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Molecular Identification of *Ophiocordyceps sinensis* Genotypes and the Indiscriminate Use of the Latin Name for Multiple Genotypes and the Natural Insect-fungi Complex

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Abstract

Seventeen genotypes of *Ophiocordyceps sinensis* have been identified in molecular mycology studies of natural *Cordyceps sinensis*, comprising multiple fungi and a dead larva from the Hepialidae family. However, these multiple genotypes belonging to independent fungi share the same Latin name, *O. sinensis*, which has compounded the historical problems associated with the indiscriminate sharing of the same Latin name for both the fungus and the natural insect-fungi complex. This paper reviews the scientific findings for multiple *O. sinensis* genotypes in natural *C. sinensis* and the history of and current perspectives on indiscriminately using the Latin names *C. sinensis* and *O. sinensis* for the multiple *O. sinensis* anamorphs and teleomorphs per the nomenclature rule established by the Amsterdam Declaration, "One Fungus=One Name", and for the natural *C. sinensis* insect-fungi complex. Although some Chinese scientists have proposed the use of "Chinese cordyceps" or "Ophiocordyceps & Hepialidae" for natural *C. sinensis* to partially resolve the indiscriminate usage of the Latin name, the mycological research community is facing a scientific challenge because multiple *O. sinensis* genotypes are currently nonculturable *in vitro* (except Genotype #1 *Hirsutella sinensis*), and the mutant sequences of Genotypes #2-17 are not present in the genome of *H. sinensis*. This paper encourages cooperation among taxonomists across disciplines to resolve the taxonomic nomenclature problem by characterizing *O. sinensis* fungi with mutant genotypes and to end the centuries-old academic confusion over the indiscriminate use of the same Latin name for *O. sinensis* fungi and the natural insect-fungi complex.

Keywords: Natural *Cordyceps sinensis*, multiple genotypes of *Ophiocordyceps sinensis*, multicellular heterokaryotic structure, genetic heterogeneity, IMA nomenclature rule: One Fungus=One Name

1. Introduction

The Chinese Pharmacopeia defines natural *Cordyceps sinensis* as an insect-fungi complex, and this complex is a precious "herb" in traditional Chinese medicine (TCM), with a rich history of clinical use for health maintenance, disease amelioration, postdisease and postsurgery recovery, and antiaging therapy [1-2]. Both culture-dependent and culture-independent mycological and molecular mycological studies have identified >90 fungal species spanning >37 genera and 17 genotypes of *Ophiocordyceps sinensis* in natural *C. sinensis* [3-24]. However, the Latin name, *O. sinensis*, refers to multiple teleomorphic and anamorphic fungi that have distinct genomes and to the natural insect-fungi complex (hereafter, natural *C. sinensis*) [9-10, 22]. This issue continues and is even getting worse, with confusion spreading from the scientific community to public media and the mass market, causing a significant decrease in the whole-sale and retail prices of natural *C. sinensis*. This review paper readdresses the centuries-long problem by including a significant amount of recently published data from molecular genotyping, genome and transcriptome studies with the intention of reaching multidisciplinary scientists who are involved in biomedical research on natural *C. sinensis* and *O. sinensis* fungi.

2. Methods

Publications since the 1840s relating to the history of devolvement of natural *C. sinensis* and Latin nomenclature of this natural product and intrinsic fungus/fungi are listed in this review based on a systematic review by Lu [25] in Chinese. Information regarding intrinsic fungal species in natural *C. sinensis* was summarized by Jiang and Yao [5], and subsequent discoveries of additional species in natural *C. sinensis* were reviewed by Li et al. [9-10] and Zhu and Li [22]. Relevant molecular biology-related publications on *O. sinensis* since 1999 were carefully checked, and >600 ITS sequences of *O. sinensis* uploaded in GenBank were confirmed, or corrected in some cases, through genotyping analysis by running a BLAST search and comparisons (https://blast.ncbi.nlm.nih.gov/blast/) and phylogenetic analysis using software MrBayes v3.2.7 (the Markov chain Monte Carlo [MCMC] algorithm).

3. Results

The International Mycological Association (IMA) presented its Amsterdam Declaration, "One Fungus=One Name" (1F=1N) [26], ruling that one name shall be used for both the anamorph and teleomorph of a single fungal species (taxonomic prerequisite to avoid applying the same name to multiple fungi). Chinese mycologists Zhang et al. [27] proposed the implementation of the nomenclature rule in *O. sinensis* research, and many *O. sinensis* papers have followed this suggestion. However, the fundamental questions that must be answered prior to implementing the nomenclature rule are whether *O. sinensis* is a single fungus or represents a group of fungi and, therefore, whether 1F=1N is applicable to *O. sinensis* research. Three controversial hypotheses have been reported in the literature, none of which have been directly confirmed based on all 4 criteria of Koch's postulates [5, 7, 9-10, 22-24].

3.1 Three hypotheses regarding *O. sinensis* and implementation of the IMA nomenclature rule "1F=1N"

3.1.1 Hypothesis I: *O. sinensis* is a single fungus.

Wei et al. [28] proposed that *H. sinensis* is the sole anamorph of *O. sinensis* based on 3 sets of evidence: (1) frequent isolation and mycological identification *via* sporulation, conidial morphology and growth characteristics; (2) individual development biology studies on microcycle conidiation of the ascospores [29-30]; and (3) molecular systematic analysis *via* internal transcribed spacer (ITS) sequencing and random molecular marker polymorphism assays [28, 31-32]. Guo et al. [33] restated that *H. sinensis* is the sole anamorph of *O. sinensis* and that other intrinsic

fungi (listed in Hypothesis II below) are not anamorphs of *O. sinensis*. They recognized the findings of the multiple genotypes of *O. sinensis* (listed in Hypothesis III below) but commented on the "incorrect" idea that treats the mutant genotypes as independent fungi.

Multigene analysis and ISSR molecular marker polymorphism studies reported apparent intraspecific variations in hundreds of *H. sinensis* strains [9, 34-35]. Li et al. [9, 35] confirmed the intraspecific variations in *H. sinensis* strains in the genome, transcriptome, and amino acid sequences. These findings differentiated the intraspecific variations within species *H. sinensis* and the interspecies variations among the 17 *O. sinensis* genotypes (detailed analysis below).

Ten years later, the key authors of [28] published an industrial artificial cultivation project on cultivated *C. sinensis* [36]. They reported that the 3 anamorphic *H. sinensis* strains (130508-KD-2B, 20110514, and H01-20140924-03) were used as the inoculation agents, and their ITS1-5.8S-ITS2 sequences were 100% identical to GenBank accession #EU570957 for the *H. sinensis* strain (*O. sinensis* specimen voucher HMAS:173836). In addition, they reported the detection of teleomorphic AT-biased Genotype #4 (GenBank accession #KC305892 and #AB067749) of *O. sinensis* but not GC-biased Genotype #1 in the fruiting body and mycelial culture of the caterpillar body of cultivated *C. sinensis*. They also detected teleomorphic GC-biased Genotype #1 in a natural *C. sinensis* specimen that was used as the study control for phylogenetic analysis. Thus, Wei et al. [36] actually presented a species contradiction between the inoculants and the teleomorphic *O. sinensis* in cultivated *C. sinensis* because the sequences of Genotype #4 of *O. sinensis* are not present in the genome of Genotype #1 *H. sinensis* [9-10, 22, 37-41]. The findings of [36] also provided evidence of at least 2 teleomorphs of *O. sinensis* and raised questions regarding whether *H. sinensis* was truly the sole anamorph of *O. sinensis* and the true causal agent of natural and cultivated *C. sinensis*.

Zhang et al. [27] summarized nearly 40 years of mycological efforts on artificial cultivation of *C. sinensis*, which have achieved little success in research-orientated academic settings, regardless of culturing *in vitro* or on insects. Hu et al. [37] reported the failure of the induction of fruiting body production after inoculating 40 larvae of *Hepialus* spp. with injection of a "fungal cell suspension" of *H. sinensis* Strains Co18 and QH195-2 "through the second proleg." This inoculation strategy was applied to circumvent the natural inoculation processes through the larval skin barrier and/or the epithelial barriers of the larval intestinal tract and spiracles and to improve the extremely low infection potency of *H. sinensis* on moth larvae [11]. Although the inoculation successfully caused death and "mummification" of the infected larvae that became "stiff cadavers", Hu et al. [37] reported the failure of induction of fruiting body production of *C. sinensis* and concluded that "attempts at cultivating the fungus to produce fruiting bodies have consistently failed" [37, 42-43].

