-H2 is 99.2% homologues to LKHE01002410 of strain 1229 but absent from the genome assemblies JAAVMX000000000, LWBQ00000000, and ANOV00000000 of strains IOZ07, ZJB12195, and Co18, respectively.

Sequences of JQ277384 (OSRC11 gene) and JQ277420 (OSRC23 gene) are absent from the transcriptome assembly of strain L0106 but the genes were transcribed in natural *C. sinensis* to 35 and 352 overlapped GAGW00000000 transcripts, respectively, with similarities of 82.7%-100% [35,39,53]. The OSRC31 gene sequence JQ277444 is 99.3%-99.5% homologous to the transcriptome sequences of natural *C. sinensis* and strain

L0106.

The mRNA Sequence KP090937 of *H. sinensis* Strain L0106

Both the hexokinase-like mRNA sequence KP090937 and assembled transcriptome sequence GCQL01012008 (1←1,137) were from *H. sinensis* strain L0106 and are 100% identical (**Figure 5**) [33]. Two segments (1→326 and 328→1,137) of KP090937 are 99.7%-100% homologous to the non-overlapped transcripts 1←326 of GAGW01010481 and 1→810 of GAGW01005022, respectively, of natural *C. sinensis*.

Segments 1→104 and 130→541 of the mRNA sequence KP090937 of strain L0106 are 100% identical to the genome segments of LKHE01001829, LWBQ01000186/LWBQ01000017, JAAVMX010000002, and ANOV01015216 of strains 1229, ZJB12195, IOZ07, and Co18, respectively [26,28,33,35-36]. Segment 541→1,137 of KP090937, however, is 87.8%-90.4% similar to segments of LKHE01001829, LWBQ01000186/LWBQ01000017, JAAVMX010000002, and ANOV01003538 of strains 1229, ZJB12195, IOZ07, and Co18, respectively, with 9 deletions in the mRNA sequence KP090937, likely indicating alternative splicing [26,28,33,35-36,53]. It seems that ANOV01015216 and ANOV01003538 of strain Co18 might be incorrectly assembled.

Cross-analysis revealed that the lengthy genome sequences LKHE01001829 of strain 1229 is 97.6%-100% homologous to the genome segment sequences of JAAVMX010000002, LWBQ01000017, and ANOV01000772 of strains IOZ07, ZJB12195, and Co18, respectively [26,28,35-36]. However, segment 93,470→94,948 of the lengthy sequence LKHE01001829 is 98.1%-98.4% homologous to segments 1→1,214 of ANOV01015216 of strain Co18 and 18,187,481→18,188,936 of JAAVMX010000002 of strain IOZ07, but only 90.4%-94.7% similar to segments 174,857→176,321 of LWBQ01000186 and 186,085←187,643 of LWBQ01000017 of strain ZJB12195 with several insertions/deletions (mono-, bi-, or poly-bases), and transition and transversion point mutations.

The mRNA Sequence KP090945 of *H. sinensis* Strain L0106

The ADP-ribose pyrophosphatase-like mRNA sequence KP090945 of strain L0106 is 100% homologous to the assembled transcriptome sequences GCQL01000385 and GCQL01014864 of strain L0106 but absent from the transcriptome assembly GAGW00000000 of natural *C. sinensis* [33,53], indicating transcriptional silencing of the ADP-ribose pyrophosphatase gene in natural *C. sinensis* but anti-natural activation of the gene in strain L0106.

KP090945 is 100% homologous to genome sequences LKHE01001747 (109,170→109,895), JAAVMX010000012 (65,952→66,677), and ANOV01003103 (1←385, 435←620) of strains 1229, IOZ07, and Co18, respectively, but only 94.1% similar to segment 122,131→122,896 of LWBQ01000044 of strain ZJB12195 with 2 large segments of DNA insertions/deletions [26,28,33,35-36].

