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# Diversity, Abundance, and Distribution of Wood-Decay Fungi in Major Parks of Hong Kong

**Shunping Ding [1,2,*], Hongli Hu [2,3] and Ji-Dong Gu [2,4,*]**

1 Wine and Viticulture, California Polytechnic State University, 1 Grand Ave., San Luis Obispo, CA 93407, USA
2 Laboratory of Environmental Microbiology and Toxicology, School of Biological Sciences, Faculty of Science, The University of Hong Kong, Pokfulam Road, Hong Kong 999077, China; huhongli7905@gmail.com
3 Ministry of Agriculture Key Laboratory of Subtropical Agro-Biological Disaster and Management, Fujian Agriculture and Forestry University, Fuzhou 350002, China
4 Environmental Engineering, Guangdong Technion-Israel Institute of Technology, 241 Daxue Road, Shantou 515041, China
* Correspondence: sding01@calpoly.edu (S.D.); jidong.gu@gtiit.edu.cn (J.-D.G.)

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**Abstract:** Wood-decay fungi are one of the major threats to the old and valuable trees in Hong Kong and constitute a main conservation and management challenge because they inhabit dead wood as well as living trees. The diversity, abundance, and distribution of wood-decay fungi associated with standing trees and stumps in four different parks of Hong Kong, including Hong Kong Park, Hong Kong Zoological and Botanical Garden, Kowloon Park, and Hong Kong Observatory Grounds, were investigated. Around 4430 trees were examined, and 52 fungal samples were obtained from 44 trees. Twenty-eight species were identified from the samples and grouped into twelve families and eight orders. *Phellinus noxius*, *Ganoderma gibbosum*, and *Auricularia polytricha* were the most abundant species and occurred in three of the four parks. Most of the species were detected on old trees, indicating that older trees were more susceptible to wood-decay fungi than younger ones. More wood-decay fungal species were observed on *Ficus microcarpa* trees than on other tree species. These findings expanded the knowledge of wood-decay fungi in urban environments in Hong Kong and provided useful information for the conservation of old trees and the protection of human life and property from the danger of falling trees.

**Keywords:** wood-decay fungi; urban environment; fungal diversity; tree diseases

## 1. Introduction

Urban forests play a very important role in the urban ecological system. They provide shelter to urban animals, adsorb and accumulate anthropogenic pollutants, and can decontaminate the polluted soils and water through phytoremediation [1–3]. Well planned and managed urban forests can support high levels of biodiversity [4,5]. It is also important that urban forests add additional value to dwellings and people are willing to pay to protect urban forests [6,7]. This is especially true in cosmopolitan Hong Kong. Old trees are registered as Old and Valuable Trees (OVTs) for protection and management purposes. The old trees provide aesthetic value in addition to their ecological functions. However, urban forests are vulnerable to disturbance from weather and infection, and falling trees can be significant human hazards [8]. Failure of trees in urban forest is usually caused by microbial infection and lack of adequate soil depth because of poor soil conditions, excessive human activities, and lack of proper management. Wood-decay fungi are the most relevant and recognized pathogens to growing and dead trees.

Wood-decay fungi are capable of breaking down complex, high-molecular weight constituents in the plant cell wall into small molecules for assimilation by the fungi involved as well as other

microorganisms [9]. They can decompose plant debris and accelerate element and material cycling in the ecosystem [10]. Most of the fungi are saprotrophs living on dead wood. However, some are pathogenic, which can infect living trees, and cause rot or decay on stems and roots of the trees. According to the type of decay they cause, wood-decay fungi are classified into white-rot fungi, brown-rot fungi, and soft-rot fungi. White-rot fungi can break down cellulose, hemicelluloses, and lignin [11]. After degradation, the wood appears bleached, soft, and crumbled. White-rot fungi occur on both coniferous wood and broad-leaved trees [11]. They contribute to 90% of wood-decay fungi, and are highly diverse, particularly in tropical areas [12]. Brown-rot fungi can break down cellulose and hemicelluloses and the decayed wood shows cracks and clefts, and becomes fragile, and powdery [13]. They account for 10% of wood-decay fungi, and are mainly distributed in temperate and cold zones and occur on coniferous trees [11,12]. Both white-rot fungi and brown-rot fungi are Basidiomycetes. Soft-rot fungi are mainly Ascomycetes, and they break down cellulose and hemicelluloses [13]. Since soft rot fungi usually occur in wood, it is not considered as a problem on living standing trees.

Because of intensified anthropogenic impact, urban forests are especially vulnerable to wood-decay fungi. Despite regular maintenance, most of the trees in the urban area of Hong Kong suffer from a lack of growing space, especially below ground in the soil [14]. Air and soil pollution and improper management contribute to additional inadequate living conditions for the trees. All those environmental restrictions result in low diversity of fungal species in the urban environment. The reduced diversity of urban fungal species may cause the imbalance between plant pathogens and anti-pathogen species in the microbiome associated with plant health. Therefore, pathogenic wood-decay fungi may cause serious disease to trees, and saprotrophic fungi may become pathogenic and infect living trees. For example, *Phellinus noxius* (Corner) G. Cunn., a white-rot fungus, which can cause brown-root rot disease, was reported with no apparent occurrence in non-disturbed natural forests [15]. In addition to *Phellinus* spp., other wood-decay fungi such as *Ganoderma* spp., *Armillaria* spp., *Kretzschmaria* spp., *Polyporus* spp., and *Trametes* spp. were also commonly reported to affect urban tree species [16–18]. Stem-decay fungi, such as *Phellinus robustus* (P. Karst.) Bourdot & Galzin and *Fomes fomentarius* (Linn.) Fr., invade trees through wounds, which may be caused by improper trimming, branch crashes during the wind, animal biting, or even bark cracks on old trees [13]. Root-decay fungi invade trees through root connections, such as *P. noxius* and *Meripilus giganteus* (Pers.) P. Karst. [13,19]. With severe infection of wood-decay fungi, the mechanical support of the trees is undermined, and the trees become unstable and subject to falling. Problems of wood-decay fungi in the urban environment can be extremely dangerous, especially in highly populated areas.

