Study on Sporocarp-Inhabiting Fungi from Decaying Xylariales Ascomata and Phylogenetic Re-Evaluation of Fungicolous Fungi in Japan

クロサイワイタケ目菌の腐朽した子のう果における菌寄生菌の菌類相とその系統学的再検討

December 2021

Wasiatus SA'DIYAH

Study on Sporocarp-Inhabiting Fungi from Decaying Xylariales Ascomata and Phylogenetic Re-Evaluation of Fungicolous Fungi in Japan

A Dissertation Submitted to
the School of the Integrative and Global Majors,
the University of Tsukuba
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in
Environmental Management
(Doctoral Program in Life Science Innovation)

Wasiatus SA'DIYAH

“Reading 20 pages per day is 30 books per year. Saving ¥1,100 per day is ¥401,500 per year. Running 1 mile per day is 365 miles per year. Becoming 1 % is better per day is 37% better per year. Sometimes small habits are underestimated.”

-James Clear & Alfred Lin-

Abstract

Investigation of hidden diversity in fungicolous fungi is essential for enhancing the functions of fungi. It will leave clues for future research. Secondary metabolites of fungicolous fungi have been utilized until recently. Early studies of fungicolous fungi focused on mycoparasites growing on basidiocarps and ascocarps. The studies were more related to chemicals, ecology, and taxonomy. Fungicolous fungi taxonomy has progressed until recently, including genus *Acrodontium* due to morphological identification in the previous studies. The relationship between asexual and sexual for *Bisporella discedens with* its asexual morph based on morphological identification. On the other hand, some mycologists are still confused about *the B. discedens* and *Calycina claroflava* relationship. These taxonomic problems in fungicolous fungi need to be resolved. Despite the taxonomical problem, the later study adopted the term sporocarp-inhabiting fungi (SCIF) to describe fungi inhabiting the surface of other fungal sporocarps. The enumeration of SCIF from the fungal host is necessary. In this study, we will be a focus on fungi associated with sporocarp. The enumeration study of SCIF is less. The enumeration of SCIF only focused on mushrooms or sequestrated fungi. Furthermore, the identification of mycoflora was limited to the familial level. In the present study, we aimed to enumerate SCIF on the sporocarp of xylarialean fungi and re-evaluate the taxonomical problems of *B. discedens* and its allies. The results showed the hidden diversity of the fungal community associated with the sporocarp of xylarialean fungi. *Acrodontium*, *Acremonium*, and *Simplicillium* The morphological and molecular identification showed about 1/3 OTUs as undescribed species or new lineages in this study. Morphological identification alone could not perform well identification. Thus, molecular identification help to solve it by using ITS and LSU sequences. The methods in this study could help the taxonomical problem in genus *Acrodontium* and *Bisporella* in this study.

Contents

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background of the research

Fungi is considered one of the largest kingdoms in eukaryotes and is estimated to include more than 2.2 to 3.8 million species (Hawksworth & Lücking, 2017). Ascomycota is the most diverse group in kingdom fungi and distributes in many varied communities, they perform many functions and benefits for humans, including alcohol fermentation, biocontrol agents, and drug candidates for cancer (Maicas, 2021; Sablayrolles, 2008; Kiss, 2003; Avis and Bleanger, 2002; Turbyville et al., 2005). Although, many fungi has been utilized as drug candidates, the cases of mortality and infection caused by fungal pathogens such as *Candida* (El-Ganiny et al., 2021; Loeffler and Stevens, 2003) *Trichosporon beigelii* (Walsh et al., 1990; Arastehfar et al., 2021), *Pseudallescheria boydii* (Lutcwick et at., 1976) showed to be resistant to currently antifungal agents. Based on the level of urgency, the existence of fungi should be investigated to

leave a clue for future study. The discovery of antifungals will be giving massive impacts on the development of drug candidates especially for antifungal drug resistance (Yue et al., 2015; Jain and Sharma, 2016; Sishuba et al., 2021).