Li et al. [11] conducted inoculation/infection experiments using either conidia or mycelia of *H. sinensis*, ascospores of *C. sinensis*, or a combination of 2 wild-type fungal complexes (CH1 and CH2) as inoculants on *Hepialus armoricanus* larvae (n=100 larvae per inoculant experiment). Strains CH1 and CH2 were isolated from the intestines of healthy larvae of *Hepialus lagii* and shared the same morphologic and growth features with *H. sinensis* but contained GC-biased Genotype #1 *H. sinensis* and AT-biased Genotypes #4-6 of *O. sinensis* and *Paecilomyces hepiali*. The inoculation experiments demonstrated extremely low infection rates (1.4%-3.5%) when the inoculation was performed with the conidia or mycelia of *H. sinensis* or the ascospores of natural *C. sinensis* but significantly higher infection rates (55.2 $\pm$ 4.3%; $P<0.001$) when the inoculation was performed with the wild-type fungal complexes (Strains CH1 and CH2) on the larvae of *H. armoricanus*.

Although the sole anamorph hypothesis for *H. sinensis* is widely appreciated, these study results [11, 27, 36-37, 42-43] raised questions from different perspectives regarding whether *H. sinensis* is the sole anamorph of *O. sinensis* and the true causal fungus of infection in larval moths or of fruiting body and ascospore production in *C. sinensis*. Rather, the true causal fungus is likely a combination of several fungal species acting in a symbiotic manner.

3.1.2 Hypothesis II: *O. sinensis* is a collective term for multiple fungi.

Jiang and Yao [5] summarized the literature findings up to 2003 concerning 22 fungal species spanning 13 fungal genera identified from natural *C. sinensis*: *Cephalosporium acremonium*, *Cephalosporium dongchongxiacae*, *Cephalosporium sinensis, Cephalosporium* sp., *Chrysosporium sinense*, *H. hepiali*, *H. sinensis*, *Isaria farinose*, *Isaria* sp., *Metarhizium anisopliae*, *Mortierella hepiali*, *P. hepiali*, *Paecilomyces lingi*, *Paecilomyces sinensis*, *Scydalium* sp., *Scytalidium hepiali*, *Sporothrix insectorum*, *Stachybotrys* sp., *Synnematium sinense*, *Tolypocladium sinense*, *Verticillium sinensis*, and *Verticillium* sp. Additional fungal species have since been identified from natural *C. sinensis* [13-15, 18, 44]. In addition to the sole anamorph hypothesis for *H. sinensis* (Hypothesis I) based on the 3 sets of evidence described above, Wei et al. [28] also concluded that *Cephalosporium dongchongxiacaonis*, *Hirsutella hepiali*, and *Synnematium sinense* are synonyms of *H. sinensis*, although the conclusions were debatable because of the problematic methods used in the study [9-10, 22]. Other fungi might not be considered the anamorphs of *O. sinensis* but instead *C. sinensis*-associated, *C. sinensis*-related, *C. sinensis*-colonized, "passing-by" fungi, or "endophytic" parasites [14, 28, 33].

Shao et al. [13], Xia et al. [15], and Zhang et al. [18, 44] reported the identification of >90 fungal species spanning >37 fungal genera in the stromata and caterpillar bodies of natural *C. sinensis* specimens. Zhang et al. [18] reported that *Pseudogymnoascus roseus* and *Penicillium chrysogenum* were the dominant fungi in the caterpillar body and stroma of *C. sinensis*, respectively. Xia et al. [15] reported that "Geomyces, Phoma, and Trichocladium were the dominant genera in the larval sample (*i.e.*, caterpillar body), while Geomyces and Cladosporium were the dominant genera in the stromal sample". Neither study reported the detection of multiple genotypes of *O. sinensis* (including Genotype #1 *H. sinensis*), *T. sinensis*, or *P. hepiali* in natural *C. sinensis*.

Li et al. [9, 35] analyzed the metatranscriptome sequence GAGW00000000 of natural *C. sinensis* [46] and found that several metatranscriptomic sequences of natural *C. sinensis*, such as GAGW01000014, GAGW01012749, GAGW01012431, and GAGW01013943, had 45-99 highly homologous (97%-100%) and overlapping transcript sequences (>90% query coverage). The shotgun genome sequence JM973748 of *H. sinensis* Strain YN07-8 [47] is 100% homologous to the short metatranscriptome sequence GAGW01010185 and 69.8%-96.9% similar to 118 other sequences of the metatranscriptome assembly GAGW00000000 of natural *C. sinensis* but to only 13 sequences of the transcriptome assembly GCQL00000000 of *H. sinensis* Strain L0106 [48], indicating that a majority of the GAGW00000000 transcript repeats were likely from multiple fungi coexisting in natural *C. sinensis* [9, 35, 46, 48].

In contrast to the sole anamorph hypothesis for *H. sinensis* (Hypothesis I) [28], Barseghyan et al. [3] concluded that both *H. sinensis* and *Tolypocladium sinensis* were the anamorphs of *O. sinensis*, and Engh [4] reported molecular identification of the *Cordyceps*-*Tolypocladium* complex. Chen et al. [49] and Leung et al. [50] reported the molecular identification of *Tolypocladium sinense* or *Tolypocladium* sp. in natural *C. sinensis*.

To address whether those fungi identified in natural *C. sinensis* are the correct anamorph(s) of *O. sinensis* or naturally occurring contaminants, Jiang and Yao [5] summarized the various methods for determining the correct *O. sinensis* anamorph and restressed that the most reliable method is to obtain direct evidence by strictly following Koch's postulate; herein, a singular "postulate" was used in the sentence, and the 3rd criterion of Koch's postulates was described in the paper. They categorized the 3 sets of research findings cited by Wei et al. [28] and Guo et al. [33] as auxiliary evidence for concluding that *H. sinensis* is the correct *O. sinensis* anamorph. Unfortunately, no evidence has been provided to date that strictly meets all criteria of Koch's postulates, particularly the 3rd and 4th postulates, or validates any of the postulated anamorphic fungi, including *H. sinensis* and *T. sinensis*, as the correct anamorph(s) of *O. sinensis*. In particular, both Hypothesis I (*H. sinensis* as a sole anamorph) and Hypothesis II (*H. sinensis* and *T. sinensis* as dual anamorphs) lack direct evidence meeting the 3rd and 4th criteria of Koch's postulates.

P. hepiali has been frequently isolated and identified from natural *C. sinensis*, and its ITS1-

5.8S-ITS2 sequences were amplified using *P. hepiali*-specific primers and molecular cloning techniques from genomic DNA isolated from the stroma, caterpillar body, ascocarps, and ascospores of natural *C. sinensis* [9-10, 20-24, 49, 51-54]. *P. hepiali* was often found to be closely associated with *H. sinensis* in natural *C. sinensis*, representing the technical difficulties associated with completely isolating these 2 fungi even by top-notch mycologists [11]. Li et al. [11] reported that 2 wild-type fungal complexes (Strains CH1 and CH2 featuring *H. sinensis*-like morphologic and growth characteristics) consisted of Genotypes #1 (*H. sinensis*), #4-6 of *O. sinensis* and *P. hepiali*. The combined use of these 2 wild-type strains as the inoculant exhibited significantly higher infection rates of 55.2 ± 4.3% on the larvae of *H. armoricanus*, representing 15- to 39-fold higher inoculation potency ($P<0.01$) than the biologically insignificant infection rates of 1.4%-3.5% after inoculation with the mycelia and conidia of pure *H. sinensis* (Genotype #1 of *O. sinensis*). Although *P. hepiali* is often considered a symbiotic fungus, Qiu et al. [54], a committee of 7 distinguished mycology and zoology professors, concluded in the written expert statement that "*Paecilomyces hepiali* sp. nov. has a close relationship with natural *C. sinensis* and is one of the dominant fungi in *C. sinensis* that constitute the fruiting body of *C. sinensis* at least in some production areas of *C. sinensis*."