In Hong Kong, tropical monsoons occur frequently from April to October each year. The severely infected trees are likely to fall because of the strong wind during monsoon seasons. Cases of injury or even death caused by unexpected falling of trees have happened occasionally. Therefore, proactive actions towards wood-decay fungi are necessary from the perspectives of both nourishing urban ornamental plants and protecting people. Despite their ecological and security importance, the taxonomy of wood-decay fungi in Hong Kong is poorly developed. In Fungi of Hong Kong, Griffiths (1977) described a number of wood-decay fungi [20]. However, the collection was barely enough. Later on, Hyde et al. (2002) studied the diversity of fungi in Hong Kong, during which a number of saprotrophic fungi, rather than macro-wood-decay fungi, were included [21,22]. In order to better manage trees in Hong Kong for both aesthetic and ecologic values, it is necessary to identify wood-decay fungi and illustrate their diversity and distribution on trees.

Therefore, this study was conducted to investigate the diversity, abundance, and distribution of wood-decay fungi associated with standing trees and stumps and to explore the potential driving factors of species-richness of wood-decay fungi in four different parks of Hong Kong.

## 2. Materials and Methods

### *2.1. Study Sites*

Hong Kong Park (HKP), Hong Kong Zoological and Botanical Garden (ZBG), Kowloon Park (KP), and Hong Kong Observatory Grounds (HKO) were chosen as the study sites (Table 1). HKP and ZBG are located in Central on Hong Kong Island, with Garden Road in between; KP and HKO are located in Tsim Sha Tsui, with Nathan Road in between (Figure 1 and Table 1). HKP has been open since 1991, is 8 hectares in area, with around 2280 trees, among which 14 are Old and Valuable Trees (OVTs) registered by the Leisure and Cultural Services Department (LCSD) of Hong Kong Government (http://ovt.lcsd.gov.hk/ovt/index.jsp?lang=en) and the major tree species are *Ficus microcarpa* L. f., *Mangifera indica* L., and *Bombax ceiba* L. [23]. ZBG is among the oldest parks in Hong Kong, which was first opened in 1864, is 5.6 hectares in area, possessing around 850 trees, among which 25 are registered OVTs representing 25 unique tree species. KP has been open since 1970, is 13.3 hectares in area, with around 1000 trees, among which 49 are registered OVTs. The major tree species are *F. microcarpa*, *Cinnamomum camphora* (L.) J. Presl, *Albizia lebbeck* (L.) Benth, and *Celtis sinensis* Pers. HKO is a non-public location and has approximately 2.5 hectares and around 300 trees. HKO was defined as a park for description convenience in this study. The trees in HKO are densely planted and the major tree species are *F. microcarpa*, *Lophostemon confertus* (R. Br.) Peter G. Wilson & J.T. Waterh, *Aleurites moluccana* (L.) Willd, and *A. lebbeck*. In these four parks, tree diseases caused by wood-decay fungi are a major problem in tree management (Figure S1).

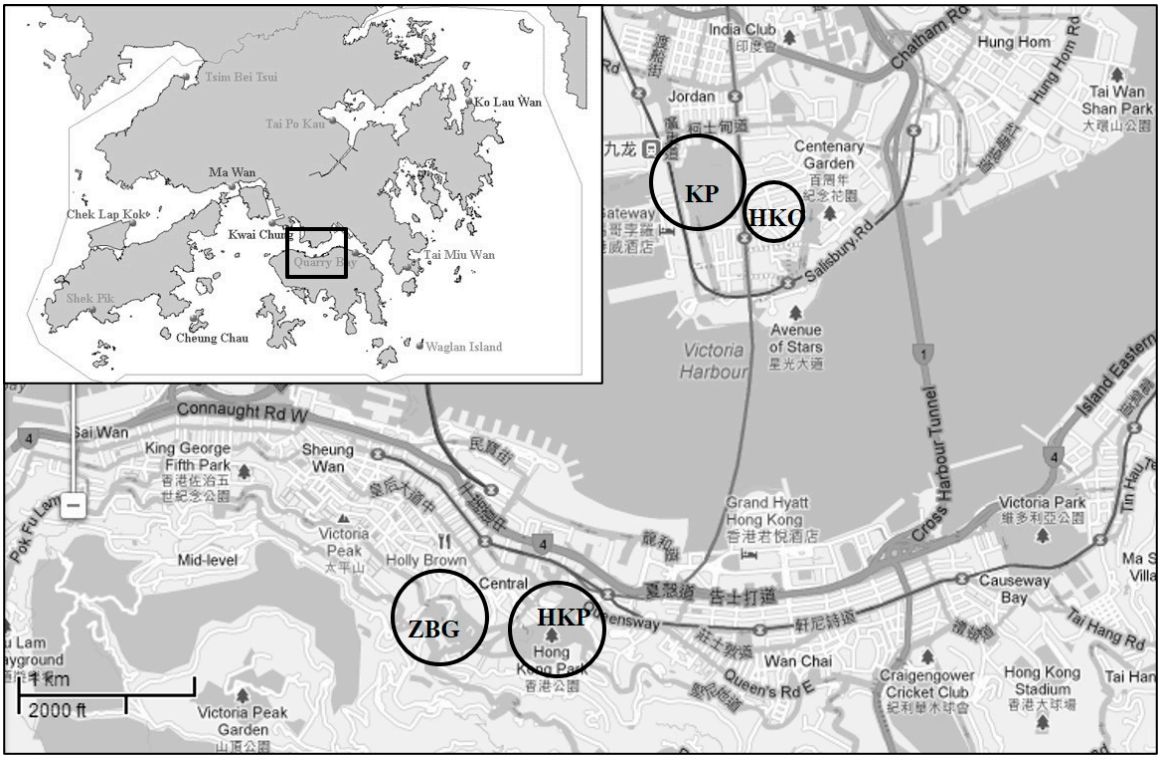


**Figure 1.** A map of Hong Kong and locations of the four parks, Hong Kong Park (HKP), Hong Kong Zoological and Botanical Garden (ZBG), Kowloon Park (KP), and Hong Kong Observatory Grounds (HKO) sampled in this study. (http://www.hko.gov.hk/tide/marine/hko.htm, https://maps.google.com.hk/).