Fungi have a widespread ecological niche. They are associated with organisms (plant, algae, animal, and fungi) and with rock, freshwater, marine, and soil. Fungi associated with plants have been mostly studied due to their benefit for medical purpose (Jain and Sharma, 2016; Helaly et al., 2018), agricultural (Jaschke et al., 2010; Poveda et al., 2020; Sishuba et al., 2021), and ecological purposes (Dissanyake et al., 2018; Zeng at al., 2021; Iqbal et al., 2012). So far, 13,000 species were reported associated with plants, including ectomycorrhizal and endophytic fungi (Hawksworth & Lücking, 2017), however little is studied about the diversity of fungi associated with the fungi (fungicolous fungi). Only 1,500 investigation fungicolous fungal taxa have been recorded (Sun et al., 2019). This number is less compared with the total number of fungal diversities and seems underestimated. Fungicolous fungi are widespread in taxon groups, especially in Ascomycota and

Basidiomycota (Gams et al., 2004). Among these groups, Dothideales, Sordariales, Hypocreales, and Hypomycetes were reported to be mostly dominated in fungicolous fungi (Gams et al., 2004). The term fungicolous fungi refers to fungal species associated with other fungi. This lifestyle classifies as natural, parasite, and saprotroph (Gams et al., 2004; Hawksworth, 1981; Ellis and Ellis, 1998; Læssoe and Petersen, 2019). Among these terminologies, sporocarp-inhabiting fungi (SCIF) was adopted to describe fungi inhabiting the surface of other fungal sporocarps or slime molds. This term is restricted for fungal associated with sporocarp (Gams et al., 2004; Hawksworth, 1981). Studies of fungicolous fungi have been studied since the early 1900s (Kern, 1910; Young and Bennett, 1922; Gilman and Tiffany, 1952). they have been progressing with some related studies such as chemical study (Shibata et al., 1957; Cheng et al., 2021), ecological study (Hargreaves et al., 2018), and taxonomical study (Sun et al., 2019; Hawksworth, 1981; Paul and Masih, 2000). Related studies will be explained in the next paragraph.

Firstly, a Chemical study that influences the discovery of secondary metabolite (Shibata et

al., 1957; Cheng et al., 2021). The interaction between the fungicolous fungi and the fungal host requires a defense mechanism by producing secondary metabolites. For example, some studies of secondary metabolites in mycoparasite provided many benefits for pharmaceutical purposes, for example, antibiotics lambertellols were produced by mycoparasites *Lambertella corni-maris*. This species is associated with *Monilinia fructigena* in Japan (Murakami et al., 2007). *Penicillium crustosum,* a fungus collected from the fruiting body of *Ophiocordyceps sinensis* were reported to produce many polyketides for anti-inflammatory (Feng et al., 2021). Secondly, the ecological study using the metabarcoding technique were studied to show niche of fungicolous fungi (Hargreaves et al., 2018; Maurice et al., 2021)

Thirdly is the taxonomical study of the fungicolous study that considers as a link to applied mycological research. Before the molecular era, morphological characteristics determined the identity of fungi. Identification by using morphology alone may not perform well classification for species level (Raja et al., 2017). Subsequently, Molecular studies have changed mycologists to

identify fungi based on genetic information. The molecular information is beneficial for reliable identification (Nilsson et al., 2008). The identification problem by using morphological identification alone is happening in one of the fungicolous fungi of genus *Acrodontium.* Genus *Acrodontium* has a wide ecological niche and accommodated 17 species *using phenotypic characters*, with type species *Acrodontium crateriforme*. Seifert (2011) classified ten species became part of the family Teratosphaeriaceae containing ten species by using a molecular approach. The seven species were placed in different order of Sordariomycetes (Videira et al., 2016). However, the proper analyses about these seven species were not studied, including *A. antarticum, A. abietis, A. griseum, A. hydnicola, A. salmoneum, A. simplex,* and *A. virelum.* On the other hand, the taxonomical study of genus *Bisporella* has been remaining unresolved. In 2013, some members of *Bisporella* were transferred into a member of the genus *Calycina* because assigned species grouped in *Calycina* type species *Calycina herbarium* clade (Baral et al., 2013). Furthermore, *Bi. discedens,* which is the early name of *Bi. claroflava* is still used in recent studies

by Zhuang et al. (2017) and Ekanayaka et al. (2019). The difference or similarity among *Calycina claroflava, Bi. discedend,* and its anamorph *Bl. Truncate* not yet disclosed. Interestingly, both genera are associated with other fungi. These taxonomical problems are necessary to re-evaluate.

