Current Biology, Volume 31

Supplemental Information

Ancestral predisposition

toward a domesticated lifestyle

in the termite-cultivated fungus Termitomyces

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Figure S1: Phylogenetic tree resulting from coalescent-based ASTRAL analysis based on the same dataset used for Figure 1.



Figure S2: Correlation between read depth and predicted CAZymes and PCA of CAZyme content corrected for phylogeny. Related to Figure 1. Plots showing no significant correlation between the number of reads used in the assemblies and the content of CAZymes (A), BUSCO genes (B) and predicted proteins (C). Plot D shows the relationship between the number of predicted proteins and CAZyme content. Plot E shows a principal component analysis on CAZyme content corrected for phylogeny. Species of *Arthromyces* are represented by red circles, *Blastosporella zonata* is represented by a green triangle, species of *Termitomyces* by purple plus signs and all other lyophylloid taxa are represented by blue squares.



Figure S3: Insect faecal substrate of Arthromyces matolae and mushroom of Tephrocybe rancida. Related to Figure 2. Macroscopic picture of faecal substrate (A). Microscopic picture showing cork-like structure (B), vessel elements (C) and (bordered) pits in the cell walls (D). These elements are characteristic for plant xylem indicating a woody origin of the substrate. Mushroom of Tephrocybe rancida with tapering pseudorhiza (E). After careful excavation of the pseudorhiza we could not find any attachment to a buried substrate as the pseudorhiza tapered into a thin thread which would eventually break (F).



Figure S4: Production of asexual spores (conidia) in laboratory cultures. Related to Figure 3. Dapi staining of the arthroconidia produced in a culture of *Arthromyces matolae* (FLAS-F-62734) showing two nuclei per conidium (A). Dikaryotic arthroconidia (B and C) and dikaryotic blastoconidia (D) produced in a culture of *Blastosporella zonata* (Bzo9).