**Table 1.** Number of Old and Valuable Trees (OVTs), area, opening year, common tree species, and location of Hong Kong Park (HKP), Hong Kong Zoological and Botanical Garden (ZBG), Kowloon Park (KP), and Hong Kong Observatory Grounds (HKO).

| Parks [a] | Number of OVTs | Area (Hectares) | Opening Year (Since) | Tree Species (Partial) | Location |
|---|---|---|---|---|---|
| HKP | 14 | 8 | 1991 | *F. microcarpa*, *M. indica*, *B. ceiba*, *Ficus virens* W.T. Aiton, *Ficus elastic* Roxb. ex Hornem, ect. | Central, north of Garden Road |
| ZBG | 25 | 5.6 | 1864 | *F. microcarpa*, *Araucaria bidwillii* Hook, *Nauclea orientalis* (L.) L., *Sophora japónica* (L.) Schott, *Podocarpus neriifolius* D. Don, ect. | Central, south of Garden Road |
| KP | 49 | 13.3 | 1970 | *F. microcarpa*, *C. camphora*, *A. lebbeck*, *Cassia fistula* L., *C. sinensis*, ect. | Tsim Sha Tsui, west of Nathan Road |
| HKO | 0 | 2.5 | Non-public | *F. microcarpa*, *L. confertus*, *A. moluccana*, *A. lebbeck*, *C. camphora*, etc. | Tsim Sha Tsui, east of Nathan Road |

[a] The information was retrieved from https://www.gov.hk/en/residents/.

### 2.2. Field Sampling

The infected trees could be visually identified based on symptoms, such as dieback, defoliation, discoloration, hollows, or cavities on tree trunks. These trees were selected for disease diagnosis. Fungal fruiting bodies, if present, were sampled for further identification. Otherwise, the roots of suspected trees were dug out from the soil to check for fungal colonization, and wood tissues were sampled aseptically and sent back to the laboratory for further analysis.

Besides the infected trees, all the OVTs in these parks (except HKO) were all checked regardless of the status they displayed. Two rounds of samplings were carried out in May 2012 (for the wet season) and in November 2012 (for the dry season) to obtain as many samples as possible, especially of the non-perennial fruiting bodies.

### 2.3. Culturing and Isolation

Samples were transported back to the laboratory for processing within hours. The wood tissues with fungal mycelia were cut aseptically into several pieces (approximately 3 $mm^3$) under aseptic conditions. Each piece was submerged into 75% ethanol for 2 min, rinsed three times with sterile water, and placed on potato dextrose agar (PDA) (BD DifcoTM) plates adjusted with 0.5% streptomycin, then incubated at room temperature. Fungal hyphal tips were transferred to fresh PDA plates for isolation and purification.

### 2.4. PCR Amplification

Total genomic DNA of approximately 5 mg of fruiting bodies or pure isolates were extracted with an E.Z.N.A.® Forensic DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the protocol provided for PCR amplification. One pair of universal primers ITS1F (5′-CTTGGTCATTTAGAGGA AGTAA-3′) [24] and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [25] was used to amplify the internal transcribed spacer (ITS) regions (including ITS1, 5.8S and ITS2) of the pure cultures or fruiting bodies. The PCR amplification reaction system included 5 µL of 5× PCR buffer, 2 µL of 25 mM $MgCl_2$, 1 µL of 10 mM deoxyribonucleotide triphosphate (dNTPs), 1 µL of 10 µM of each primer (ITS1F and ITS4), approximately 10 ng template DNA, 0.15 µL of 5 U/µL GoTaq® DNA Polymerase (Promega, Madison, WI, USA), 1 µL of 1% BSA (bovine serum albumin) (*w*/*v*) and finally made up to 25 µL with sterile water. The thermal cycling protocol included an initial denaturation at 95 °C for 3 min, followed by 32 cycles consisting of denaturation at 95 °C for 45 s, annealing at 52 °C for 30 s, extension at 72 °C for 45 s, and a final extension at 72 °C for 10 min. Amplified products were visualized on 1% agarose electrophoresis gels stained with GelRed™ Nucleic Acid Gel Stain (Biotium, Fremont, CA, USA) to check for product purity and size. PCR products were purified with GFX™ PCR RNA and a Gel Band Purification Kit (Amersham Biosciences, Waltham, MA, USA). Purified PCR products were sequenced at the Genome Research Centre of The University of Hong Kong. The nucleotide sequences have been deposited in GenBank (accession numbers KU194304-KU194347).

### 2.5. Phylogenetic and Statistical Analysis

The obtained sequences were checked and revised with BioEdit v7.0.5.3 [26]. Basic Local Alignment Search Tool (BLAST) searches were performed using the GenBank database (http://www.ncbi.nlm.nih.gov). The sequences with highest similarity and coverage (Table S1) were downloaded for alignment using ClustalW in BioEdit and then conducted phylogenetic analysis [27]. The neighbor-joining tree with 1000 replications of bootstraps was built with the alignment in MEGA 5.05 after a minor manual adjustment [28]. To confirm the accuracy of the phylogenetic analysis, Bayesian Posterior Probability was calculated with BEAST v1.5.4 [29]. The best model for Bayesian analysis was selected with MrModeltest 2.2 [30]. Both branch support numbers were shown at the nodes of the neighbor-joining trees.

The isolation frequency of major wood-decay fungi was estimated by calculating the percentage of each fungus in all the samples collected in the parks (number of isolates/n, where n = 52). The occurrence rate of each species was calculated as the frequency of occurrence of a species in the four parks (e.g., *P. noxius* was detected in three out of four parks, and therefore the occurrence rate was 75%). To study the species assemblage of the wood-decay fungi in each park, relative occurrence of each wood-decay fungal species was calculated with the occurring frequency of each species in each park. The colonization rate of wood-decay fungi on old trees was compared with that on the younger trees. The colonization rate on OVTs and non-OVTs was compared with a paired *t* test in GraphPad Prism 5.0.

### 2.6. Morphological Characters

The identification of fungal fruiting bodies and fungal cultures was conducted mainly based on molecular techniques stated above, but morphological characters were also used to confirm the results from molecular methods. For morphological characteristics, pictorial guides were used, and microscopic observations were conducted following the authorized descriptions [12,20,31–33]. Fruiting bodies were selectively dried in the drying oven (70 °C) and kept as specimens and cultural isolates were inoculated onto slants containing PDA medium and kept at 4 °C for storage.

## 3. Results

### 3.1. Diversity of Wood-Decay Fungi in Parks

During this survey, around 4430 trees from four major parks in Hong Kong were examined. A total of 52 fungal isolates were obtained from 44 trees. The colonization rate of trees by wood-decay fungi was approximately 0.7% in the surveyed parks. The ITS rDNA PCR amplification, together with morphological characterization of the fruiting bodies and culture isolates, retrieved 28 fungal species, which were affiliated with 12 families from 8 orders (Table 2).