Cross-analysis revealed that the lengthy genome segment of LWBQ01000044 of strain ZJB12195 is 99.3%-99.6% homologous to segments of JAAVMX010000001/JAAVMX010000012, LKHE01000716/LKHE01001747, and ANOV01000098/ANOV01005573 of strains IOZ07, 1229, and Co18, respectively [26,28,35-36]. However, segment 120,984→122,545 of LWBQ01000044 is 93.5%-95.2% similar to segments 108040→109544 of LKHE01001747, 64,802→66,326 of JAAVMX010000012, and 1←1,151 of ANOV01003103 of strains 1229, IOZ07, and Co18, respectively, with several DNA insertions/deletions and some transition and transversion point mutations (**Figure S11**) [26,28,35-36].

The mRNA Sequence KP090946 of *H. sinensis* Strain L0106

The ribose-phosphate pyrophosphokinase-like mRNA sequence KP090946 of strain L0106 is 99.8%-100% homologous to the transcriptome assemblies GCQL01011182 and GAGW01003914 of strain L0106 and natural *C. sinensis*, respectively (**Figure S12**) [33,53].

KP090946 is 96.4%-96.5% similar to the genome segments 4,761→6,137 of LKHE01001673, 5,597,720←5,599,096 of JAAVMX010000006, and 20,514←21,890 of ANOV01001719 of strains 1229, IOZ07, and Co18, respectively, with a 48-nt. deletion occurred in the sequence of KP090946, as well as in GCQL01011182 and GAGW01003914 (**Figure S12**), indicating alternative splicing during transcription [26,28,33,35-36,53]. KP090946 is only 89.6% similar to LWBQ01000045 (385,652→386,959) of strain ZJB12195 with 4 segment insertions/deletions and scattered transversion and transition point mutations [33,35].

Cross-analysis revealed that segments 1→10,761 and 10,706→23,317 of ANOV01001719 of strain Co18 is 99.7%-99.8% homologous to segments 15,855←26,615 and 23,334←15,917 of LKHE01001673 of strain 1229 and segments 5,577,280→5,588,040 and 5,587,978→5,600,523 of JAAVMX010000006 of strain IOZ07 [26,28,36]. Segment 6,493→10,761 of ANOV01001719 is 99.1% homologous to segment 397,504←401,738 of LWBQ01000045 of strain ZJB12195, but many other ANOV01001719 segments are 93.2%-96.6% similar to the segments of LWBQ01000045 [26,35].

The mRNA Sequence KP090949 of *H. sinensis* Strain L0106

The amidophosphoribosyl transferase-like mRNA sequence KP090949 is 100% homologous to the 2←952 of GCQL01009009 transcriptome assembly of strain L0106 [53]. In contrast to the full-length transcript in strain L0106, the gene was partially transcribed in natural *C. sinensis* to a segment 176→514 of GAGW01013335 with a 613-nt. deletion corresponding to segment 339→951 of KP090949, indicating alternative transcription or posttranscriptional change in response to the natural and unnatural conditions [33,53].

KP090949 is 93% similar to genome sequences 424,486←425,462 of LWBQ01000048, 15,195,213←15,196,189 of JAAVMX010000003, 7,439←8,415 of LKHE01001105, 9,085←10,061 of ANOV01001694 of strains ZJB12195, IOZ07, 1229, and Co18, respectively [26,28,33,35-36]. The most variable segment 403→448 of KP090937 consists of multiple tran-

sition, transversion, and DNA segment deletions (mono-, bi- or poly-bases) in the KP090946 mRNA sequence, some of the deletions are not in multiples of 3 (**Figure S13**). Cross-analysis revealed high homologies (98.8%-99.7%) among the 4 assembled genome sequences LKHE01001105, LWBQ01000048, JAAVMX010000003, and ANOV01001694 of *H. sinensis* strains 1229, ZJB12195, IOZ07, and Co18, respectively [26,28,35-36].

The mRNA Sequence KP090959 of *H. sinensis* Strain L0106

The 5'-nucleotidase-like mRNA sequence KP090959 of strain L0106 is 100% and 99.8% homologous to transcriptome sequences GCQL01011621 of strain L0106 and GAGW01006965 of natural *C. sinensis* (**Figure S14**) [33,53].

