

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

## Opinion

Sung et al.<sup>1</sup> 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*,<sup>2</sup> although Dr. Hywel Jones<sup>1</sup> believed in a Research Gate discussion the selection of strain EFCC7287 was arbitrary. Among the 5 genes examined by Sung et al.<sup>1</sup> and Quandt et al.<sup>2</sup> 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,<sup>3</sup> LKHE00000000 of Strain 1229,<sup>4</sup> and LWBQ00000000 of Strain ZJB12195,<sup>5</sup> 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.<sup>6-8</sup> The genomic sequence analysis indicated that Sung et al.<sup>1</sup> & Quandt et al.<sup>2</sup> 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.<sup>6-13</sup>

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.<sup>7,8,11,12</sup> 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.<sup>6-13</sup> Bushley et al.<sup>14</sup> demonstrated the multicellular heterokaryotic hyphae and ascospores of natural *C. sinensis* with mono-/bi-/tri-nucleate structures. Barseghyan et al.<sup>15</sup> 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.<sup>6,8,11,12,16,17</sup> 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*.<sup>6</sup>

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

Volume 3 Issue 3 - 2018

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**Received:** April 27, 2018 | **Published:** May 11 2018

*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.<sup>6-8,11,12,15</sup>

## Acknowledgements

None.

## Conflict of interest

Authors declare that there is no conflict of interest.

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