The previous studies in fungicolous fungi raised the question about the fungicolous communities that consist on sporocarp. The enumeration of fungicolous fungi from other fungi is less investigated. This enumeration is beneficial to leave clues for future research (Hosoya et al., 2015; Korf, 2005), such as taxonomy, pharmacy, and ecology. In 2014, the diversity of fungicolous fungi were obtained from sclerotia of *Cenococcum geophilum.* The result shows 85 operational taxonomic units (OTUs). OTUs appeared to be distantly related to any described fungal species (Obase et al., 2014). Enumeration of fungicolous fungi was firstly reported by Marletto (1969), which isolated yeasts and yeast-like fungi from the surface of *Tuber* spp. Studies of mycoflora in fungal tissue have focused on sequestrate fungi (Buzzini et al., 2005; Pacioni et al., 2007; Perlinska-

Lenart et al., 2020). In these habitats, unique phylogenetic groups of Hypocreales and Dothideomycetes were previously obtained (Gams et al., 2014).

Mycologists have attempted to adopt several approaches to enumerate SCIF from the fungal host, the culture-dependent, and the culture-independent. The Culture-dependent provides a pure culture for the identification and taxonomical purpose. This approach can identify fungi at genus to species level. In the previous studies, the isolated fungi were identified at the species level through this approach (Dimkpa and Orikoha, 2021; Buzzini et al., 2005; Pacioni et al., 2007; Perlinska-Lenart et al., 2020). On the other hand, a culture-independent approach to show the fungal diversity of SCIF from sporocarp (Perlinska-Lenart et al., 2020; Maurice et al., 2021). Although, two methods could show the diversity of fungi (Dissanayake et al., 2018; Perlinska-Lenart et al., 2020). However, culture-independent methods are more suitable to assess fungal diversity due to showing a better representation of sample diversity (Shokralla et al., 2012; Taberlet et al., 2018). The isolation

process has included dilution plating (Paulitz and Menge, 1986; Tondje et al., 2007) or surface sterilization (Whipps and Budge, 1990; Pacioni et al., 2007).

In previous studies, the enumerations of SCIF were collected from sporocarp of hypogeous fungi and mushrooms. Alternatively, xylarialean fungi as a diverse group of ascomycetes were selected in the present study. These fungi are often associated with plants and distributed worldwide. Xylariales fungi were selected in this study because they were found associated with some fungi such as *Chlorostroma vestlandicum (*Nordén et al., 2014), *Hypoxylon invadens* (Fournier, 2014; Becker et al., 2020), *Simplicillium lamellicola* (Zare and Gams, 2001), and *Bisporella discedens* (Johnson, 1988). Furthermore, several mycoparasite helotialean species were reported on the surface of ascomata or stromata (Ellis and Ellis, 1998; Gams et al., 2004; Læssoe and Petersen, 2019). Thus, we hypothesized that fungal communities will be found from sporocarp of xylarialean fungi.

1.2 The problem of the fungicolous fungi

The problems of the previous research about fungicolous fungi are listed below.

1. Only a few studies have been conducted on sporocarps of fungi. The enumeration of fungicolous fungi focused on mushrooms or sequestrates fungi.

2. The problem of taxonomical studies among fungicolous fungi has been remaining until recently. Fungicolous fungi in genus *Acrodontium* and *Bisporell*a had several issues due to multi treated by several mycologists.

1.3 The purpose of the research

The purposes of the research are.

1. The present study aims to enumerate SCIF associated with the sporocarp of xylarialean fungi using a combination of direct observation and dilution methods. This enumeration will leave clue for future study such as taxonomical and chemical study.