**Table 2.** A list of wood-decay fungi detected during the study, together with their host trees, locations and significances [12,13,32–34].

| Fungal Species | Host Trees (Codes If Applicable and Location) | Notes |
|---|---|---|
| ***Auricularia polytricha* (Mont.) Sacc.** | *F. microcarpa* (HKP CW/110) wood stumps (ZBG, KP) | white rot fungus, saprotrophic; occur on the barks |
| ***Botryobasidium conspersum* J. Erikss.** | *P. neriifolius* (ZBG CW/69) | fruiting body occurs inside the hollow on the branch |
| ***Ceriporia lacerata* N. Maek., Suhara & R. Kondo** | *Ficus variegata* (Blume) (ZBG) *F. microcarpa* (KP YTM/81) | white rot fungus, causing stem and butt decay to broad-leaved trees |
| ***Cryptococcus heveanensis* (Groen.) Baptist & Kurtzman** | *A. lebbeck* (KP YTM/94) | occur on the wounded part |

**Table 2.** *Cont.*

| Fungal Species | Host Trees (Codes If Applicable and Location) | Notes |
|---|---|---|
| *Earliella scabrosa* **(Pers.) Gilb. & Ryvarden** | *F. microcarpa* (KP YTM/65) fallen branch (HKO) | white rot fungus, fruiting body occur on branch intersection; also saprophytically live on fallen branch |
| ***Fuscoporia senex* (Nees & Mont.) Ghob.-Nejh** | *Syzygium samarangense* (Blume) Merr. & L.M. Perry (ZBG) *Sophora japonica* (L.) Schott (ZBG CW/62) | white rot fungus, cause stem decay, discovered in the cavities on the tree trunk |
| ***Fomes* sp.** | *F. microcarpa* (KP YTM/97) | white rot fungus, cause heart wood-decay; fruiting body discovered on fallen branches, |
| ***Ganoderma applanatum* (Pers.) Pat.** | *F. microcarpa* (KP YTM/92) | white rot fungus, cause stem, root, and butt rot; fruiting body occur on wounded part, causing cavity on branch intersection |
| ***Ganoderma gibbosum* (Nees) Pat.** | *F. microcarpa* (HKP CW/110, CW/107; KP YTM/93, YTM/74; HKO) wood stump (HKP) | white rot fungus, fruiting body discovered both on standing trees and wood stumps, usually observed on wounded part of the trees, and cavities occur in most cases |
| ***Ganoderma lucidum* (Curtis) *P. Karst.*** | dead *A. lebbeck* (HKO) | white rot fungus, cause root rot, fruiting bodies occur around the dead tree |
| ***Ganoderma webrianum* Bres. & Henn.** | wood stump (ZBG) | white rot fungus, cause butt rot; fruiting bodies were detected when the tree was declined and the wood stump after the tree was cut down |
| ***Gymnopus gibbosus* (Corner) A.W.Wilson, Desjardin & E.Horak** | *F. microcarpa* (KP YTM/91) | the fruiting bodies were occurred in small clusters on the branch, and there was a noticeable hollow on the branch |
| ***Helicobasidium mompa* Nobuj. Tanaka** | *F. microcarpa* (KP) | on root |
| ***Hypoxylon fendleri* Berk. ex Cooke** | *F. microcarpa* (HKP CW/107) | around the tree |
| ***Hypoxylon* sp.** | *F. microcarpa* (HKO) | detected from culturing |
| ***Hypoxylon vinosopulvinatum* Y.M. Ju, J.D. Rogers &H.M. Hsieh** | *F. microcarpa* (KP YTM/81) | detected from culturing |
| ***Hexagonia tenuis* (Hook.) Fr.** | *F. microcarpa* (KP YTM/67 ect.) | white rot fungus, live on fallen branches, or dead part of the tree |
| ***Kretzschmaria deusta* (Hoffm.) P.M.D. Martin** | *C. sinensis* (KP) | White rot fungus, cause root decay |
| ***Kretzschmaria* sp.** | *F. microcarpa* (KP YTM/74) | white rot fungus, cause stem and root rot; patches of fruiting body occur on the trunk |
| ***Lopharia* sp.** | *F. microcarpa* (KP) | saprotroph, fruiting body occur on dead part of the tree |
| *Marasmiellus palmivorus* (Sharples) Desjardin (comb. prov.) | *Asplenium nidus* L. (ZBG) | can cause bunch rot on palm [35], discovered in rhizosphere soil of a palm |
| ***Phellinus noxius*** | *F. microcarpa* (HKP CW/110; KP YTM/65; HKO) *L. confertus* (HKO) *A. moluccana* (HKO) wood stumps (HKO) | white rot fungus, cause brown root rot disease |
| ***Physisporinus vitreus* (Pers.) P. Karst.** | *M. indica* (HKP CW/111) *Lysidice rhodostegia* Hance (ZBG CW/58) | white rot fungus, cause white rot and root decay |
| ***Psathyrella candolleana (Fr.) Maire*** | *C. sinensis* (KP YTM/69) | white rot fungus [36], fruiting bodies on the butt |
| ***Rigidoporus vinctus (Berk.) Ryvarden*** | *Drypetes roxburghii* (Wall.) Hurus (ZBG CW/67) | white rot fungus, inside and on the brim of the cavity on the tree trunk |
| ***Trametes hirsute (Wulfen) Lloyd*** | dead *Paulownia fortunei* (Seem.) Hemsl. (ZBG) dead wood (HKO) | white rot fungus, saprophyte |
| ***Xylaria escharoidea* (Berk.) Fr.** | *F. microcarpa* (HKP CW/107) | around the root |
| ***Xylogone sphaerospora* Arx & T. Nilsson** | *A. lebbeck* (KP YTM/94) | detected from culturing |

The phylogeny based on multiple sequence alignment showed that the dominant wood-decay fungi detected in this study were affiliated with the orders of Hymenochaetales (Figure 2) and Polyporales (Figure 3). Within the order of Hymenochaetales, two genera, *Phellinus* and *Fuscoporia*, were identified and both are important pathogenic wood-decay fungi affiliated with the family of

Hymenochaetaceae (Figure 2). Within in the order of Polyporales, multiple fungal species were identified, and they belong to four families: Ganodermataceae, Polyporaceae, Phanerochaetaceae and Meripilaceae (Figure 3).