Two segments (1←333 and 333←1,971) of KP090959 of strain L0106 are 86.9%-96.1% similar to segments 9,371,542←9,373,237 and 9,373,304←9,373,686 of JAAVMX010000005; 88,957←90,652 and 90,719←91,101 of LKHE01002574; and 15,089←16,784 and 16,851←17,233 of ANOV01001374 of strains IOZ07, 1229, and Co18, respectively, with scattered transition, transversion, and insertion/deletion point mutations (**Figure S14**) [26,28,33,35-36]. However, KP090959 is 86.9%-100% similar to 5 segments of LWBQ01000131 of strain ZJB12195: 206,165→206,547; 206,614→207,046; 207,045→207,352; 207,353→207,564; and 207,574→208,657 [33,35]. As the result of transcription splicing process, a 67-nt. deletion occurred between the 2 segment sequences of KP090959, locating between the genome segment sequences of JAAVMX010000005, LKHE01002574, and ANOV01001374 and between nucleotides 206,547 and 206,614 of LWBQ01000131.

Cross-analysis revealed that the genome sequence of ANOV01001374 of strain Co18 is 99.2%-99.9% homologous to the genome sequences of JAAVMX010000005 and LKHE01002574 of strains IOZ07 and 1229, respectively [26,28]. However, Table S8 shows many segment sequences of ANOV01001374 with 90.9%-96.6% similarities to many segments of LWBQ01000131 of strain ZJB12195, in addition to 70 other high-similarity LWBQ01000131 segments (>97%) with large segment insertions/deletions and scattered transversion and transition point mutations.

The mRNA Sequence KP090961 of *H. sinensis* Strain L0106

The purine nucleosidase-like mRNA sequence KP090961 is 97.9% and 100% homologous to the overlapped transcriptome sequences GCQL01017603 and GCQL01014666 of strain L0106 but absent from the GAGW00000000 transcriptome assembly of natural *C. sinensis* [33,53], indicating transcriptional silencing of the gene in natural *C. sinensis* and anti-natural transcriptional activation in strain L0106.

KP090961 is 98.2%-98.9% homologous to segments 64,831←65,280 of LKHE01002043 and 15,187,215←15,187,928 of JAAVMX010000003 of strains 1229 and IOZ07, only 93.0% similar to segment 416,051←416,805 of LWBQ01000048 of strain ZJB12195, but absent from the ANOV00000000 genome assembly of strain Co18 [26,28,33,35-36]. Comparing to the 3 genome sequences, there is an 8-nt. deletion between nucleotides 539 and 540 of the mRNA sequence KP090961. There is a 41-nt. insertion in the LWBQ01000048 genome sequence, locating between nucleotides 378 and 379 of the KP090961 sequence. Upstream of the inserted segment in LWBQ01000048, 3 genome segment sequences of strains share 100% sequence homology.

Cross-analysis revealed that LKHE01002043 of strain 1229 is 97.8%-99.4% homologous with multiple overlapped segments of ANOV01005037/ANOV01006908/ANOV01012844, LWBQ01000048, and JAAVMX010000003 [26,28,35-36]. LKHE01002043 is 74.6%-96.4% similar to numerous other segments of ANOV01000934/ANOV01003645/ANOV01005037/ANOV01005038/ANOV01005521/ANOV01006908/ANOV01007855/ANOV01012844, LWBQ01000048/LWBQ01000085, and JAAVMX010000003 of strains Co18, ZJB12195, and IOZ07, respectively, with multiple insertions/deletions and scattered transversion and transition point mutations, the majority of which form 2 overlapped groups of the transcripts (**Table S9**).

DISCUSSION

Transcription of *H. sinensis* Genes

Liu et al [33]. reported the dynamic transcriptional alteration in the mycelia of *H. sinensis* strain L0106 following 3, 6, and 9 days of liquid fermentation without an insect host, featuring with nonlinear reductions in the total number of transcriptomic unigenes (25,511→25,214→16,245), nonlinear increases (681-nt.→682-nt.→994-nt.) in the average unigene length, and reductions (58.2%→57.9%→57.0%) in the GC content. Such dynamic alteration indicates switching on or off of multiple genes in response to continuous in vitro fermentation. Our cross-analysis of the transcriptome sequences of *H. sinensis* strain L0106 and natural *C. sinensis* confirmed differential transcriptional activation and deactivation and posttranscriptional modifications of many genes.