In addition to supporting the sole anamorph hypothesis of *O. sinensis* for *H. sinensis*, Guo et al. [33] encouraged scientists to consider the complex symbiotic relationships of the multiple intrinsic fungi colonizing natural *C. sinensis*. However, to date, the symbioses among the multiple fungi in natural *C. sinensis* are poorly understood, except the enhancement of the symbiotic inoculation potency of Genotype #1 *H. sinensis* with Genotypes #4-6 of *O. sinensis* and *P. hepiali* [11].

3.1.3 Hypothesis III: *O. sinensis* is a collective term for multiple mutant genotypes of *O. sinensis* fungi.

Kinjo & Zang [6] first reported genetic variants of *O. sinensis* in 2001 and showed 2 major phylogenetic groups (GC- and AT-biased) and 3 phylogenetic clades, which, however, were believed to be intraspecific variants based on their morphological and growth features, although the first sequence of AT-biased Genotype #4 was discovered by Engh [4] in a graduate study in 1999 at the University of Oslo. Stensrud et al. [14] analyzed 71 ITS sequences of *O. sinensis* belonging to 3 phylogenetic clades (GC-biased Group A and AT-biased Groups B-C) and 2 unrelated clades (Groups D-E). They confirmed that the sequences of Groups A-C were the correct sequences of *O. sinensis*. Chinese mycologists Zhang et al. [17] analyzed 397 ITS sequences in 2013 and confirmed that Groups A–C were the correct *O. sinensis* sequences.

To date, >600 ITS1-5.8S-ITS2 sequences of *O. sinensis* have been uploaded to GenBank under GenBank Taxid: 72228, representing 17 genotypes of *O. sinensis* (Table 1) with numerous, scattered transition, transversion, or insertion/deletion mutant alleles or hereditary variations with reciprocal substitutions of large DNA segments and genetic material recombination [9-10, 22]. Figure 1 shows the sequence alignment of the transition mutant genotypes, including Genotypes #1-6 and #15-17 of *O. sinensis*. Figure 4 of Li et al. [10] shows the sequence alignment of the transversion and insertion/deletion mutant genotypes, including Genotypes #7-14 of *O. sinensis*. Genotypes #1-3 and #7-14 are GC-biased, and Genotypes #4-6 and #15-17 are AT-biased [6-12, 14, 16, 19-20, 22-24, 55-56]. The topology of the Bayesian tree (Figure 2) shows the phylogenetic relationship of the 17 genotypes of *O. sinensis*. Genotypes #13-14 are hereditary variants (offspring) with reciprocal substitutions of large DNA segments and genetic material recombination between the chromosomes of 2 parental fungi: Genotype #1 *H. sinensis* and the AB067719-type Group-E "*O. sinensis*" fungus (Table 2) [9-10, 14, 22].

Five sets of genome assemblies are available in GenBank, namely, ANOV00000000, JAAVMX000000000, LKHE00000000, LWBQ00000000, and NGJJ00000000, for the *H. sinensis* Strains Co18, IOZ07, 1229, ZJB12195, and CC1406-203, respectively [37-41]. The nrDNA ITS1-5.8S-ITS2 sequences of the *H. sinensis* genomes are 99.6%-100% homologous to the ITS sequences (AB067721) of Genotype #1 *H. sinensis*, but 78.5%-96.1% similar to the sequences of GC-biased Genotypes #2-3 and #7-14 of *O. sinensis* and 85.5%-89.9% similar to the sequences of AT-biased

Genotypes #4-6 and #15-17 of *O. sinensis* (Table 1). No additional DNA segments in ANOV00000000, JAAVMX000000000, LKHE00000000, LWBQ00000000, or NGJJ00000000 show >90% similarity to any of the AT-biased genotypes [7, 9-10, 22-24]. These *O. sinensis* genotypes are differentially present in the stroma, caterpillar body, ascocarps, and ascospores of natural *C. sinensis* [7-10, 12, 16, 19-24, 36, 55-56].

Table 1: Percent similarities between each nrDNA ITS1-5.8S-ITS2 sequence of 5 whole genomes of Genotype #1 *H. sinensis* strains and multiple *O. sinensis* genotype sequences

Geno-type	GenBank Accession #	nrDNA ITS1-5.8S-ITS2 Segments of the Whole-Genomes of *H. sinensis*				
		ANOV01021709	LKHE01000582	LWBQ01000008	JAAVMX010000017	NGJJ01000799
	ANOV01024851	—	100%	—	100%	—
	LKHE01000582	99.9%	—	—	—	—
	LWBQ01000008	97.5%	98.3%	—	—	—
	JAAVMX010000017	99.9%	99.9%	98.3%	—	—
	NGJJ01000799	99.7%	100%	99.6%	100%	—
#1	AB067721	99.7%	100%	99.6%	100%	100%
#2	MG770309	94.4%	94.7%	94.7%	94.7%	94.7%
#3	HM595984	95.9%	96.1%	95.6%	95.3%	95.3%
#4	AB067744	89.1%	89.4%	89.3%	89.4%	89.4%
#5	AB067740	86.3%	86.7%	86.2%	86.7%	86.7%
#6	KJ720572	85.5%	85.5%	85.5%	85.5%	85.5%
#7	AJ488254	94.7%	95.0%	95.3%	95.0%	95.0%
#8	GU246286	89.5%	89.5%	89.2%	89.5%	89.5%
#9	GU246288	95.0%	95.2%	94.6%	95.2%	95.2%
#10	GU246287	83.0%	83.2%	83.0%	83.2%	83.2%
#11	JQ695935	78.9%	79.1%	78.5%	79.1%	79.1%
#12	GU246296	94.3%	94.5%	93.9%	94.5%	94.5%
#13	KT339190	87.5%	87.5%	87.2%	87.5%	87.5%
#14	KT339178	89.3%	89.6%	89.1%	89.6%	89.6%
#15	KT232017	89.6%	89.9%	89.4%	89.9%	89.9%
#16	KT232019	87.4%	87.7%	87.6%	87.7%	87.7%
#17	KT232010	87.7%	88.1%	87.5%	88.1%	88.1%