### 3.2. Abundance and Distribution of Wood-Decay Fungi in Different Parks

Nine fungal species, accounting for 60% of the wood-decay fungi detected in this study were considered as most abundant wood-decay fungi in the four parks investigated (Figure 4). Among the nine fungal species, *P. noxius* was the most abundant, followed by *G. gibbosum* and *A. polytricha*. These three species were also detected in most parks.

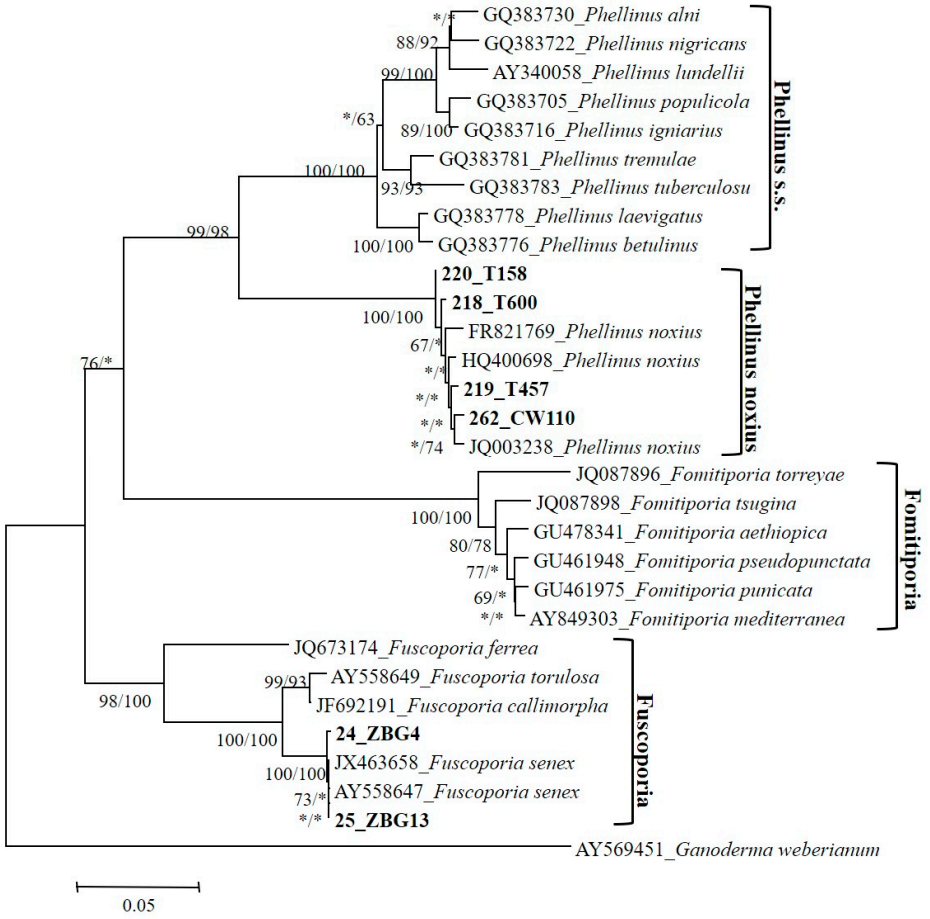


**Figure 2.** A neighbor-joining tree generated with ITS rDNA using MEGA 5.05 showing the phylogeny of Hymenochaetaceae species detected in this study. The numbers in front of the slashes show bootstrap (1000 replications) values (%) of the branches, and the numbers behind the slashes show Bayesian posterior probability (%) generated with BEAST v1.5.4. Values that are less than 50% were substituted with asterisk marks. The best model for Bayesian analysis was GTR + I + G, selected using MrModeltest 2.2. Sequences in bold indicate fungal species obtained from this study.

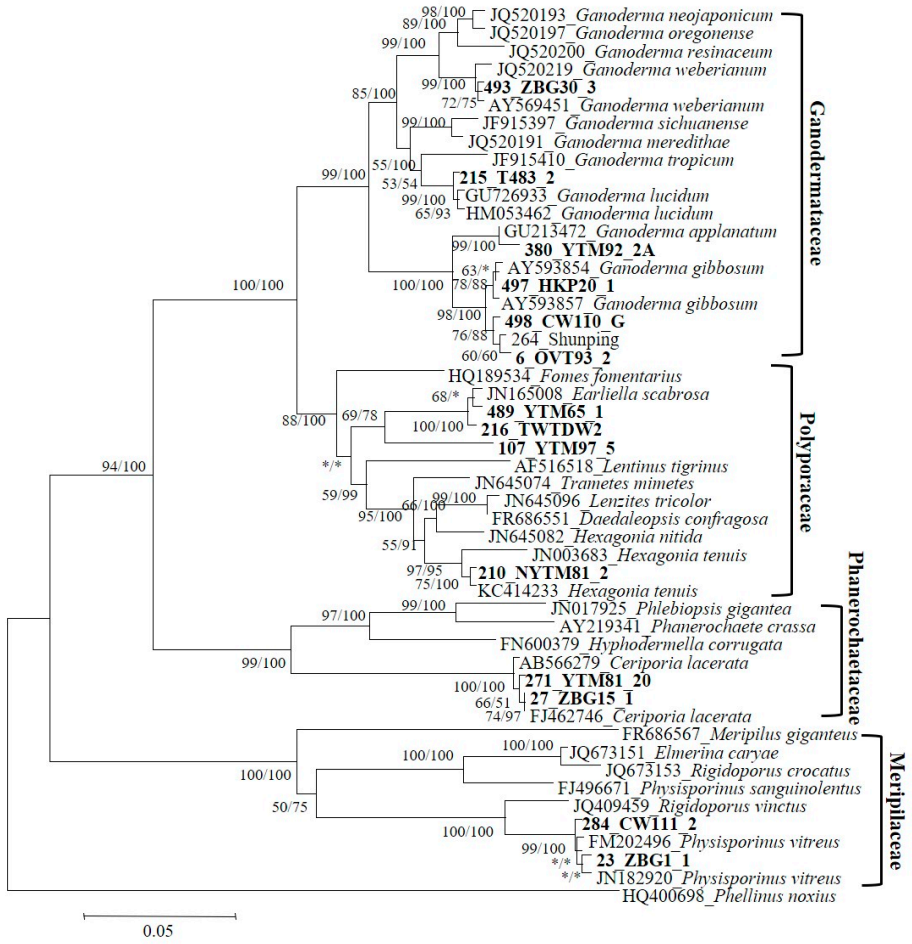


**Figure 3.** A neighbor-joining tree generated with ITS rDNA using MEGA 5.05 showing the phylogeny of Polyporales species detected in this study. The numbers in front of the slashes show bootstrap (1000 replications) values (%) of the branches, and the numbers behind the slashes show Bayesian posterior probability (%) generated with BEAST v1.5.4. Values that are less than 50% were substituted with asterisk marks. The best model for Bayesian analysis was HKY + I + G, selected using MrModeltest 2.2. Sequences in bold indicate fungal species obtained from this study.