A) Many genes were differentially transcribed partially or in full length in natural *C. sinensis* but not in *H. sinensis* strain L0106 (Sections III.5-III.6, III.10, III.12, III.14-III.17, III.21), or vice versa (Sections III.5, III.11-III.13, III.16, III.18-III.19, III.23, III.27). Some transcripts might be derived from the colonized fungi in natural *C. sinensis*, other than *H. sinensis*. Some naturally silent genes were anti-naturally activated in *H. sinensis* strain L0106 in respond to the 3-9 days of liquid fermentation without an insect host. Many other genes were naturally transcripted but silent unnaturally in strain L0106 [33];

B) Some of the segment sequences of the transcriptome GAGW00000000 and GCQL00000000 are highly variable [33,53]. Many transcripts from strain L0106 are 2-3-fold longer in legth than those from natural *C. sinensis*, or vice versa, indicating differential transcription or post-transcriptional modification;

C) Some of the insertion/deletion mutations in the genome sequences are present in multiples of 3 (Figures 3-4, S2, S9-S10), which are representative of the protein codons in the open reading frames for derivation of the amino acid sequenc-

es. However, other mutations do not show this pattern (Figures 2, 5, S1, S3-S6, S8, S11-S14), indicating possibilities of translational disruption of the coding sequences, causing irreversible arrest of protein translation.

The transcriptome assembly GAGW00000000 was derived from a natural C. sinensis specimen (unknown maturation status), which was purchased from the market of Kangding of Sichuan Province [53]. Liu et al [63]. constructed 2 cDNA libraries from total mRNA of the stroma and caterpillar body of a *C. sinensis* specimen (unknown maturation status) also collected from Kangding, Sichuan and found apparent differences between the cDNA libraries. Xia et al [62]. reported a transcriptome project of the fully mature natural *C. sinensis* specimens that were collected from Deqin of Yunnan province. Zhong et al [60]. reported a transcriptome project of natural *C. sinensis* "in the early teleomorph stage without ascus forming" that was collected from Yushu of Qinghai province. Li et al [64]. reported another transcriptome project of artificial *C. sinensis* specimens and described dynamic transcriptional alterations of many *C. sinensis* genes in the different development phases. However, Liu et al. [63], Xia et al. [62], Zhong et al. [60], and Li et al [64]. did not upload their assembled transcriptome sequences in GenBank. Some of them uploaded the assembled cDNA sequences to www.plantkingdomgdb.com/Ophiocordyceps_sinensis/ but the database is unaccessable for our cross-analysis. Dong et al. [65] reported significant differences and dynamic alterations in proteomic polymorphisms between the stroma and caterpillar body of *C. sinensis* during maturation, indicating the diversified and dynamically altered transcriptome profiles in the *C. sinensis* stroma and caterpillar body, reflecting the occurrence of transcriptional, posttranscriptional, and translational alterations and posttranslational modifications in the compartments of *C. sinensis* during maturation.

The Mating-Type Genes

Bushley et al. [24] reported detection of the MAT1-1-1, MAT1-1-2, and MAT1-1-3 genes of *MAT1-1* idiomorph and the MAT1-2-1 gene of *MAT1-2* idiomorph in strain CS68-2-1229 and hypothesized pseudohomothallism for *H. sinensis* (Genotype #1 of *O. sinensis*). Hu et al. [26] reported detection of the MAT1-1-1 and MAT1-2-1 genes in the genome of strain Co18 and hypothesized homothallism for *H. sinensis*. Zhang et al. [66], however, reported detection of MAT1-2-1 gene, but not genes of *MAT1-1* idiomorph, in *H. sinensis* strains CS2 and SCK05-4-3. We summarized in **Table 3** the differential occurrence of the mating genes in *H. sinensis* strains. Because of the differential occurrence of homothallic and heterothallic reproduction strategies, Zhang and Zhang [47] proposed a facultative hybridization hypothesis for *H. sinensis*. We found that the mating genes of *MAT1-1* idiomorph were absent from the genome assembly LWBQ00000000 of strain ZJB12195 and the MAT1-2-1 gene was absent from JAAVMX000000000 of strain IOZ07. Such inconsistent occurrence of the mating genes of the *MAT1-1* and *MAT1-2* idiomorphs in the genomes of *H. sinensis* strains fails to support genetic capability of self-fertility but supports heterothallic reproduction [67].