2. To solve the current taxonomical problem of some genera, proper identification and phylogenetic analysis will be used in this study.

1.4 The research designs

The present study has two objectives that will be separated into different chapters. Chapter 2 will discuss enumerating SCIF on the sporocarp of xylarialean fungi on the ground. Chapter 3 will discuss re-evaluating problematic genera.

To achieve the first objective of this study needs a combination of direct observation and dilution methods to get isolated SCIF. Obtaining evidence of SCIF on the sporocarp using direct observation is necessary. Afterward, the dilution method will be performed to separate SCIF from the fungal host, and culture-dependent methods are selected to identify the fungal at the genus to species level. Molecular and morphological approaches are used to identify SCIF from genus to species level. In this

study, the internal transcribed spacer (ITS), large subunit nuclear ribosomal (LSU), and V9G/LR5 are performed to amplify SCIF strains. The phylogenetic tree is used for further analysis.

CHAPTER 2.

MYCOBIOTA FROM DECAYING XYLARIALES ASCOMATA: NOTES ON SOME INTERESTING SPOROCARP-INHABITING FUNGI ISOLATED IN JAPAN

2.1 Introduction

Fungi are one of the largest kingdoms in eukaryotes and are estimated to include more than 2.2 to 3.8 million species (Hawksworth and Lücking, 2017). Fungal species are distributed worldwide and grow on a wide variety of substrates, such as soil, litter, living organisms (higher plants, algae, animals, fungi, and lichens), house dust, and rock surfaces. Additionally, hidden underestimated diversity has been reported in extreme environments, such as soda soils detected using a culture-independent method (Grum-Grzhimaylo et al., 2015), deep-sea using direct DNA sequencing method (Le Calvez et al., 2009). Xerophile fungi, such as *Aspergillus halophilicus* and *Wallemia sebi* (Sklenář et al., 2017; Zalar et al., 2005), thermophiles (Morgenstern et al., 2012), and hyperparasitic fungi (Sun et al., 2019) were also reported.

Fungicolous fungi are also known to dominate a unique niche (Sun et al., 2019; Gams et al.,

2004). The term refers to species of fungi that are associated with other fungi and used even when the biological nature of the association and its trophic relationship are obscure (Gams et al., 2004; Jeffries, 1995). These fungi have been studied intermittently by various researchers. Early studies of fungicolous fungi focused on mycoparasites growing on basidiocarps and ascocarps (Gilman and Tiffany, 1952; Seeler, 1943). *Acremonium*/*Verticillium*-like fungi from the old stromata of xylarialean fungi or the tissues of *Puccinia* sp. were previously reported (Gams, 1975; Gams and Zare, 2001; Zare et al., 2000; Zare and Gams, 2001), while fungicolous fungi occurring in mushrooms in Japan were detected previously (Tubaki, 1955). Antagonists are different from mycoparasites in terminology on the other hand, studies on antagonistic fungi initially focused on pathogenic soil fungi using isolates from the same environment/substrate or stocked cultures in laboratories (Porter, 1924; Waksman, 1936) and then shifted to research on specific parasites and host fungi, such as *Tilletiopsis* on *Sphaerotheca* (Hoch, 1979) and *Trichoderma* on *Sclerotinia* (Santos and Dhingra, 1982). However, few studies have been conducted on the enumeration of fungi

in fungal tissue. An early study of mycoflora on truffle sporocarps was reported by Marletto (Marletto, 1969), who isolated yeasts and yeast-like fungi from the surface of *Tuber* spp. Studies of mycoflora in fungal tissue have focused on sequestrate fungi (Buzzini et al., 2005; Pacioni at al., 2016; Perlińska-Lenart et al., 2020) or on sclerotium-forming fungi (Danon et al., 2010; Jones and Stewart, 2000; Tu, 1980; Whipps and Budge, 1990). Recently, Obase et al. (Obase et al., 2014) obtained fungal isolates from the sclerotia of *Cenococcum geophilum* isolated in the USA and assigned them to 85 operational taxonomic units (OTUs) according to the sequences of nuclear internal transcribed spacer (ITS) and large subunit (LSU) rDNA regions. They mentioned that the identification of many fungal OTUs was challenging because most of them appeared to be distantly related to any described fungal species.

