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Phylogenetic analysis of *Ganoderma australe* complex in a Bornean tropical rainforest and implications for mechanism of coexistence of various phylogenetic types

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ABSTRACT

We hypothesized that different phylogenetic types of the *Ganoderma australe* complex can coexist and that their coexistence is promoted by resource partitioning among them. Our field survey and phylogenetic analysis revealed six phylogenetic types coexisting within a 3 ha primary forest plot in a Bornean tropical rainforest. Two of the six have been reported previously, whereas the remaining four are new. Fruit bodies of all dominant phylogenetic types appeared more frequently from fresh coarse woody debris than expected. Comparison of resource use patterns between the observed fungal community and the null community did not provide significant evidence of niche partitioning. Although we found high genetic diversity within the plot, the phylogenetic types at the site share resources. Resource partitioning on phylogenetic types of host trees or stochastic processes during colonization of pieces of coarse woody debris might play an important role in forming the community structure of phylogenetic types of *G. australe*.

1. Introduction

Wood-decaying fungi compete with each other within pieces of coarse woody debris (CWD) (Boddy, 2000), and there is substantial fungal diversity at the forest stand level. Different resource use patterns among polypore species have been observed in relation to tree phylogenetic type, size, decay stage, and condition of CWD (e.g., stump or fallen logs) in the temperate and boreal zones (Heilmann-Clausen et al., 2005; Yamashita et al., 2010). As a result, in tropical rainforests with a hyperdiversity of fungi (Lodge, 1997; Yamashita et al., 2015a), niche partitioning is expected to play an important role in the maintenance of diversity (Lodge and Cantrell, 1995).

Ganoderma australe is a cosmopolitan white rot fungus of tropical regions (Ryvarden and Johansen, 1980; Corner, 1983). It is a dominant wood-decaying fungus in primary forest of East Malaysia (Yamashita et al., 2009). The species is morphologically and phylogenetically closely related to *Ganoderma applanatum*, which is distributed in northern temperate regions (Ryvarden and Johansen, 1980; Corner, 1983; Smith and Sivasithamparam, 2000). The *G. australe* species complex, which includes *G. applanatum*, contains at least eight phylogenetic types: the Malaysia group, the Thailand group, the Asia group, the Southern Hemisphere and Asia group, and another four groups (Moncalvo and Buchanan, 2008). Multiple phylogenetic types may coexist within small areas.

Because the strength of competition is expected to increase as phylogenetic relatedness increases (Violle et al., 2011), competition is expected to be stronger within the *G. australe* complex than between it and other species. At our study site, members of the *G. australe* complex have been reported from CWD ranging from thin twigs (≤5 cm) to thick stems (≥40 cm) (25.7 ± 31.9 cm, mean ± s.d.), as well as in logs with intact bark and partly decayed logs (Yamashita et al., 2009). However, the degree to which different phylogenetic types of the complex coexist at this site, and the ecological traits of the individual phylogenetic types is not currently known.

In this study, we hypothesized that some phylogenetic types of the *G. australe* complex coexist in primary Bornean tropical

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rainforest and that their coexistence is promoted by resource partitioning among them. We conducted a field survey within a 3 ha plot in the Lambir Hills National Park and analyzed molecular data from samples. We compared the resource use patterns of the phylogenetic types of the observed *G. australe* complex community with those of a null community.

2. Materials and methods

2.1. Study site

Our study site is located in a tropical rainforest in the Lambir Hills National Park, Sarawak, Malaysia (4.2°N, 113.5°E; 150–250 m a.s.l.). This park is covered mostly by dipterocarp-dominated primary forest. A 4 ha plot was established in the park in 2000 (Yumoto and Nakashizuka, 2005).

A field survey was conducted from 29 May to 27 June 2009. All the CWD with a diameter of >10 cm was inspected for the appearance of fresh fruit bodies of *G. australe* within a 3 ha plot within the 4 ha plot. If we found any, we collected up to three from each piece of CWD; if four or more were present, we collected fruit bodies from the two endpoints and the middle. The decay stage and diameter at the center of the CWD were recorded. We defined the decay stage as (1) fresh, (2) medium, or (3) old (for further description, see Yamashita et al., 2009). The diameter was classified into size classes of 10–19, 20–29, 30–39 cm, 40–49 cm, and ≥50 cm. Fruit bodies of *G. australe* appeared from pieces of CWD at decay stages 1 and 2. We also recorded the number of pieces of CWD with a diameter of >10 cm at stages 1 or 2.