In contrast to the occurrence of mating genes in the genomes of *H. sinensis* strains, the presence of the mating genes of *MAT1-1* and *MAT1-2* idiomorphs has been reported in several studies of natural *C. sinensis* that contains multiple co-colonized fungi. The mating genes were detected (1) in the whole-genome of fully matured natural *C. sinensis* [62]; (2) in the early-developed stroma and caterpillar body of natural *C. sinensis* with very low read count values and in 31 other *C. sinensis* specimens [60], and (3) in artificial *C. sinensis* of different development phases [64]. Although the hyphae of artificial *C. sinensis* were included as a development stage of artificial *C. sinensis*, no methodology for fungal purification was described [64], indicating that the hyphae were wild-type fungi as previously reported by Zhang et al. [16] and Xia et al. [34]. Because of the co-extence of multiple colonized fungi in natural and artificial *C. sinensis*, the results from these studies may not be specifically used to accurately estimate the genetic capability of the self-fertility of *H. sinensis* (Genotype #1 of *O. sinensis*).

Technically self-fertility may come true when the mating genes of both *MAT1-1* and *MAT1-2* idiomorphs are transcribed and translated, and both mating proteins are fully activated within a single fungal cell. However, our analysis herein demonstrated that the MAT1-1-1 gene transcript was absent, but MAT1-2-1 gene transcript precent, in the GCQL00000000 transcriptome assembly of strain L0106 [33,53]. Notably, Zhang and Zhang [47] observed the 4.9%-6.1% intraspecific variations of MAT1-1-1 and MAT1-2-1 genes in various *H. sinensis* strains, which probably cause the coding sequence disturbance of the genes and translation arrest and reproductive impotence of *H. sinensis*. These findings are inconsistent with the homothallic/pseudohomothallic mating hypotheses that were underpinned solely by the genome data but indicate functional heterothallic behaviors.

Studies have reported variable transcription of the mating genes in natural and artificial *C. sinensis* that contains multiple co-colonized fungi.

1. MAT1-1-1 gene was expressed in all 5 specimens of natural *C. sinensis* (maturing stage of development), but MAT1-2-1 gene expressed in only 2 of 5 *C. sinensis* specimens and MAT1-1-3 gene expressed in only one specimen [68].
2. MAT1-1-3 and MAT1-2-1 genes, but not MAT1-1-1 and MAT1-2-1 genes, were expressed with very low read count values in the early-developed stroma and caterpillar body of natural *C. sinensis* [60].
3. The 4 mating genes were incoordinately expressed in all development phases of artificial *C. sinensis* [64]. They were expressed in primordium differentiation and mature fruiting body of artificial *C. sinensis*, with expression highest in the fertile part and lowest in the caterpillar body of mature artificial *C. sinensis*.
4. Zhao et al. [69] reported nearly no expressions of the MAT1-1-1 (transcripts per million reads, TPM of 0-2.27) and MAT1-2-1 (TPM of 0-1.74) genes and concluded that these genes may not play roles in the fruiting body initiation stage of *C. sinensis*.

The differential expression of the mating genes of both *MAT1-1* and *MAT1-2* idiomorphs in natural and artificial *C. sinensis* is then insufficient to prove that the transcripts were from a single fungal cell of *H. sinensis*, nor to support the homothallic/pseudohomothallic mating hypotheses for *H. sinensis*.