The term "sporocarp-inhabiting fungi (SCIF)" was used for fungicolous fungi that restricted to those existing on sporocarps of other fungi and trophic relationship with host has been widely recognized by Gams et al. (Gams et al., 2004). In these habitats, unique phylogenetic groups of

Hypocreales and Dothideomycetes were previously obtained. Although these previous studies were conducted under different research objects and methods, the results implied the existence of taxonomically interesting species or new lineages of SCIF. Most of these studies used sequestrate or sclerotium-forming fungi, and a few studies have been conducted on mycoflora on sporocarps above the ground.

The order Xylariales is a diverse group of ascomycetes, often associated with plants and is distributed worldwide (Petrini and Petrini, 1985). Several mycoparasitic helotialean species have been reported to occur on the surface of ascomata or stromata of xylarialean fungi [Gams et al., 2004, Ellis and Ellis, 1998; Læssoe and Peterson, 2019; Becker et al., 2020; Nordén et al., 2014). However, a survey of SCIF that do not sporulate on their hosts has not been conducted. In this study, we enumerated the SCIF mycobiota on xylarialean ascomata using direct observation and dilution plate methods.

2.2 Materials and Methods

2.2.1 Field sampling of ascomata

Xylariales ascomata were collected from two different forests in Ibaraki Prefecture, Japan: the Sakuragawa area on 16 August 2020 (sample nos. AM002, AM003, and AM007), and the Tsukuba area on 28 August 2020 (sample nos. WS34, WS35, and WS36) (Table 2), these fungal hosts were identified based on morphological features using references by Læssoe and Peterson (2019) and Vasilyeva et al. (2007). Both sampling sites were typical secondary forests where *Aucuba japonica*, *Cryptomeria japonica, Quercus serrata,* and *Zelkova serrata* were dominant. The Sakuragawa area is a valley forest that is always in a moist environment. On the other hand, the Tsukuba area is an urban forest that is always in a dry environment. Fallen or trapped twigs on which xylarialean fungi (i.e., *Annuloxypoxylon*, *Hypoxylon*, and *Nemania*) grew on were collected at each site (Figure 1) to isolate SCIF. Samples were placed in a paper bag, transported back to the laboratory within the day, and kept in a refrigerator (4 °C). The isolation procedure of SCIF was

performed within four days.

2.2.2 Isolation of SCIF

Three small pieces of fungal tissues (ca. 2–3 mm^3) from each sampled ascomata or stroma of Xylariales fungi was carefully cut using a sterile scalpel. These tissues were then submerged in 100 µL of sterilized MilliQ (Merck, Darmstadt, Germany) and vortexed for ten minutes. The supernatant was diluted with sterile MilliQ at three concentrations (i.e., 10^{-3}, 10^{-4}, and 10^{-5}) and plated onto Yeast Malt Extract Agar consisting of 20 g of malt extract (Thermo Fisher Scientific, Detroit, MI, USA), 2 g of yeast extract (Becton, Dickinson and Company, Erembodegem, Belgium), 20 g of agar (Fujifilm Wako Pure Chemical Corporation, Tokyo, Japan), and 1000 mL of MilliQ with 0.05 g/L chloramphenicol (Nacalai Tesque, Kyoto, Japan). The plates were incubated at 20 ± 0.5 °C. The plates were examined regularly for three to seven days, and emerging fungal colonies were axenically transferred onto fresh potato dextrose agar (PDA; Becton, Dickinson, and Company, Sparks, MD, USA) plates for morphological observation and DNA extraction.

