

Delimitation of cryptic species within *Hypholoma fasciculare* complex based on parallel MiSeq sequencing of mitochondrial and nuclear loci

Hirotohi Sato¹), Ryoma Ohta²), Noriaki Murakami²)

¹Kyoto University, Japan

²Tokyo Metropolitan University, Japan

This study aims to delimit cryptic species within *Hypholoma fasciculare* complex in Japan based on DNA taxonomy. From September 2008 to November 2016, 100 fruiting bodies of the *H. fasciculare* complex were collected in 28 areas in Japan. The large and small subunit of the mitochondrial rDNA (mtLSU and mtSSU), the internal transcribed spacer region of the nuclear rDNA (ITS), and the 20 single-copy genes selected from FunyBase (<http://genome.jouy.inra.fr/funybase/>) were amplified. The Illumina MiSeq platform for sequencing with 2 x 250 bp read length. Mitochondrial sequences indicated that *H. fasciculare* complex contains four haplotypes. The monophyly of each haplotype was not strongly supported in the maximum likelihood (ML) tree of ITS sequences, but it was strongly supported in that of combined dataset of ITS and 20 single-copy genes. The analysis of molecular variation (AMOVA) for the single nucleotide polymorphisms (SNPs) of the combined nuclear dataset indicated that the genetic variation observed between haplotypes was much higher (77.96%; $\Phi_i=0.780$; $P=0.001$) than that observed between local populations within haplotypes (2.07%; $\Phi_i=0.094$; $P=0.001$), that between samples within local populations (0.67%; $\Phi_i=0.0337$; $P=0.349$) and that within samples (19.29%; $\Phi_i=0.193$; $P=0.001$). Those results suggest that four haplotypes observed in *H. fasciculare* complex represent reproductively isolated species. Our findings suggest that molecular phylogeny inferred from single nuclear locus (e.g., ITS) is not necessarily sufficient for delimiting sibling species of macro-fungi. Parallel sequencing of mitochondrial loci and multiple nuclear loci using MiSeq and subsequent SNP analyses would allow for more accurate detection of species boundaries.