

Molecular evidence not supporting the use of *Hirsutella sinensis* strain EFCC7287 as the taxonomic standard for multiple *Ophiocordyceps sinensis* fungi

Opinion

Sung et al.¹ reported the sequences of five EFCC7287 genes (nrSSU, nrLSU, tef1, rpb1, rpb2) in the taxonomic-nomenclature study and renamed *Cordyceps sinensis* (Berkeley) Saccardo to *Ophiocordyceps sinensis* (Berkeley), according to the molecular systematics rules for fungi. It seems that *Hirsutella sinensis* strain EFCC7287 became a taxonomic standard for studying *O. sinensis*,² although Dr. Hywel Jones¹ believed in a Research Gate discussion the selection of strain EFCC7287 was arbitrary. Among the 5 genes examined by Sung et al.¹ and Quandt et al.² EF468827 (nrLSU) of 28S gene aligns with 99.5-99.7% homology to the ribosomal DNA segments (ANOV01021709, LKHE01000582, and LWBQ01000008) of the genome sequences of 3 *H. sinensis* strains: ANOV00000000 of Strain Co18,³ LKHE00000000 of Strain 1229,⁴ and LWBQ00000000 of Strain ZJB12195,⁵ while EF468971 (nrSSU) of 18S gene with 98.7-99.5% homology to the segments (ANOV01024851, LKHE01000582, LWBQ01000008) of the *H. sinensis* genome sequences. But EF468827 and EF468971 have low similarities to Genotypes#3-17 of *O. sinensis*; and no other segments with high similarity homology were identified within ANOV00000000, LKHE00000000, or LWBQ00000000.⁶⁻⁸ The genomic sequence analysis indicated that Sung et al.¹ & Quandt et al.² worked exclusively on *H. sinensis*, Genotype#1 of *O. sinensis*, without considering Genotypes#2-#17 of *O. sinensis* that have been deposited in GenBank and described in many research papers since 2002.⁶⁻¹³

Studies with using PCR amplicon-sequencing, cloning sequencing, Southern-blotting and RFLP following EcoRI restrictive digestion, and SNP mass spectrometry genotyping reported that the GC-biased Genotype#1 *H. sinensis* was either non-detectable or a minor *O. sinensis* component within the *C. sinensis* insect-fungi complex, whereas the AT-biased genotypes of *O. sinensis* predominated.^{7,8,11,12} Molecular examinations demonstrated that the genotypes of *O. sinensis*, including Genotype#1 *H. sinensis*, are differentially present and quantitatively and asynchronously altered in the compartments of natural *C. sinensis* during maturation. The molecular data indicate that the sequences of Genotypes#2-#17 *O. sinensis* reside not in the genome of *H. sinensis* but rather in the genomes of other independent fungi.⁶⁻¹³ Bushley et al.¹⁴ demonstrated the multicellular heterokaryotic hyphae and ascospores of natural *C. sinensis* with mono-/bi-/tri-nucleate structures. Barseghyan et al.¹⁵ conclude that *H. sinensis* and *Tolyptocladium sinensis* are “the anamorphs of *Ophiocordyceps sinensis*”. Close association of *Paecilomyces hepiali* with several genotypes of *O. sinensis* have been characterized in natural *C. sinensis*, forming species (fungal) complexes.^{6,8,11,12,16,17} Genotypes#13 and #14 of *O. sinensis* were identified from the heterokaryotic ascospores of natural *C. sinensis* as genetic variants (offspring) with large DNA segment reciprocal substitutions between 2 parental fungi, Genotype#1 *H. sinensis* and AB067719-type *O. sinensis*.⁶

The molecular evidence indicates that (1) the name *O. sinensis* represents a group of fungi, including the 17 mutant genotypes of

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O. sinensis and probably other *C. sinensis* associated fungi that have been considered as and biologically associated to the anamorphs and/or teleomorphs of *O. sinensis*, and (2) Strain EFCC7287 may be considered as the “taxonomic standard” solely of the GC-biased *H. sinensis*, but not of Genotypes#2-#17 *O. sinensis* or other *C. sinensis* associated fungi.^{6-8,11,12,15}

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Conflict of interest

Authors declare that there is no conflict of interest.

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