To resemble the functional heterothallic reproduction, *H. sinensis* (the postulated anamorph and Genotype #1 of *o.sinensis*) needs an opposite mating partner capable of expression of the mating genes of the *MAT1-1* and *MAT1-2* idiomorphs, meaning that the telemorphic *O. sinensis* may have more than one anamorphic fungus. The candidates of mating partner are the AT-biased *O. sinensis* genotype fungi or other fungal species within one of heterokaryotic cells [24], triggering revisit of the following academic puzzles observed previously:

A) Hu et al. [26] concluded that the fruiting body and ascospore productions have been consistently failed after inoculating ghost moth larvae of Hepialidae family with pure *H. sinensis*. Many such inoculation strategies could induce death and mummification of larvae, but no stromal formation;

B) Wei et al. [70] reported a species contradiction between the inoculant *H. sinensis* (GC-biased Genotype #1 of *O. sinensis*) and the sole teleomorph of AT-biased Genotype #4 of *O. sinensis* in the fruiting body of artificial *C. sinensis*;

C) Co-occurrence of the multiple AT-biased genotypes of *O. sinensis* and GC-biased *H. sinensis* (Genotype #1 of *O. sinensis*) in the stroma, caterpillar body, ascocarps, and ascospores of natural *C. sinensis* [6,8-11,17-19,21-22]. The sequences of the AT-biased genotypes do not reside in the genome of GC-biased *H. sinensis* but belong to the genomes of independent fungi;

D) Mao et al. [32] observed "H"-type fusions of hyphae during germination, containing AT-biased Genotype #4 or #5 of *O. sinensis* fungi without GC-biased *H. sinensis* in natural *C. sinensis* specimens collected from different production areas;

E) The biomasses of the AT and GC-biased genotypes of *O. sinensis* underwent dynamic alterations in an asynchronous manner in the caterpillar body and stroma of *C. sinensis* during maturation, whereas the AT-biased genotypes of *O. sinensis* always predominate in the *C. sinensis* stroma [6,17-19];

F) Two Genotypes #13-14 of *O. sinensis* were found in the multicellular heterkaryotic ascospores of natural *C. sinensis* with mono-, bi-, and tri-nuclear structure. They feature with reciprocal substitutions of large DNA segments between 2 parental fungi, Group-A *H. sinensis* and a Group-E fungus [6,9-11,19,24];

G) Barseghyan et al. [20] reported that Tolypocladium sinensis and *H. sinensis* were the dual anamorphs of *O. sinensis*;

H) The close relationship and tight association of Paecilomyces hepiali with *H. sinensis* in the stroma, caterpillar body, ascocarps, and ascospores of natural *C. sinensis* and in the intestines of healthy larvae of Hepialidae family [6,8-9,17-19,30].

Marker Genes Used in Multigene Analysis

Multigene examination strategy has been used in phylogenetic studies of *O. sinensis* and the sequences of many DNA loci, nrSSU, nrLSU, MAT1-1-1, MAT 1-2-1, tef1α, rpb1, rpb2, csp1, and β-tub1, are highly homologous to the corresponding segments of the assembled genomes. MAT1-1-1, MAT1-2-1, β-tub1, csp1, rpb1, and rpb2 are sensitive in distinguishing *H. sinensis* from other fungal species. However, tef1α may be less sensitive and nrSSU and nrLSU are the least sensitive. For instance, EF468971 (nrSSU) and EF468827 (nrLSU) are highly homologous (97.4%-99.5%) to the sequences of other taxa: Cordyceps multiaxialis (AJ309359), Cordyceps nepalensis (AJ309358), Hirsutella leizhouensis (KY415580), Hirsutella rhossiliensis (MH872887, NG_064109, EF546655), Ophiocordyceps acicularis (EF468805), Ophiocordyceps arborescens (NG_060238; AB968414), Ophiocordyceps brunneanigra (MF614654, MF614653), Ophiocordyceps geometridicola (MF614647, MF614648), Ophiocordyceps macroacicularis (MH461122, NG_060239; AB968416; MF614655), Ophiocordyceps multiperitheciata (NG_064462), Ophiocordyceps spataforae (MG831747).

