

2-002-1

## Environmental DNA metabarcoding reveals the most comprehensive phylogenetic diversity and invisible lineages of early diverged wood-decomposers

Takashi Shirouzu<sup>1)</sup>, Shunsuke Matsuoka<sup>2)</sup>, Nobuaki Nagata<sup>3)</sup>, Masayuki Ushio<sup>4)</sup>,  
Kentaro Hosaka<sup>5)</sup>, Hideyuki Doi<sup>2)</sup>

<sup>1)</sup>Graduate School of Bioresources, Mie University, Japan

<sup>2)</sup>Graduate School of Simulation Studies, University of Hyogo, Japan

<sup>3)</sup>Collection Center, National Museum of Nature and Science, Japan

<sup>4)</sup>Hakubi Center, Kyoto University, Japan

<sup>5)</sup>Department of Botany, National Museum of Nature and Science, Japan

---

**Purpose:** We aimed to conduct the most comprehensive diversity survey of early diverged wood-decaying basidiomycetes, Dacrymycetes, in cool-temperate forests. The diversity of Dacrymycetes has not been thoroughly investigated, despite their significant functions as brown rotters. To obtain a more reliable phylogenetic hypothesis for the early-evolution of wood-decomposers, we conducted a comprehensive diversity survey of Dacrymycetes using a combined approach comprising a fruiting body survey, culture isolation, and eDNA analysis.

**Methods:** The study site was a mixed forest of *Pinus densiflora* and broad-leaved trees at an elevation of ca. 1,300 m at the Sugadaira Research Station (N 36.521, E 138.350), University of Tsukuba, Nagano, Japan. At this site, we surveyed Dacrymycetes diversity for 3 years by fruiting body collection, culture isolation using a dilution-to-extinction method, and eDNA analysis with DNA metabarcoding.

**Results and Conclusions:** The 3-year investigation revealed a total of 28 OTUs, of which 10 were collected as fruiting bodies, 10 were isolated as cultured mycelia, and 27 were detected as eDNA sequences. The eDNA metabarcoding revealed various lineages across the Dacrymycetes phylogeny, including previously undiscovered early branches that could not be obtained as fruiting bodies. The fruiting body survey and culture isolation could uncover only half of the OTUs estimated from eDNA metabarcoding data, suggesting that a large number of invisible lineages are latent in the environment. This complemented survey approach allowed us to detect previously invisible early branches. These findings contribute to our understanding of the evolutionary history of mushroom-forming fungi.