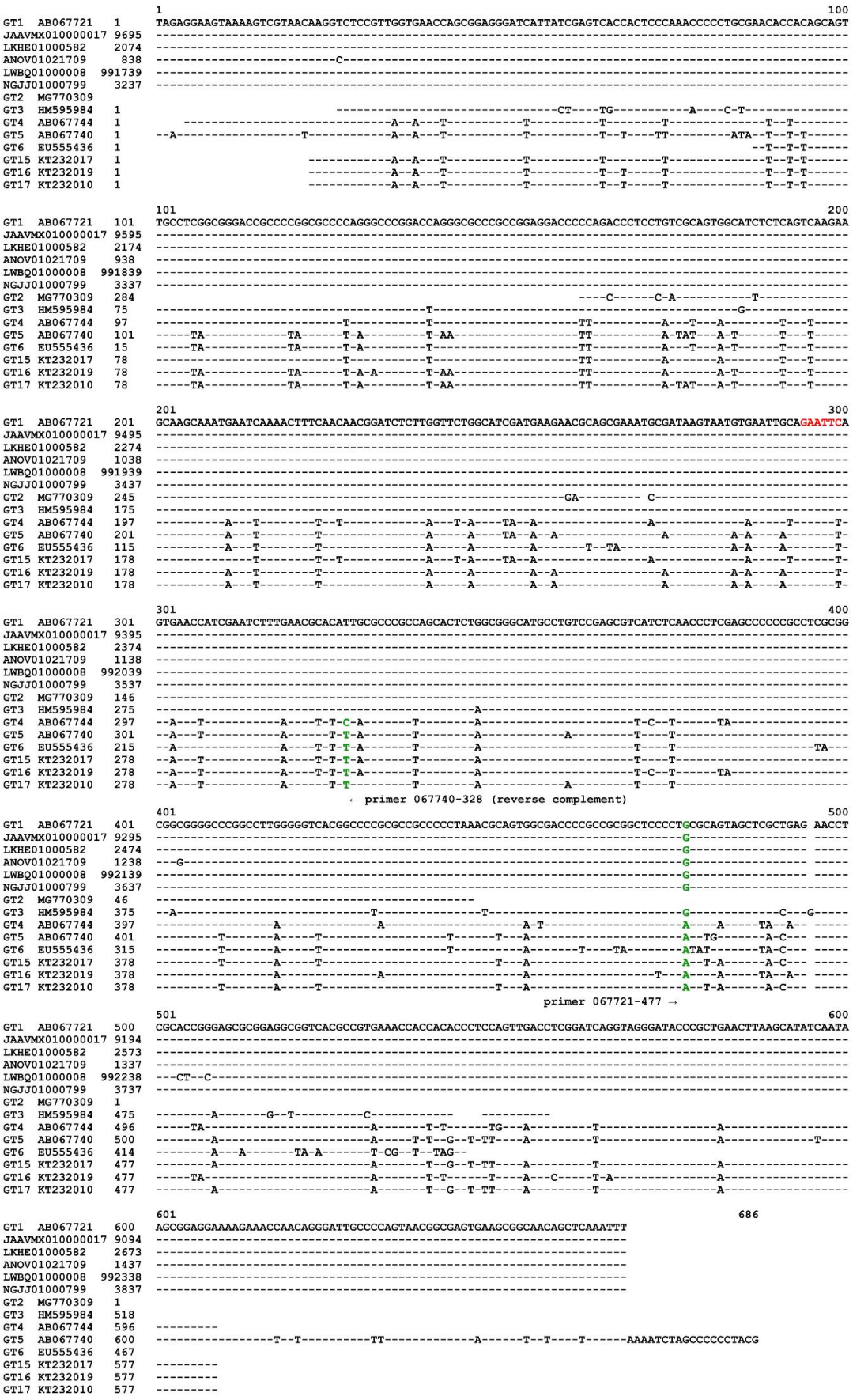

Figure 1: Sequence alignment of the transition mutant genotypes of *O. sinensis*

The sequences contain complete or partial segments of ITS1-5.8S-ITS2 nrDNA and partial segments of the 18S and 28S genes. "GT" stands for *O. sinensis* genotype. AB067721, MG770309, and HM595984 stand for the GC-biased Genotypes #1-3 of *O. sinensis*, respectively. AB067744, AB067740, EU555436, KT232017, KT232019, and KT232010 represent AT-biased Genotypes #4-6 and #15-17 of *O. sinensis*, respectively. ANOV01021709, JAAVMX010000017, LKHE01000582, LWBQ01000008, and NGJJ01000799 are the ITS1-5.8S-ITS2 nrDNA segments of the genome assemblies of Genotype #1 *H. sinensis* Strains Co18, IOZ07, 1229, ZJB12195, and CC1406-203, respectively. "GAATTC" in red (294→299 of AB067721) is the enzymatic site of endonuclease *Eco*RI occurring in the GC-biased genotype sequences and the genome assembly sequences of *H. sinensis* strains. A single-base mutation to "GAATTT" in AT-biased Genotypes #4-6 and #15-17 results in the loss of the enzymatic site. 067721-477 is the SNP extension primer and was designed based on the sequence of GC-biased Genotype #1 AB067721 for SNP extension toward position 477 (in green) of the AB067721 sequence. 067740-328 (the reverse complement) is the SNP extension primer that was designed based on the sequence of AT-biased Genotype #5 AB067740 for SNP extension toward position 328 (in green) of the AB067740 sequence. The arrows "→" and "←" indicate the primer extension directions. Hyphens indicate identical bases, and spaces denote unmatched sequence gaps.

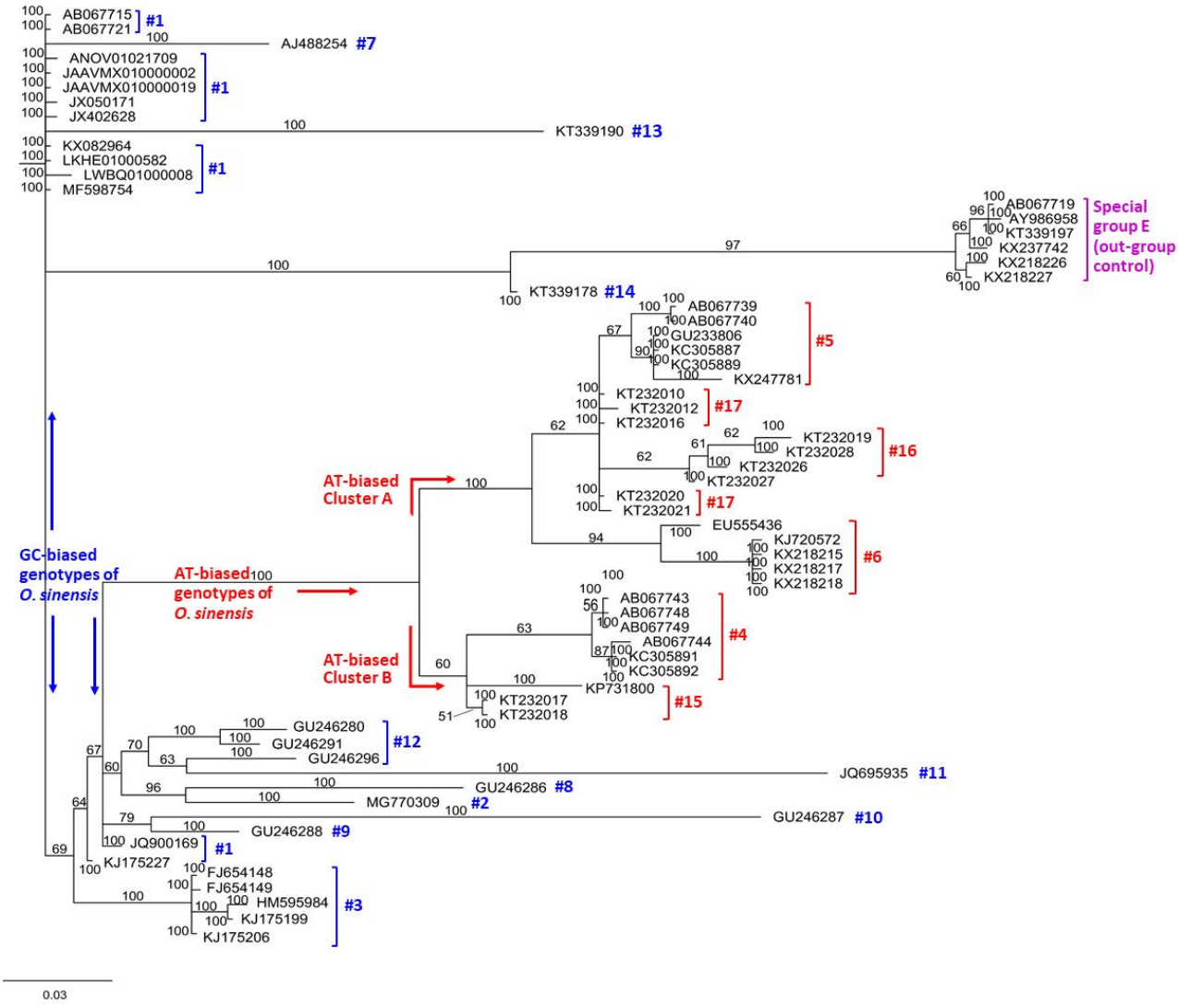

Figure 2: Bayesian phylogenetic tree of multiple genotypes of *O. sinensis*

Four nrDNA sequences of the whole genomes of *H. sinensis* strains and 59 ITS sequences of 17 genotypes of *O. sinensis* were analyzed. The Bayesian majority-rule consensus tree was inferred using MrBayes v3.2.7 software (the Markov chain Monte Carlo [MCMC] algorithm) from $3x10^4$ samples with a sampling frequency of 10^3 iterations after removing the first 25% of the samples of a total of $4x10^6$ iterations. GC-biased Genotypes #1-3

and #7-14 are side-noted in blue, and AT-biased Genotypes #4-6 and #15-17 are side-noted in red. Genotypes #13-14 are the genetic variants (offspring) with reciprocal substitutions of large DNA segments between the 2 parental fungi, Genotype #1 *H. sinensis* (Group-A) in the "blue" clade and a special AB067719-type Group-E fungus [14] in the "purple" clade as the outgroup control. ANOV01021709, LKHE01000582, LWBQ01000008, JAAVMX010000002, and JAAVMX010000019 are the nrDNA segments of the whole-genome sequences for *H. sinensis* Strains Co18, 1229, ZJB12195, and IOZ07, respectively [37-40].