2.2. Specimens

The fruit bodies were used for molecular study. We cut each into several pieces and removed context tissue using flame-sterilized forceps and preserved it in 99% ethanol. We dried the rest of the material for 48 h and deposited it at the Research, Development, and Innovation Division of the Forest Department Sarawak, Kuching, Malaysia (see list of specimens in Supplementary Appendix 1). We identified fruit bodies by the color of the pileus and context from keys provided by Ryvarden and Johansen (1980). After molecular study, we measured the size of spore samples in aqueous 1% KOH by microscopy and confirmed that the size ranged from 7.7–12.5 × 3.7–7.5 μm, which is close to the size of this species reported by Ryvarden and Johansen (1980).

2.3. Molecular experiments

Genomic DNA was extracted from the context tissue with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The rDNA internal transcribed spacer (ITS) region was amplified using the primer pair ITS1-F/ITS4-B (Gardes and Bruns, 1993). Polymerase chain reaction (PCR) was performed using a HotStarTaq Plus Master Mix (Qiagen). Each PCR reaction contained a 50 μl mixture (16 μl of distilled water, 25 μl of master mix, 3 μl of ~0.5 ng/μl template DNA, 5 μl of Coral Load PCR buffer, and 0.5 μl of each primer (final, 0.25 μM)). Each DNA fragment was amplified in a PCR thermal cycler (PTC-0200 DNA Engine Cycler; Bio-Rad, Hercules, CA, USA) using an initial 5 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 54 °C, and 1 min at 72 °C; and a final 10 min at 72 °C. The reaction mixture was then held at 4 °C for 5 min. PCR products were purified with a QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions.

The purified PCR products were then sequenced by Macrogen Japan Inc. (Tokyo, Japan) in a PTC-225 Peltier Thermal Cycler (MJ Research, Waltham, MA, USA) using ABI PRISM BigDye Terminator Cycle Sequencing kits with AmpliTaqR DNA polymerase (FS enzyme) (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturers' protocols. The fluorescent-labelled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were then resuspended in distilled water and electrophoresed in an ABI 3730xl sequencer (Applied Biosystems).

Our PCR amplifications always resulted in a single discrete band, but clear sequencing data of a few PCR products could not be obtained owing to intra-strain polymorphism or faint PCR bands. These products were cloned into the vector pCR4-TOPO using the TOPO TA cloning kit (Invitrogen, Waltham, MA, USA) following the manufacturer's instructions. Recombinant One Shot DH5α-T1 *Escherichia coli* colonies were randomly picked and screened directly for inserts by colony PCR with primers for the vector. The PCR products of positive clones were purified and sequenced as described above.

The sequences determined in this study were deposited in DDBJ (accession nos. LC084660–751; http://getentry.ddbj.nig.ac.jp/top-e.html) and used for phylogenetic analysis.

2.4. Phylogenetic analysis

In addition to the sequences obtained in this study, 39 sequences used by Moncalvo and Buchanan (2008) were included in the phylogenetic analysis. Phylogenetic analyses of the rDNA ITS sequences were conducted using the neighbor-joining method (Saitou and Nei, 1987). MAFFT v. 6 software (Katoh and Toh, 2008) was used for preliminary multiple alignments of nucleotide sequences, and final alignments were manually adjusted in BioEdit software (Hall, 1999). Alignment gaps were treated as missing data, and ambiguous positions were excluded from the analysis. The best-fit evolutionary model was selected on the basis of the Bayesian Information Criterion scores generated in MEGA 6 software (Tamura et al., 2013). We chose the Kimura two-parameter model (Kimura, 1980) of nucleotide substitution with a discrete gamma distribution (shape parameter = 1) to allow for non-uniformity of rates among sites, and neighbor joining was performed in MEGA 6. Node support was evaluated by bootstrap analysis (Felsenstein, 1985) using 1000 replications.

2.5. Statistical analysis

The *G. australe* complex was divided into several phylogenetic types on the basis of the phylogenetic analysis. The number of CWD samples from which fungal fruit bodies belonging to each phylogenetic type appeared was used as an index of abundance. To reveal the resource use patterns among the phylogenetic types, we compared frequency distributions of the size classes and decay stages of CWD samples from which fruit bodies appeared among phylogenetic types by chi-squared test. The frequency distribution of decay stages of CWD samples with fruit bodies was also compared with that of pieces of CWD in the plot.

Overlap in resource use among phylogenetic types was quantitatively evaluated by Pianka's niche overlap index (Pianka, 1973), which ranges from 0 for complete difference in resource use to 1 for complete overlap. The observed resource use pattern was compared with that of a randomly assembled community to test the effect of competition. The null community was created by two randomization algorithms: RA3 retains the number of resource types available for each phylogenetic type but randomizes which resource types are used, and RA4 retains the number of resource types available for each fungal species and assigns 0 to resource types without records. These two methods were recommended by Gotelli and Ellison (2013). For each pair, 10 000 random Monte