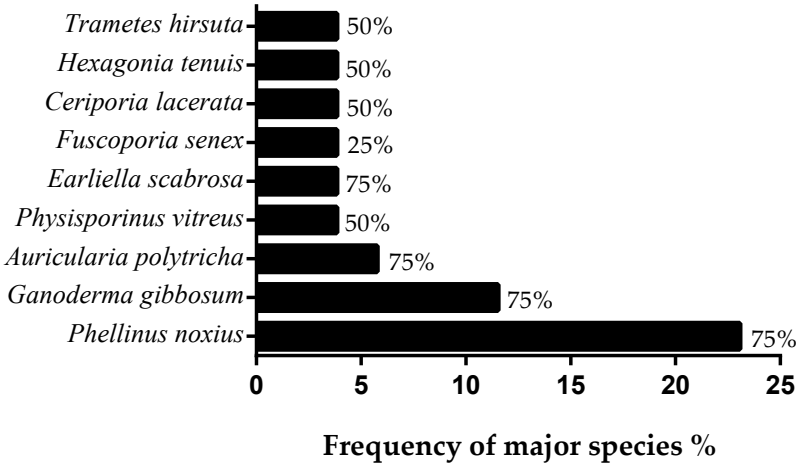


**Figure 4.** Frequency of major wood-decay fungal species in the four parks (n = 52). The percentage value by each bar indicates the occurrence rate in the parks.

To calculate the occurrence rate of each wood-decay fungal species in each park and on each tree species (Figure 5 and Table 3), fungi that were reported as root-rot pathogens or could cause serious wood-decay were listed with species names while less significant fungal species or fungi with unknown causes were categorized as "others". According to the relative occurrence, KP hosted the largest number of wood-decay fungal species, followed by ZBG, and then HKO and HKP. In KP, in addition to eight significant wood-decay fungal species (about 50% of the total samples collected from KP), around 50% of the fungi detected were not known to be pathogens, suggesting that KP may have a higher diversity of wood-decay fungi. Most of the fungi were observed in more than one park, but some fungi were only detected in one park. For example, *R. vinctus*, *G. webrianum*, and *F. senex* were only detected in ZBG. In this study, the samples were collected from 13 living tree species and dead wood or wood stumps, fallen branches, and shrubs in the four parks. More wood-decay fungal species were detected on dead trees or wood stumps and *F. microcarpa* than the rest of the wood types (Table 3).

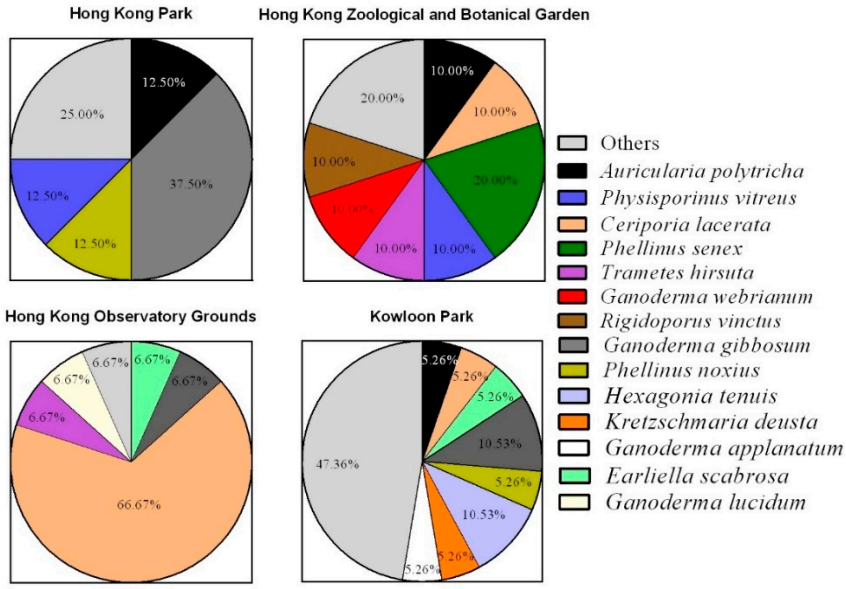


**Figure 5.** A graph showing the wood-decay fungal species assemblages in each park and the percentage of each fungal species in terms of all the samples collected from each park. Each color represents one significant wood-decay fungus, and non- or less significant wood-decay fungi are categorized as "Others".

### *3.3. The Colonization of Wood-Decay Fungi on Old Trees and Their Significance*

Colonization rate of wood-decay fungi on old trees was higher than that on the younger ones. Of the four parks, 23% of OVTs (large trees in the case of HKO) were infected with wood-decay fungi, and the infection rates were 29%, 16%, 20%, and 35% for HKP, ZBG, KP, and HKO, respectively (Figure 6A). However, the occurrence rate of wood-decay fungi in all trees in these parks was only around 0.7% (Figure 6A). More wood-decay fungi were discovered from OVTs than those from younger trees ($t$ = 2.496, df = 3, $p$ = 0.0440). Of all the fungal samples collected, 70% were detected from OVTs (or large trees in the case of HKO), and 30% from younger trees and stumps. The ratio between the numbers of wood-decay fungi samples collected from OVTs and the numbers of wood-decay fungi samples collected from non-OVTs were 7:1, 1:1, 2.8:1, and 2:1 for HKP, ZBG, KP, and HKO, respectively (Figure 6B).