2.2.3 DNA extraction, polymerase chain reaction (PCR) amplification, and DNA sequencing

DNA was extracted from the cultures using a rapid preparation procedure for DNA extraction (Izumitsu et al., 2012). The internal transcribed spacer (ITS) was amplified using the primer pairs ITS5/ITS4 (White et al., 1990) and the large subunit nuclear ribosomal DNA (LSU) using the primer pairs LR0R/LR5 (Vilgalys and Hester, 1990). Alternatively, a primer set of V9G/LR5 (de Hoog et al., 1998) was used to amplify five isolates that failed using LR0R/LR5. A 25 μL reaction mixtures that contained 3 μL of MilliQ, 12.5 μL of 5×buffer, 5 μL of 2 mM dNTPs, 1 μL of each primer at 20 pM, and 0.5 μL KOD FX Neo polymerase (TOYOBO, Tokyo, Japan) were prepared. Then, the PCR was carried out on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) as follows: initial denaturation at 94 °C for 2 min, followed by 38 cycles of 98 °C for 10 s; 61.5 °C (ITS5/ITS4), 46 °C (LR05R/LR5), or 55 °C (V9G/LR5) for 30 s of annealing, 68 °C for 1 min of extension, and 68 °C for 7 min of final extension. The amplified

PCR products were purified using ExoSAP-IT Express (Thermo Fisher Scientific) following the manufacturer's instructions. Purified DNA was sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) with the same primer pair for the ITS and LSU regions. Sequencing was performed on SeqStudio using default settings (Thermo Fisher Scientific). DNA sequences were manually assembled using ChromasPro version 2.1.8 (Technelysium Pty Ltd, Helensvale, Australia).

3.2.4 Morphological observation

Eight isolates obtained from fungal substrates were prepared for microscopic morphological identification using the methods described by Zare et al. (2000) with reference to Gams et al. (1987). Fungal structures were observed in preparations mounted using MilliQ. Field and macroscopic images were obtained using a X-M1 mirrorless digital camera (Fujifilm, Tokyo, Japan) with a QZ-35M lens (TAMRON, Saitama, Japan), a COOLPIX 4500 compact digital camera

(Nikon, Tokyo, Japan), and a SMZ-10A stereomicroscope (Nikon) with a DP12 digital camera (Olympus, Tokyo, Japan). The morphological characteristics of the isolates were observed and recorded using an OPTIPHOT2 microscope with a differential interference contrast device and a DS-L2 digital camera (Nikon). To characterize colony morphology, PDA and 2% malt extract agar (Becton, Dickinson, and Company) were used.

2.2.5 Phylogenetic analyses

DNA sequences were compared using a megablast-search integrated into BLAST (Altschul et al., 1997) based on the GenBank database (https://www.ncbi.nlm.nih.gov, accessed 18 October 2021). ITS sequences were used for ascomycetous isolates, and only a 98%–100% match with reliable sources (ex-type sequences or taxonomically validated sequences) was accepted as proof of identification, except for Eurotiomycetes genera *Aspergillus*, *Penicillium*, *Taralomyces,* and Dothideomycetes species *Cladosporium cladosporioides* complex sensu Bensch (Bensch et al.,

2012) LSU sequences were used for basidiomycetous isolates, and proof identification was conducted using the same criteria as ascomycetous fungi, except for *Microporus*, *Sistotrema,* and *Trametes*. Sequences were deposited in the DNA Data Bank of Japan (DDBJ; Table 3).

To determine the novelty of the ascomycetous fungi, the candidates for new taxa were compared with those present in GenBank using the ITS and/or LSU sequences. Since several ITS sequences of environmental DNA and endophytic fungi that were deposited in GenBank were phylogenetically related to those of our samples, ITS data were used for alternative analysis. According to proof identification, known *Acremonium*, *Acrodontium*, and *Simplicillium* species were included in the alignment. Sequences for each dataset were aligned using MAFFT version 7.429 in the default setting (Katoh and Standley, 2013). Ambiguously aligned portions of the alignments were manually removed using MEGA7 (Kumar et al., 2016). A maximum-likelihood (ML) analysis was performed using IQ-TREE 2.1 (Minh et al., 2020) with 1000 standard nonparametric bootstrap replicates. The substitution model was estimated ModelFinder

incorporated in IQ-TREE 2.1

2.3 Results

Six samples were collected from the two study areas (Table 2). Microscopic observations of the asexual structures of SCIF on Xylariales ascomata were as follows: ascomycetous fungi different from xylarialean fungi (Figure 3F, H, O, P), unidentified-fungus conidium (Figure 3Q), *Acrodontium*-type conidium (Figure 3AA), Ustilaginomycotina (Figure 3Z), exogenous mycelium (Figure 3M, V, W, AC, AD), and mycelial structures suggestive of Polyporales basidiomycetes (Figure 3AF, AG).