The "ITS Pseudogene" Hypothesis for *H. sinensis*

Li et al. [27] proposed the "ITS pseudogene" hypothesis for the AT-biased genotypes of *O. sinensis*, based on (1) the detection of GC-biased Genotype #1 (*H. sinensis*) and AT-biased Genotype #5 of *O. sinensis* in 8 of 15 cultures of a mono-ascospore of natural *C. sinensis* and (2) the detection of the 5.8S cDNA of Genotype #1, but not Genotype #5, in cDNA libraries constructed from 2 *H. sinensis* cultures. However our study herein found that the genome JAAVMX000000000 of strain IOZ07 contains 17 overlapped sequences of 5.8S genes, all of which belong to GC-biased *H. sinensis*, Genotype #1 of *O. sinensis*. The sequences of the AT-biased Genotypes #4-6, #15-17 of *O. sinensis* resides not in the genome of *H. sinensis* but belong to the genomes of independent fungi [8]. No 5.8S gene transcript cDNA could be identified in the transcriptome assembly GAGW00000000 of natural *C. sinensis*, indicating transcriptional silencing of 5.8S genes consistently naturally occurred in all colonized *C. sinensis* fungi. These findings provide solid evidence contradicting against the "ITS pseudogene" hypothesis for the AT-biased genotypes of *O. sinensis* fungi [6,10-11].

Intraspecific Variations of *H. sinensis* Genes

In contrast to the interspecies genetic variations among the 17 genotypes of independent *O. sinensis* fungi [6-7,9-11,19,31,38,71], intraspecific variations at the genome and transcriptome levels within the species *H. sinensis* have been accessed herein through comprehensive cross-analysis among the assembled and unassembled genome/mitogenome sequences, the PCR-amplified sequences of the *H. sinensis* genes, and the transcriptome sequences of *H. sinensis* strains and natural *C. sinensis*, while the taxonomic position of these *H. sinensis* strains (Genotype #1 of *O. sinensis*) was determined by the authors of the original studies [4,15,24,26,28-29,33,35-36,39-41,49-52,53-60]. The cross-analysis did reveal intraspecific genetic variations (similarities <97%) in a substantial number of segment sequences of the *H. sinensis* genomes and transcriptomes, although some technical errors might have occurred during sequencing and assembling of the shotgun genome and transcriptome sequences with using the second and third generations of technologies.

Multigene analyses reported high genetic diversity of *H. sinensis*, Genotype #1 of *O. sinensis* (a few of the diversified sequences belonging to Genotype #3), isolated from natural *C. sinensis* specimens collected in southern Tibet or in western margin areas and the central region of the Hengduan Mountains and

lower genetic diversity of *H. sinensis* obtained from northern and eastern margin areas of the Mountains [39-42,47]. Accordingly these authors from the same research group proposed hypotheses of "the center of origin" and "the fungal evolutionary geography" for *H. sinensis*. In contrast to these hypotheses of the geography-associated *H. sinensis* variations, Xiao et al. reported large diversity in ISSR molecular marker polymorphism among the 40 *H. sinensis* strains isolated from *C. sinensis* [44] specimens collected from the same production area, Qingshashan of Qinghai province, China. The cross-analysis of genome, mitogenome, and transcriptome sequences presented herein has verified intraspecific genetic variations in *H. sinensis*, Genotype #1 of *O. sinensis*, which is distinct from the interspecies variations among Genotypes #1-17 of *O. sinensis* [6-11-15,17-19,21-23,30].

Among the 33 marker genes (OSRC1-OSRC33) [41], we found that the OSRC14, OSRC19, OSRC27, and OSRC32 contained more mutant alleles in *H. sinensis* strains. In addition, 61 of the 254 unassembled genome sequences are genetically variable when comparing to the assembled genome sequences of *H. sinensis* strains. Many of OSRC marker genes and the unassembled genome sequences were differentially transcribed in natural *C. sinensis* and strain L0106. Multiple overlapped sequences of the 18S and 28S genes exist in the genomes of *H. sinensis* strains and multiple transcripts of the 18S and 28S genes were found in natural *C. sinensis*. These overlapped gene sequences contain scattered insertion/deletion, transition, and transversion mutant alleles, some of which may cause translation interruptions due to nonsense, frame shift, or missense mutations of the genes. Some of the overlapped transcripts of the mutant 18S and 28S genes might be possibly derived from the fungi colonized in natural *C. sinensis* [16,34].