Table 2: Percent similarities among the ITS1-5.8S-ITS2 sequences of parental fungi, AB067721 of Genotype #1 *H. sinensis* (Group-A) and an AB067719-type fungus (Group-E), and their offspring, Genotypes #13-14 of *O. sinensis*

Genotype		ITS1	5.8S	ITS2	ITS1-5.8S-ITS2
		vs. AB067721 sequence of Genotype #1 (Group-A)			
#13	KT339190	**100%**	94.8%	62.5%	86.3%
#14	KT339178	66.0%	94.9%	**100%**	87.7%
		vs. AB067719 sequence of Group-E			
#1	AB067721	65.4%	94.8%	66.5%	76.6%
#13	KT339190	60.9%	**100%**	**99%**	88.2%
#14	KT339178	**100%**	**100%**	64.0%	89.2%

Southern blot analysis using an *H. sinensis*-specific probe that was designed based on the ITS1 sequence of AB067721 (GC-biased Genotype #1) [20] confirmed the coexistence of both GC-biased (the *Eco*RI-sensitive, faster-migrating DNA moiety in the upper panel of Figure 3) and AT-biased (the *Eco*RI-resistant, slower-migrating DNA moiety) genotypes of *O. sinensis* fungi in the stroma and caterpillar body of *C. sinensis*. The natural biomasses (without PCR amplification) of the GC- and AT-biased genotypes underwent dynamic alterations in an asynchronous, disproportional manner in the stroma and caterpillar body of *C. sinensis* during maturation. The biomass of *Eco*RI-sensitive, GC-biased Genotype #1 *H. sinensis* (the faster-migrating DNA moiety) was extremely low in the stroma of premature *C. sinensis* (the "Pre" lane in Figure 3) and increased with *C. sinensis* maturation (the "M" lane) but was never the predominant fungal species in the stromata.

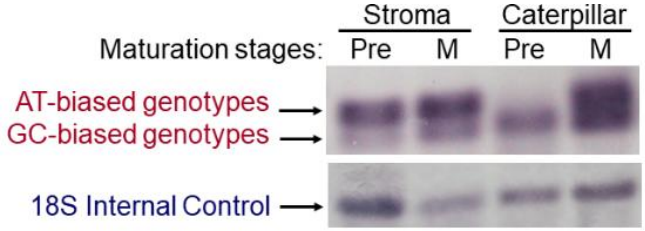

Figure 3: Southern blot of *C. sinensis* nrDNA in the stromata and caterpillar bodies of natural *C. sinensis* during maturation
(Reproduced with permission from *Am. J. Biomed. Sci.* 2010, 2(3), 217-238) [20]. Genomic DNA was isolated from the stromata or caterpillar bodies of premature (Pre) or mature (M) natural *C. sinensis* collected from Kangding County of Sichuan Province in China and prepared using the restriction enzymes *Eco*RI, *Dra*I and *Ava*I. Upper panel: An *H. sinensis*-specific probe was used for Southern blotting. Lower Panel: A nonspecific 18S internal control probe was used for Southern blotting. Caterpillar refers to the caterpillar body of natural *C. sinensis*.

MassARRAY SNP MALDI-TOF mass spectrometry genotyping also confirmed the cooccurrence of GC- and AT-biased genotypes of *O. sinensis* fungi in the stroma of natural *C. sinensis* (Figure 4). SNP mass spectrometry genotyping, as well as restriction fragment length polymorphism assays, also confirmed the dynamic, disproportional alterations of the abundance of *O. sinensis* genotypes in the stromata of *C. sinensis* during maturation [20, 53, 56]. In addition, Genotypes #5-6 and

#16 (AT-biased Cluster A shown in Figure 2) were detected in the *C. sinensis* ascocarps and ascospores, while Genotypes #4 and #15 (AT-biased Cluster B shown in Figure 2) were detected in the ascocarps but not in the ascospores [8-10, 22]. These findings, plus the differential existence of Genotypes #13-14 in the semi- and fully ejected *C. sinensis* ascospores, respectively, indicate the genomic independence of Genotypes #2-17, the sequences of which reside in the genomes of independent *O. sinensis* fungi rather than in the genome of Genotype #1 *H. sinensis* [4, 6-10, 12, 16, 19-20, 22-24, 36-41, 52-53, 55-56].

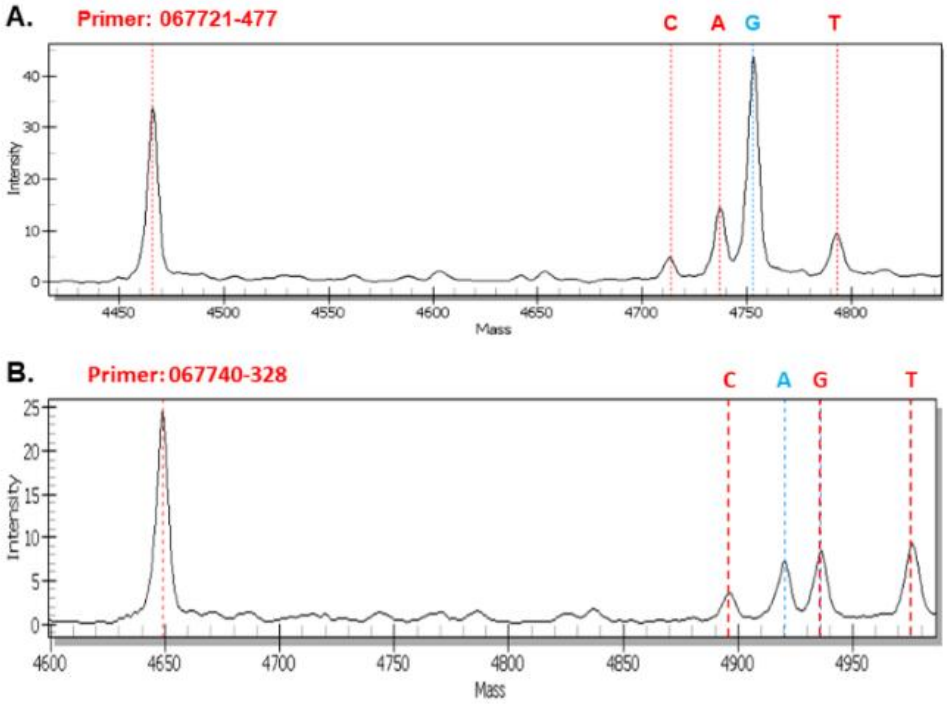

Figure 4: Representative MALDI-TOF mass spectra used to distinguish between the GC- and AT-biased genotypes (Panel A) and between the AT-biased genotypes (Panel B)
(Reproduced with permission from *Am. J. Biomed. Sci.* 2010, 2(3), 217-238) [20]. Genomic DNA for both panels was isolated from the stroma of premature natural *C. sinensis* collected from Kangding County of Sichuan Province in China. Panel A: Genomic DNA templates were amplified using primers designed based on the ITS sequence AB067721 of GC-biased Genotype #1 *H. sinensis*. The PCR amplicons were used as templates for the second PCR for SNP genotyping using biochip-based MassARRAY MALDI-TOF mass spectrometry. The SNP extension primer 067721-477 (5' CGCCGCGGCTCCCCT 3') was designed based on the alignment of the GC-biased AB067721 sequence and extended to the SNP at position 477 in the AB067721 sequence (*cf.* Figure 1). The allele peaks are marked with "A", indicating the primer with an extended adenine and representing the AT-biased genotypes of *O. sinensis*, or "G", denoting the primer with an extended guanine and representing GC-biased Genotype #1 *H. sinensis*. The allele peaks are marked with "C" or "T", referring to the primer with an extended cytosine or thymine, which represent unknown transversion point mutations at the sequence position. Panel B: Genomic DNA templates were amplified using primers designed based on the AT-biased sequences AB067744 and AB067740 of *O. sinensis*. The PCR amplicons were used as templates for the second PCR for SNP genotyping using MassARRAY MALDI-TOF mass spectrometry. The SNP extension primer 067740-328 (5' GTGCTAGCGGGCGTA 3', reverse complement) was designed based on the Genotype #5 (AB067740) sequence and extended to the SNP at position 328 of AB067740 (*cf.* Figure 1). The allele peaks are marked with "A", indicating the primer with an extended adenine and representing AT-biased Genotype #5 of *O. sinensis*, or "G", indicating the primer with an extended guanine and representing AT-biased Genotype #4 of *O. sinensis*. The allele peaks are marked with "C" or "T", referring to the primer with an extended cytosine or thymine, which represent unknown transversion point mutations at the sequence position.