A total of 139 isolates were obtained from the six ascomata. Of these, 12, 38, 7, 50, 26, and 6 isolates were obtained from AM002, AM003, AM007, WS34, WS35, and WS36, respectively (Table 3). Eighty-eight isolates with identical ITS sequences and/or the same colony characteristics within the same samples were excluded. Forty-four representative isolates were shown in Table 3.

Sequencing of the ITS and LSU regions of the fungal isolates was performed, except for *Dactylospora* sp. and several Basidiomycota (Table 3). A BLAST search using the LSU or ITS sequences facilitated the genus-level identification of fungal isolates in many cases. The isolates belonged to six orders of Ascomycota (Chaetothyriales, Cladosporiales, Eurotiales, Hypocreales, Mycosphaerellales, and Sclerococcales) and eight of Basidomycota (Cantharellales, Corticiales, Entylomatales, Exobadisiales, Hymenochaetales, Polyporales, Robbauerales, and Ustilaginales) (Table 3). Some of the isolates (approximately 30%) have not yet been identified or are unidentifiable at the species level; for example, *Talaromyces* sp., *Penicillium* sp. 1, *Penicillium* sp. 2, and *Penicillium* sp. 3 are unidentifiable due to lower identities (<98% in ITS) with known species. Although the ITS sequence similarities were approximately 98–99%, several species were treated as unidentified species as "sp." for *Acremonium*, or tentatively identified as *Acrodontium* sp. aff. *crateriforme*, and *Simplicillium* sp. aff. *sympodiophorum* or *Acrodontium* cf. *salmoneum* based on morphological observations (Figure 2–4). As a result, 33 different fungal OTUs were identified in

this study (Table 3).

Table 2. List of samples from the moist forest (Sakuragawa area) and the urban dry forest (Tsukuba area).

Sample no.	Species	Substrate	Sampling site
AM002	*Hypoxylon* sp.	*Cryptomeria japonica*	JAPAN, Ibaraki, Sakuragawa, near Ibaraki prefecture road route 41, 36°14'26.195"N 140°03'57.412"E
AM003	*Hypoxylon* sp.	*Alnus* sp.	JAPAN, Ibaraki, Sakuragawa, near Ibaraki prefecture road route 41, dried river, 36°14'54.103"N 140°05'58.300"E
AM007	*Nemania* sp.	*Cryptomeria japonica*	JAPAN, Ibaraki, Sakuragawa, around Ibaraki prefecture road route 41, near Otoko-river, 36°14'13.736"N 140°06'05.799"E
WS34	*Annulohypoxylon annulatum*	*Zelkova serrata*	JAPAN, Ibaraki, Tsukuba, near Takasaki Nature Park, 36°00'10.4"N 140°06'49.5"E
WS35	*Nemania* sp.	*Zelkova serrata*	JAPAN, Ibaraki, Tsukuba, near Takasaki Nature Park, 36°00'10.4"N 140°06'49.5"E
WS36	*Annulohypoxylon annulatum*	*Zelkova serrata*	JAPAN, Ibaraki, Tsukuba, near Takasaki Nature Park, 36°00'10.4"N 140°06'49.5"E

The fungal communities were compared between the two study sites. *Acrodontium* spp., *Robbauera albescens*, and *Moesziomyces antarcticus* were isolated from the Sakuragawa and Tsukuba areas. *Acrodontium* spp. and polypores or corticioid immature mushrooms (*Neoantrodiella*-like or *Sistotrema*-like) were detected in Tsukuba area. The species compositions showed a similar trend among the three isolated sources (WS34, WS35, and WS36) in this study

area. For example, *Neoantrodiella gypsea*, *Robbauera albescens*, *Simplicillium* spp., and *Sistotrema* sp. were detected in at least two samples. Although the number of isolated species in the Sakuragawa area was lower than that in the Tsukuba area (10 spp. vs. 24 spp.), the species composition differed among samples. For example, *Acremonium* sp. was dominant in AM003, and few corticioid mushrooms (e.g., *Sistotrema* spp.) were detected among the three samples (AM002, AM003, and AM007).