A large number of *H. sinensis* strains have been arbitrarily selected in the studies of *O. sinensis* fungi and natural *C. sinensis* insect-fungi complexes [4,8,12-13,15,17-19,21-22,26-28-30,33,35-36,39-40,44,72]. According to the ResearchGate discussion with Dr. Nigel Hywel-Jones, Sung et al [4]. arbitrarily selected strain EFCC7287 of *H. sinensis* (Genotype #1 of *O. sinensis*) as the reference strain for fungus *Cordyceps sinensis* molecular taxonomy and nomenclature project and renamed it to *Ophiocordyceps sinensis*. According to the sequences of 5 DNA loci, strain EFCC7287 is proven belonging to species *H. sinensis* [6-7,71]. Sung et al. [4] therefore actually defined *H. sinensis* (Genotype #1 of *O. sinensis*) as the sole anamorph of teleomorph *O. sinensis* and did not expand the taxonomy-nomenclature project to other 16 genotypes of *O. sinensis* that belong to independent fungi, because of unavailability of pure cultures of the mutant genotypes. Accordingly the rename of *C. sinensis* to *O. sinensis* is only restricted to *H. sinensis*, Genotype #1 of *O. sinensis*. Moreover, many arbitrarily selected *H. sinensis* strains have been used in industrial fermentation for manufacture of commercial products, but studies are lacking in exploring differences in pharmacological and toxicological profiles between these strains and the type strain HMAS 55469 of *H. sinensis* [73]. Although Wei et al. [72] defined Cephalosporium dongchongxiacaonis, Hirsutella hepiali, and Synnematium sinensis as the synonyms of *H. sinensis* through molecular systematic approaches, the *H. sinensis* type strain HMAS 55469 was not included in the study as the standard strain and the problematic molecular and bioinformatics methods used in the study lead to uncertainty in the study conclusion [6,9,38,43-44]. Consequently the possible intra- or interspecies variations may need to be re-accessed at genome and transcriptome levels among these so called "synonymic fungi" by the owners of these fungal strains. Furthermore, direct comparisons in various scientific disciplines (molecular mycology, genome/mitogenome, transcriptome, proteome, metabolome and chemistry, pharmacology, toxicology, etc.) are also absent between *H. sinensis* strains that were submitted to government regulatory bodies for product registration and the arbitrarily selected *H. sinensis* strains for industrial use are often replaced later with new strains because of unfortunate degeneration of the registered strains with time. Our findings of significant intraspecific genome and transcriptome variations in *H. sinensis* strains may serve as an admonishment in academic and industrial use of *H. sinensis* strains and encourage scientists to establish a genome standard for the *H. sinensis* type strain HMAS 55469 and even transcriptome, proteome, and metabolome standards of the type strain under various and standard fermentation conditions.

CONCLUSION

In conclusion, our findings present herein demonstrate alternative transcriptions of many *H. sinensis* genes and apparent intraspecific variations at the genome, mitogenome, and transcriptome levels among *H. sinensis* strains. The results may serve as a precaution for possible significant differences in metabolome/chemical constituents, proteome, and pharmacology between natural *C. sinensis* and mycelial fermentation products of *H. sinensis* and possible alterations in the safety profiles of *H. sinensis*-fermented products after arbitrarily exchanging *H. sinensis* strains for academic and industrial uses. Inconsistent co-existence and alternative transcriptions of multiple *H. sinensis* mating-type genes in the *H. sinensis* strains and natural *C. sinensis* are exactly the opposite of the homothallic and pseudohomothallic mating previously hypothesized for *H. sinensis* [24,26], and heterothallic mating may have to be considered.

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Xiu-Zhang Li and Yu-Ling Li have contributed equally.

CONFLICT OF INTERESTS

None

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