**Table 3.** A list of number of trees from each species (or wood types) that were infected by different wood-decay fungi.

| Tree Species or Types | Wood-Decay Fungal Species | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***A. polytricha*** | ***C. lacerata*** | ***E. scabrosa*** | ***G. gibbosum*** | ***P. noxius*** | ***P. senex*** | ***P. vitreus*** | ***T. hirsuta*** | ***H. tenuis*** | ***G. lucidum*** | ***G. webrianum*** | ***K. deusta*** |
| *Ficus microcarpa* | 1 | 1 | 1 | 5 | 3 | | | | 2 | | | |
| dead tree/wood stump | 2 | | | 1 | 3 | | | 2 | | | 1 | |
| *Aleurites moluccana* | | | | | 4 | | | | | | | |
| *Celtis sinensis* | | | | | | | | | | | | 1 |
| *Albizia lebbeck* | | | | | | | | | | 1 | | |
| *Ficus variegata* | | 1 | | | | | | | | | | |
| fallen branch | | | 1 | | | | | | | | | |
| *Lophostemon confertus* | | | | | 1 | | | | | | | |
| shrub | | | | | 1 | | | | | | | |
| *Syzygium samarangense* | | | | | | 1 | | | | | | |
| *Sophora japonica* | | | | | | 1 | | | | | | |
| *Mangifera indica* | | | | | | | 1 | | | | | |
| *Lysidice rhodostegia* | | | | | | | 1 | | | | | |

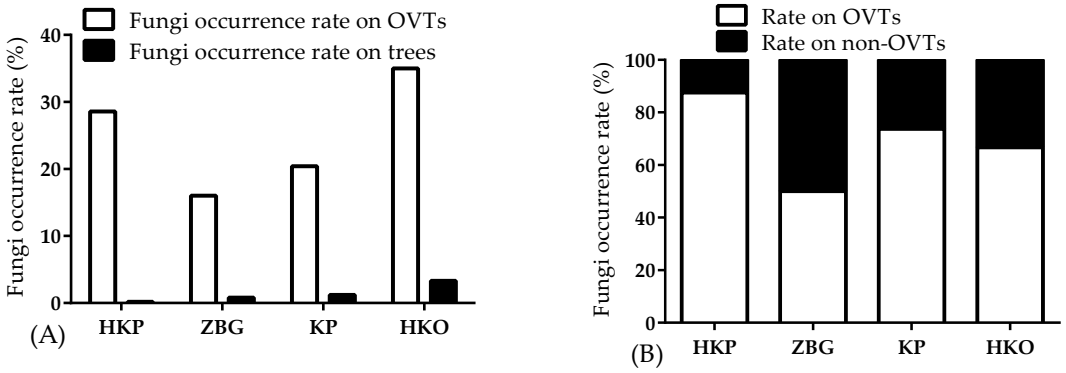


**Figure 6.** A comparison of wood-decay fungi occurrence rates on old trees and young trees. (**A**). A comparison of wood-decay fungi occurrence rate on OVTs and over all fungi occurrence rate. (**B**). A comparison of wood-decay fungi detected from OVTs and non-OVTs. (large trees in the case of HKO; HKP = Hong Kong Park, ZBG = Hong Kong Zoological and Botanical Garden; KP = Kowloon Park; HKO = Hong Kong Observatory Grounds).

## 4. Discussion

In this study, we surveyed four major parks in Hong Kong for detection of common wood-decay fungi that affected the urban forests in the cosmopolitan Hong Kong. The major wood-decay fungi found in this study were species in Hymenochaetaceae and Polyporales. The family of Hymenochaetaceae contains many species that may cause diseases in broad-leaved and coniferous trees, causing heart rot, canker, and root rot diseases. *Phellinus noxius* and *F. senex* from this group were detected in this study and both are known for causing white rot [15]. Other species in this family, such as the *Phellinus igniarius* group, are usually found as parasites on broad-leaved wood plants, cause white-rot of heartwood [37–41]. The Polyporales were mostly saprophytes and contain a large number of wood-decay fungi, in which a portion of them could be pathogenic [42,43]. Species within this order such as *Daedaleopsis confragosa* (Bolton) J. Schröt. and *Fomes fomentarius* (L.) Fr., can cause white trunk rot, and *Ganoderma Austral* N. Maek., Suhara & R. Kondo and *G. webrianum*, can cause white-rot and butt-rot [37]. *Ganoderma* spp., *Earliella* spp., *Hexagonia* spp., *Ceriporia* spp., and *Physisporinus* spp. Were detected in this study, which were also common wood-decay fungi affects urban trees [18,44,45]. *Fungi* within Polyporales accounted for 50% of all the wood-decay fungi in this study, suggesting the role as the main wood rotter in the four parks of Hong Kong. This was in line with previous surveys on wood-decay fungi in urban trees in that Polyporales were dominant fungi in urban tree failures [18,44]. The highest diversity of wood-decay fungal community was observed in KP, which could be due to the existence of the largest number of OVTs among the four surveyed parks. Interestingly, ZBG seemed to host a unique group of wood-decay fungi comparing to the other parks. This could be a result of the unique plant community of ZBG that might provide substrates for a different wood-decay fungal community [46].

Plant diseases were traditionally detected with the observation of symptoms and signs. However, some diseases do not present obvious symptoms or fruiting bodies at the early stage of infection. In this case, instead of diagnosing based on observations, molecular techniques could be applied [47]. For the ITS sequences generated in this study, 86% of them could be identified to the species level. The ITS sequences was also commonly used in other wood-decay fungi studies [18,48]. It is applicable in this type of study, but additional genetic regions that are less conservative, such as β-tubulin and elongation factor, could be used for a more accurate speciation [49].

*P. noxius* contributed to a large percentage of wood-decay fungi in the four parks investigated, especially in HKO. The pathogen is highly virulent in a number of tree species [50,51] and trees may show discoloration in foliage within 2 months and significant decline over periods of one year or more [15]. Trees with observable decline and dieback were more likely to be diagnosed and therefore *P. noxius* ended up with a high frequency of detection. The main spreading strategy of

*P. noxius* was through root contacts, which enables the pathogen to cause an overwhelming break out of brown-root-rot disease to a large area of densely planted trees [15]. For the same reason, a cluster of infection was observed at the entrance in HKO. On the other hand, the pathogen can also survive as a saprophyte and could remain infectious on the wood debris for up to a decade, making it difficult to eliminate the pathogen once it was established [52]. Therefore, between the cluster of infected *A. moluccana* at the entrance of HKO and the infected *A. moluccana* 50 m away inside HKO, a number of stumps, bushes, and trees were found to be infested with *P. noxius*. If these infected trees were not completely removed and infested soil untreated, more trees in HKO would be infected [53].