Wei et al. [36] reported that AT-biased Genotype #4 fungus was the sole *O. sinensis* teleomorph in cultivated *C. sinensis*, which was distinct from the GC-biased Genotype #1 that was detected in natural *C. sinensis* and in 3 anamorphic inoculants (*H. sinensis* Strains 130508-KD-2B, 20110514, and H01-20140924-03) used in the industrial artificial cultivation project. Bayesian phylogenetic analysis placed GC-biased Genotype #1 and AT-biased Genotype #4 in isolated clades (*cf.* Figure 2), and the sequences of Genotype #4 were not present in the genome of Genotype #1 *H. sinensis* [6-10, 16, 22-24, 37-41]. Stensrud et al. [14] stated that "Groups B and C (AT-biased Genotypes #4-5) ⋯ showed highly accelerated evolution of the 5.8S gene compared with Group A (GC-biased Genotype #1) ⋯ Such a large sequence variation of the 5.8S nrDNA far exceeds what is normally observed in fungi (and other organisms), even at higher taxonomic levels (genera and family)." The apparent species contradiction between the inoculants and the fruiting body of cultivated *C. sinensis* may imply that Wei et al. [36] overlooked the Genotype #4 sequences among the anamorphic inoculation strains, which would confirm the findings of [11] that the actual causal agent is a fungal (species) complex containing several *O. sinensis* genotypes and *P. hepiali* or that secondary or sequential infections by the true causal fungus/fungi (at least Genotype #4 of *O. sinensis*) occurred during artificial cultivation. A third possibility is that a preprogrammed, nonrandom mutagenic conversion of GC-biased Genotype #1 to AT-biased Genotype #4 occurred precisely without exception in all cultivated *C. sinensis* pieces during cultivation; however, this possibility seems unlikely.

Wei et al. [36] discovered the teleomorphic AT-biased Genotype #4 of *O. sinensis* in cultivated *C. sinensis* but the teleomorphic GC-biased Genotype #1 in natural *C. sinensis*. Because the sequences of AT-biased Genotype #4 are not present in the genomes of GC-biased Genotype #1 *H. sinensis* Strains Co18, 1229, ZJB12195, IOZ07, and CC1406-203 [37-41] but instead belong to the genome of independent fungus [7-10, 16, 22-24, 37-41], Wei et al. [36] actually raised a dual-teleomorph hypothesis for *O. sinensis*, which is distinct from the sole teleomorph hypothesis for GC-biased Genotype #1 that they raised 10 years ago [28].

Li et al. [57] reported differential occurrence and transcription of the mating-type genes of *MAT1-1* and *MAT-1-2* idiomorphs in 237 *H. sinensis* strains, inconsistent with the self-fertilization under homothallism and pseudohomothallism hypotheses [37, 58]. Obviously, sexual reproduction of *O. sinensis* requires mating partners, which may be GC-biased Genotypes #1, #2 or #7 of *O. sinensis*, either monoecious or dioecious, or AT-biased genotypes of *O. sinensis* for physiological heterothallism although the taxonomic positions of *O. sinensis* Genotypes #2-17 need to be determined, or heterospecific fungal species for hybridization if the species are able to break the interspecific reproductive barriers, or for facultative hybridization [9, 34, 57].

Collective results of molecular mycology studies confirm that *O. sinensis* is a collective name for multiple genotypes of *O. sinensis* fungi rather than a single fungus, and *H. sinensis* is unlikely to be the sole anamorph of *O. sinensis*. Although Li et al. [8, 59] hypothesized that the sequences of multiple AT-biased *O. sinensis* genotypes were pseudogenic components of the genome of Genotype #1 *H. sinensis*, which was based on the detection of Genotypes #1 and #5 of *O. sinensis* in 8 of 15 cultures of *C. sinensis* monoascospores and the presence of the 5.8S cDNA of Genotype #1 but not Genotype #5 in a cDNA library, scientific findings do not support the "ITS pseudogene ⋯ in a single genome" hypothesis [7, 9-10, 16, 20, 22-24, 56]. Facing the scientific challenge of the "ITS pseudogene" hypothesis, Li et al. [8, 59] changed the phrasing that the mutant sequences were from "a single genome" [8] to from a "single ascospore" [59]. This switch in wording indicates the authors' acknowledgment that multicellular heterokaryotic ascospores may contain various genomes belonging to multiple fungi in different mono-/bi-/tri-nucleated ascosporic cells [16, 34, 58]. Thus, the One Fungus=One Name nomenclature rule may not be applicable to *O. sinensis* research because multiple *O. sinensis* fungi do not meet the prerequisite (One Fungus) of the rule [23].

3.2 History and current perspectives on indiscriminate use of the same Latin name(s)

The Chinese Pharmacopeia states that natural *C. sinensis* consists of the *O. sinensis* fruiting body

and a dead larval Hepialidae moth, *i.e.*, natural *C. sinensis* ≠ *O. sinensis* fungus/fungi [9-10, 22]. However, the same Latin name, either *C. sinensis* or *O. sinensis*, has been indiscriminately applied to the *C. sinensis* host-fungi complex and *O. sinensis* fungi [3-4, 6-10, 12, 14-15, 17-19, 22-25, 28, 36, 46, 49, 55, 58-60, 62-65]. Although the implementation of the 1F=1N nomenclature rule in *C. sinensis* research proposed by Zhang et al. [27] did not initiate indiscriminate use of the same Latin name in *C. sinensis* studies, it contributes to further confusion in the centuries-long history of this indiscriminate practice [9-10, 22]. The term "*O. sinensis*" is currently used to refer to the natural insect-fungi complex as well as the teleomorphic *O. sinensis* fungi and the postulated anamorphic *H. sinensis* and multiple mutant *O. sinensis* genotypes, obscuring the Latin name's specific meaning and causing confusion in *C. sinensis*/*O. sinensis* research and even in the natural *C. sinensis* markets [8-10, 12, 14, 16-17, 19-20, 22, 28, 36-37, 58-59, 61].

Natural *C. sinensis* was originally called DōngChóngXiàCǎo or DongChongXiaCao in Chinese (also known as XiàCǎoDōngChóng, HiaTsaoTomChom, HiaTsaoTomTchom, HiaTsaoTongTchong, HeaTsaonTsongChung, DōngChóngCǎo, ChóngCǎo, དབྱར་རྩྭ་དགུན་འབུ, Yartsa Gunbu, Yarchagumba, Yarsagumba, Yarshagumba, Totsu-Kaso, Tochukaso, etc.) [25]. Early records of this folk medicine appear in "Man ngag bye ba ring bsrel" by the Tibetan doctor Zur Mkhar Mnyam Nyid Rdo Rje (1439-1475) and several ancient TCM books (1694-1765), including "Essentials of Materia Medica" written by Ong Wang, "Light of Embers for Materia Medica" written by Fang-Yi Tang, "New Compilation of Materia Medica" written by Yi-Luo Wu, and "A Supplement to the Compendium of Materia Medica" written by Xue-Min Zhao (Figure 5).