Stumps and dead trees were expected to host a large number of wood-decay fungi because these fungi have a role as saprophytic decomposers. Unfortunately, *F. microcarpa* was also found to host a variety of different fungi. *Ficus microcarpa*, especially the ones that were identified as OVTs, had widely spread branches and numerous large aerial roots that were exposed to the humid air. There might be a greater chance of microbial colonization on these *F. microcarpa* than other trees. In addition, the spaces among aerial roots and the main stems may trap water for an extended time, making it a conducive environment for fungal colonization. According to Table 3, *P. noxius* was detected on five types of host in this study, indicating a potential wide host range, which was in line with previous reports from Taiwan that *P. noxius* could infect more than 200 tree species [15,51]. The other wood-decay fungi with more than one occurrence were also found in different tree species. Although there was no obvious evidence to prove the relationship between the host trees and specific pathogens in this study, some tree species such as *F. microcarpa*, were more likely to be colonized by wood-decay fungi in general than others [54].

The severity of rot disease of trees depended on the aggressiveness of wood-decay fungi, susceptibility of the host tree species, as well as the environmental conditions. Old trees could be more vulnerable to decay fungi than young trees under environmental and senescent stresses [55]. Compared to young trees, more old trees were observed with wood-decay fungi but with less significance. The most aggressive pathogenic wood-decay fungus discovered from this study was *P. noxius* (Figure S2). The pathogen infected OVTs in HKP, KP, and several large trees in HKO, which were still alive at the time of the second sampling despite the declining leaves and rot at the base of the stem, whereas bushes and smaller trees infected in HKO were already dead. Old and large trees may have extended root systems or plenty of aerial roots, as for *F. microcarpa*, to support the trees for a certain period of time, while for small trees, the infection of the main stems resulted in the blocking of nutrients and water supplies from the soil.

A variation in disease severity and progression was observed with each fungal species affecting a different tree species. *Physisporinus vitreus* could also cause root-rot diseases, but the severity was observed to be dependent upon the tree species (Figure S3). An *M. indica* tree in HKP infected by *P. vitreus* had been removed already during the study period because of the severe rot in the roots, while a *L. rhodostegia* tree in ZBG only showed fruiting bodies of *P. vitreus* without any obvious symptoms of root-rot. This may be because of host specificity, or due to different stages of infection. *Fuscoporia senex* is a white-rot fungus which usually causes stem-rot [56]. It had been discovered more on Fabaceae trees in Hong Kong, but fruiting bodies of fungi were also discovered on stumps. Though *F. senex* was only discovered in ZBG but, for both trees, it caused significant cavities on the tree trunks. Similar cavities were also discovered on other old trees, either on the stem or the base trunk, caused by *Ganoderma* species, such as *G. applanatum*, which was found in HKP and KP, laterally attached to the surface of the cavity of the trees, and *G. gibbosum*, which was found at HKP, KP, and HKO, laterally attached to old trees or old wood stumps (Figure S4). However, pathogenic fungi, such as the above-mentioned *P. noxius*, was found in both small and large trees in HKO, presenting high aggressiveness and causing tree decline in a rather short time. In contrast, trees colonized by less aggressive wood-decay fungi such as *Ganoderma* spp., did not cause quick decline of trees even though cavities formed in the tree trunk. The pathogenicity of a wood-decay fungus was also affected by its environment and hosts, which may cause a shift in their trophic mode. *Earliella scabrosa* were found in

both KP and HKO, but their trophic modes were different. In HKO, *E. scabrosa* was found on a fallen branch, as a saprobe, while in KP, the fruiting body of *E. scabrosa* was found laterally attached to a *F. microcarpa* YTM/65, as a rot pathogen (Figure S3).

Though for most of the trees, only one wood-decay fungal species was detected, it was possible that one tree could be simultaneously colonized by two or more wood-decay fungal species [39]. The *F. microcarpa* YTM/65, located in KP, saw both *E. scabrosa* and *P. noxius* detected. It was assumed that *P. noxius* could result in a more serious problem for the tree since the decayed roots were already penetrated by mycelia of *P. noxius*. Another case was the *F. microcarpa* CW/110 in HKP, of which the root was mostly colonized by *P. noxius*, while the cavity of the base trunk was more likely to be caused by *G. gibbosum*. The interactions between the two parasites on a single tree were unclear, but they may collectively facilitate the wood-decay process, or they may not interact at all due to their colonization on different parts of the trees.

Urban trees are valuable in many ways and a better understanding of common wood-decay fungi in an area facilitates the protection and management of trees against rot and risk. To better protect the trees, regular inspection should be conducted for proactive management [23]. Early detection of fungal infection makes it possible to save the tree by removing infected tissues and to replace highly hazardous trees gradually [5]. HKP, ZBG, and KP receive a large number of visitors daily, so weak trees can be dangerous if not identified and removed in a timely manner. When it is necessary to remove a diseased tree, it is crucial to clean up the infected area for replanting trees, especially if it is a root rot fungus. Trees should be managed by following an appropriate protocol. Pruning should be avoided during fungal sporulation seasons and pruning wounds should be protected to avoid wood-decay fungi infections [18].

## 5. Conclusions

This survey, emphasizing the urbanized ecosystem by including trees from four public parks, provided a basis for the baseline determination of fungal infection, conservation, protection, and management of urban trees. In this study, 28 wood-decay fungal species were detected in HKP, ZBG, KP and HKO. Among them *P. noxius*, *G. gibbosum*, and *A. polytricha* were the most abundant species and occurred in three of the four parks. Across the tree species, *F. microcarpa* were most vulnerable to wood-decay fungi with different species, while dead wood and wood stumps were more likely to be colonized by saprotrophic wood-decay fungi. The colonization rate of wood-decay fungi on older trees was much higher than that on the younger ones, resulting in infections and the subsequent problems were more likely shown in the older trees.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/1999-4907/11/10/1030/s1, Figure S1: Wood-rot on trees. Figure S2: Fruiting bodies of *P. noxius* and *F. senex*. Figure S3: Fruiting bodies of *E. scabrosa* and *P. vitreus*. Figure S4: Fruiting bodies of *Ganoderma* spp. Table S1: A list of reference sequences from GenBank.

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