记载冬虫夏草的古代中医学著作

Ong Wang 汪昂.《本草备要》：**Essentials of Materia Medica, 1694.**

Fang-Yi Tang 唐方沂.《青藜馀照》：**Light of Embers for Materia Medica, 1712–1722.**

Yi-Luo Wu 吴仪洛.《本草从新》：**New Compilation of Materia Medica, 1757.**

Xue-Min Zhao 赵学敏.《本草纲目拾遗》：**A Supplement to the Compendium of Materia Medica, 1765.**

Figure 5: English translations of the titles of ancient Chinese medical books on *C. sinensis*

After natural *C. sinensis* was introduced to Western countries by the French missionary Dominicus Parennin in 1723, its intrinsic fungus was identified by Jonathan Pereira in 1843 as belonging to the *Sphaeria* genus, and the insect portion was identified by Edward Doubleday as belonging to Agrotis [25, 66-67], indicating the scientific recognition of natural *C. sinensis* as an insect-fungal complex. Miles Joseph Berkeley described the fungus as *Sphaeria Sinensis* Berkeley in 1843 and then renamed it *Cordyceps Sinensis* in 1857 [60, 68]. Pier Andrea Saccardo renamed it *Cordyceps sinensis* (Berkeley) Saccardo in 1883 [69-70].

Sung et al. [71] renamed it *Ophiocordyceps sinensis* (Berkeley) G.H. Sung et al. in 2007 when a fungal Strain EFCC 7287 was arbitrarily selected as the nomenclature standard (according to Dr. Hywel-Jones) [23]. Derived from Strain EFCC 7287, the sequences from 5 nuclear loci in phylogenetic analyses, EF468827 for nrLSU (large subunit of nrDNA), EF468971 for nrSSU (small subunit of nrDNA), EF468767 for tef1 (transcription elongation factor-1α), and EF468874 and EF468924 for rpb1 and rpb2 (the largest and second largest subunits of RNA polymerase II), are 98.7%-100% homologous to the sequences of the whole genome of the *H. sinensis* Strains Co18, 1229, ZJB12195, IOZ07, and CC1406-203 [37-41], indicating that Strain EFCC 7287 is Genotype #1 *H. sinensis* and that the renaming of *C. sinensis* to *O. sinensis* by Sung et al. [71] was applicable only to Genotype #1 without extending the taxonomy-nomenclature project to the diverse *O. sinensis* genotypes belonging to independent mutant fungal genomes [9-10, 16, 22-24].

The initial intention and continuous efforts to connect the fungal species to the insect-fungi complex led to indiscriminate use of the same Latin name(s) for the insect-fungi complex and for multiple *O. sinensis* fungi. Lu [25] reviewed that

(1) In 1846, Lindley J [62] "mentioned XiàCǎoDōngChóng as a TCM herb ⋯ labeled it with the Latin name *Sphaeria Sinensis* given by Berkeley, and attached 2 photos of XiàCǎoDōngChóng;"

(2) In 1857, Berkeley MJ [60] stated that "the Latin name for XiàCǎoDōngChóng ⋯ was renamed *Cordyceps Sinensis* ⋯ and noted that XiàCǎoDōngChóng was one of the herbal medicines";

(3) In 1892, Pratt AE [65] "reported exporting TCM herbs from Sichuan and Tibet ⋯ [and] used the Latin name *Sphaeria Sinensis* for TchongTsao".

Etc.

Indiscriminate use of the same Latin name has continued to the present. For instance, Bushley et al. [58] stated in 2013 that "*Ophiocordyceps sinensis* ⋯ is an economically and medicinally valuable fungus that parasitizes the Himalayan ghost moth (Hepialidae) to form the combination of insect and fungus known as Dong Chong Xia Cao (winter worm, summer grass) in Chinese" but also stated "*O. sinensis* has been overharvested to the point of becoming an endangered species in China", and Xiang et al. [46] stated in 2014 that "*Ophiocordyceps sinensis* ⋯ is an Ophiocordycipitaceae entomoparasitic fungus that has been used as a tonic and roborant for thousands of years in Asia". The same Latin name has also been indiscriminately used in public databases such as GenBank and HMAS (Herbarium Mycologicum and Academiae Sinicae) and in nonscientific literature.

Based on the definition of natural *C. sinensis* ≠ *O. sinensis* fungi by the Chinese Pharmacopeia as well as research findings, botany-TCM systematics describe *C. sinensis* as the entire insect-fungi complex, including the *C. sinensis* stroma and caterpillar body. Ren et al. [72] described the caterpillar body of *C. sinensis* as a pseudosclerotium complex consisting of the fungal sclerotium and fragments of larval tissues. Dissection observations revealed some intact larval organs/tissues in the caterpillar body of *C. sinensis*, including an intact larval intestine, an intact body wall of fair thickness with numerous bristles, head tissues, and fragments of other larval tissues (Figure 6), reflecting the host's active immunity-defense during fungal infection and ensuring success in extracting insect genomic and mitochondrial DNA and RNA and sequencing larval genes and transcripts [9, 73-74]. Lei and Shui [75], Meng et al. [76], and Wang et al. [77-78] reported host innate immunity and acquired immunological responses during *O. sinensis* fungal infection and other insect functions in the ghost moth, distinct from the *in vitro* culture medium that passively provides nutrients for fungal growth. In addition, studies of the stroma and caterpillar body of *C. sinensis* found distinct profiles of the mycobiota/microbiota, genomic DNA pools, RAPD random molecular marker polymorphisms, transcriptomic cDNA pools, proteomic expression polymorphisms, HPLC fingerprints of chemical constituents, distinct pharmacological functions and even opposite therapeutic effects of the stroma and caterpillar body of the natural product [9-10, 13, 15-16, 18, 20-22, 44-45, 52, 62, 79-82].

Obscuring the differences between the natural insect-fungi complex and teleomorphic and anamorphic *O. sinensis* fungi leads to unavoidable confusion. For example, Hu et al. [37] disclosed that "*O. sinensis* strain Co18 ⋯ was selected for genome sequencing" but also used the term *O. sinensis* to refer to the natural insect-fungi complex, such as "The caterpillar fungus *Ophiocordyceps sinensis* ⋯ is one of the most highly valued traditional Chinese medicines" and "Figure 4 Maturation of *O. sinensis* sexual structures in the laboratory. A field-collected sample with an immature fruiting body (a) was incubated in soil (b) for up to two weeks, the fruiting body swelled (c) by producing mature sexual perithecia and asci". The corresponding author of the study clarified to the audience at an international conference in 2013 that the genome study material was the *H. sinensis* Strain Co18 determined through a mono-spore purification approach. Regardless of this clarification to the conference audience, the paper [37] has been distributed globally, accompanied by confusion regarding the study material. Consequently, Zhang & Zhang [34], Zhang et al. [83], Wei et al. [36] and many others cited this paper [37] and concluded that genome sequencing was complete for *O. sinensis*. Thus, these authors overlooked the genetic heterogeneity of the *O. sinensis* teleomorphs and anamorphs and multicellular heterokaryotic structures of the *C.*

sinensis hyphae and ascospores and the detection of insect DNA and transcript sequences in the caterpillar body of natural *C. sinensis* [7, 9-10, 22-23, 58-59, 73-74]. Therefore, the statements "the genome sequencing for *Ophiocordyceps sinensis* has been completed" [34], "Chinese medicinal fungi (*Ophiocordyceps sinensis* …) have been genome sequenced" [83], and "combined thinking of DōngChóngXiàCǎo genomic sequence data" [36] are scientifically inaccurate, causing further confusion beyond that caused by the original distribution of the paper [37].

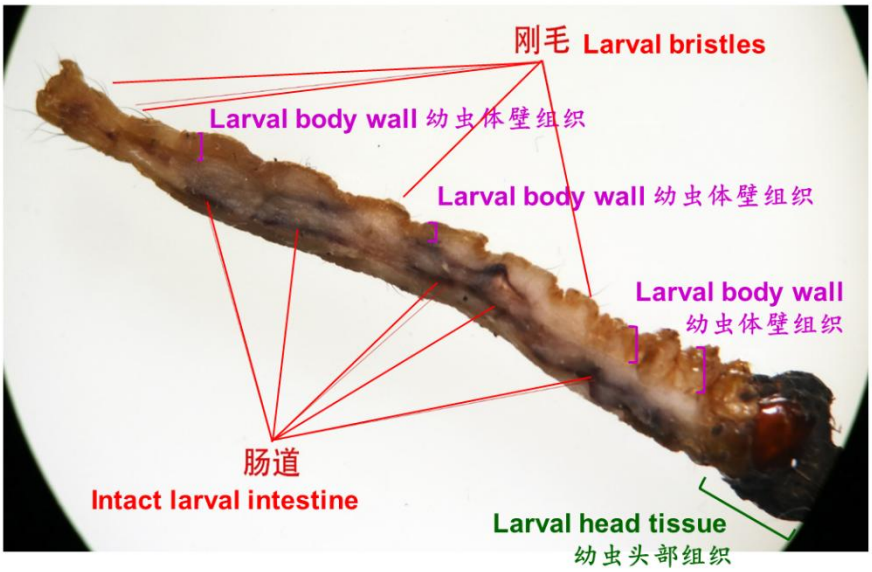

Figure 6: An anatomical image of the caterpillar body of natural *C. sinensis*. "]" in pink indicates the thickness of the larval body wall.

In addition to the many examples of confusion in *C. sinensis* studies, the paper title "Genome sequencing and analysis of the entomopathogenic fungus *Hirsutella sinensis* isolated from *Ophiocordyceps sinensis*" [38] and the paper title "Transcriptome sequencing and analysis of the entomopathogenic fungus *Hirsutella sinensis* isolated from *Ophiocordyceps sinensis*" [48] replaced the name *H. sinensis* with *O. sinensis*, thus producing the illogical "*O. sinensis* isolated from *O. sinensis*." Of course, it is not scientifically legitimate to state that the fungus "*Hirsutella sinensis*" was isolated from fungus "*Ophiocordyceps sinensis*", when Sung et al. (2007) used *H. sinensis* strain EFCC 7287 as the nomenclature reference and renamed *Cordyceps sinensis* to *Ophiocordyceps sinensis*. Obviously, the term "*Ophiocordyceps sinensis*" in the paper titles referred to natural *C. sinensis*, presenting a different level of confusion that *Ophiocordyceps sinensis* fungus = natural *C. sinensis* insect-fungi complex.

Barseghyan et al. [3] concluded that "the investigated strains were identified as *Hirsutella sinensis* and *Tolypocladium sinensis* species, which were identified as anamorphs of *Ophiocordyceps sinensis*". Here, again, the names *H. sinensis* and *T. sinensis* cannot logically and literally be replaced with the term *O. sinensis*. Otherwise, such an inappropriate replacement would be against the prerequisite "One Fungus" of the nomenclature rule "1F=1N" regardless of whether the dual anamorph conclusion was in debate.

Although the sole teleomorph hypothesis for Genotype #1 (Hypothesis I) [28] encourages the implementation of the 1F=1N nomenclature rule in *C. sinensis* research [27], this hypothesis of the sole teleomorph GC-biased Genotype #1 in natural *C. sinensis* is inconsistent with the hypothesis of the sole teleomorph AT-biased Genotype #4 in cultivated *C. sinensis* proposed 10 years later by the same key authors [36] and has been invalidated by scientific evidence supporting Hypothesis III, demonstrating that *O. sinensis* Genotypes #3, #4, #5, and #7 were individually detected in natural and cultivated *C. sinensis* but not Genotype #1 *H. sinensis* [4, 6-7, 10-12, 19, 22, 36, 55] and that *O. sinensis* Genotypes #1, #5-6, #13-14, and #16, but not Genotypes #4 and #15 of AT-biased Cluster B (*cf.*

Figure 2), were detected in the teleomorphic ascospores of natural *C. sinensis* [8-10, 22]. Thus, improperly implementing the 1F=1N rule in *C. sinensis* studies overlooks the definition set by the equation One Fungus=One Name (1F=1N) of the Amsterdam Declaration [26], in which "One Fungus" is the taxonomic prerequisite for proper implementation of the nomenclature rule "=One Name" [9-10, 22].

3.3 Suggestions for renaming natural *C. sinensis*

In 2012, mycologists Zhang et al. [47] suggested continued use of the name *O. sinensis* for the fungus/fungi and renaming the natural insect-fungi complex using the non-Latin term "Chinese cordyceps", not italicized and with lowercase "c" for cordyceps. This proposal has not been generally accepted because governmental regulations worldwide require every natural medicinal product to have a unique, exclusive Latin name.

Alternatively in 2013, botany-TCM scientists Ren et al. [72] suggested the Latin name "Ophiocordyceps & Hepialidae" for natural *C. sinensis*, reflecting the parasitic relationship between bat moth larvae and the postulated causal fungus/fungi. However, implementing this renaming proposal has been met with hesitation because the taxonomic positions of *O. sinensis* Genotypes #2-17 remain undetermined, likely due to the indistinguishable "*H. sinensis*-like" morphologic and growth features shared at least by Genotype #1 *H. sinensis*, Genotypes #3-5 and #7 of *O. sinensis* fungi [4, 6, 8, 12, 14, 19, 36, 55] and the "*Hirsutella*-like" morphology shared by numerous fungal species in the families Ophiocordycipitaceae and Clavicipitaceae and in the genera *Harposporium* and *Polycephalomyces* [84]. If *H. sinensis* and *T. sinensis* were correctly identified as anamorphs of *O. sinensis* [3] and given the proposal "for Ophiocordycipitaceae (*Hypocreales*) with new combinations in *Tolypocladium*"[84], revising the term proposed by Ren et al. [72] to the new term "Ophiocordycipitaceae & Hepialidae" may be appropriate to denote the natural insect-fungi complex, as it reflects both the dead Hepialidae larva and the multiple *O. sinensis* anamorphs and teleomorphs of the Ophiocordycipitaceae family, including the multiple *O. sinensis* genotypes with "a large sequence variation of the 5.8S nrDNA ⋯ at higher taxonomic levels (genera and family)" [14]. However, special attention is needed for the genetically variant offspring, Genotypes #13 and #14 of *O. sinensis* [9-10, 22], which were identified in the teleomorphic ascospores of natural *C. sinensis* and feature reciprocal substitutions of large DNA segments due to chromosomal intertwining interactions and genetic material recombination between the parental fungi, *H. sinensis* (Group-A of *O. sinensis*, represented by AB067721), and an AB067719-type Group-E fungus [14] (an independent fungal species represented by AB067719) (*cf.* Table 2), regardless of whether chromosomal intertwining interactions and genetic material recombination resulted from fungal hybridization or parasexuality [57]. It remains unclear why the genetically distinct Genotypes #13 and #14 of *O. sinensis* exist differentially in the semi- and fully ejected ascospores, respectively, collected from the same *C. sinensis* specimen if they are truly the offspring of the 2 parental fungi through different hybridization or parasexual reproduction processes, as well as what physiological and mycological roles Genotypes #13 and #14 play in the production, maturation, and ejection of ascospores.

The nomenclature for the natural *C. sinensis* insect-fungi complex and multiple *O. sinensis* fungi is highly academic and requires cooperation from multidisciplinary taxonomists across the fields of mycology, zoology, and botany-TCM. Unfortunately, mycologists have seemed to encounter difficulties in the taxonomic determination and nomenclature of the multiple mutant *O. sinensis* genotypes since Zhang et al. [17] declared in 2013 the taxonomy project to be urgent, probably due to the continued nonculturability of the mutant genotypes of *O. sinensis* fungi, excluding Genotype #1 *H. sinensis*.

4. Discussion and suggestions

The sequences of Genotypes #2-17 of *O. sinensis* have been demonstrated to reside in the genomes of independent fungi rather than in the genome of Genotype #1 *H. sinensis*. Improperly implementing the 1F=1N nomenclature rule has expanded the centuries-old confusion stemming from indiscriminate use of the same Latin name for

the natural *C. sinensis* insect-fungi complex and the multiple *O. sinensis* anamorphs and teleomorphs. Therefore, we suggest postponing the implementation of the 1F=1N nomenclature rule in *C. sinensis* studies until *O. sinensis* has been taxonomically demonstrated to be a single fungus and GC-biased Genotype #1 *H. sinensis* to be its sole anamorph. The long-standing academic problem of indiscriminately using the same Latin name for the natural insect-fungi complex and the multiple *O. sinensis* teleomorphs and anamorphs can be resolved by taxonomically characterizing the mutant genotypic fungi that are currently classified as *O. sinensis* to avoid compromising scientific understanding and to alleviate the socioeconomic consequences that arise from confusion in taxonomic nomenclature.

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