2.3.1 Morphological observation and molecular phylogenetic analyses of noteworthy fungal species

Noteworthy isolates were briefly described with phylogenetic analyses in this section, and some were deposited at the Japan Collection of Microorganisms (JCM), RIKEN BioResource Research Center, Tsukuba, Japan: cf., the JCM On-Line Catalogue of Strains (https://jcm.brc.riken.jp/en/catalogue_e).

Acrodontium luzulae Videira & Crous (2016) (Figure 2 A–L)

Strains examined: WS34_2_5_A_As_10000 and WS36_3_1_A_As 10000

Note: These strains were identified as *A. luzulae* (Videira et al., 2016) based on the conidial morphology: conidia hyaline, thin-walled, smooth, solitary, ellipsoid with obtuse apex, 2.8–5.3 × 1.6–2.4 µm (av. 3.7 × 1.8 µm, n = 55), l/w 1.4–2.7(–3.3) (av. 2.1, n = 55). The ITS sequences of our isolates were identical to that of the holotype strain of *A. luzulae* (CBS 839.71; KX287274) (Videira et al., 2016). The species is characterized by conidiogenous cells, hyaline, elongated, smooth, and thin-walled with straight to flexuous, proliferating sympodially, and forming rachis in the upper part.

Acrodontium luzulae has been isolated from dead leaves of *Luzula sylvatica* and rust on *Carex* sp. (de Hoog, 1972, Videria et al., 2016) and dark ice (Perini et al., 2019) in previous studies. In this study, our strains were collected from the old stroma of *Annulohypoxylon* sp. and *Hypoxylon* sp., representing a new isolation record for this species. In addition, this is the first report of *A. luzulae* in Japan. Together with the previous literature data, it is possible to characterize *A. luzulae* as a cosmopolitan saprotrophic species of fungicolous fungi or plant decomposers.

***Acrodontium crateriforme* complex**

Acrodontium crateriforme has been reported as a cosmopolitan species in invertebrate animals, fungi, humans, and plant tissues (de Hoog, 1972; Videira et al., 2016). Koukol (2010) highlighted that repeated isolation of *A. crateriforme* from invertebrates and fungi indicates a preference for more nitrogen-rich substrates. The same author pointed out that the fungus has a wide ecological range and conidial size variations among strains obtained in previous studies. In the present study, this fungus was isolated from five different samples, and these strains showed genetic and morphological variations (Figure 2M–AF, Figure 3). Further analysis based on additional gene regions, such as *rpb2* or *β-tubulin* (Videira et al., 2016), and the further discovery of hidden lineages will elucidate the diversity of their host preferences or their phylogenetic complexity.

***Acrodontium* sp. 1 aff. *crateriforme* (J.F.H. Beyma)** de Hoog (1972) (Figure 2 M–V)

Strain examined: AM002_1_2_B_As_1000

Note: Conidia in culture hyaline, thin-walled, smooth, solitary, spherical to oblong with obtuse apex, 2.4–3.1 × 1.5–1.7 µm (av. 2.6 × 1.6 µm, n = 30), l/w 1.4–1.8 (av. 1.6, n = 30). Although the ITS sequences of this strain completely matched with the ex-holotype of *A. crateriforme* (CBS 144.33; NR_152320), the conidia of this strain were slightly smaller than those of the ex-holotype strain [vs. (3–) 3.5–4.5(–5) × (1.5–) 2–3(–4) µm; de Hoog (1972)]. We treated this strain as *Acrodontium* sp. 1 aff. *crateriforme* in this paper.

Figure 1. Habitats of the Xylariales fungi used in this study. **(A–H)** Field and microscopic observation of the sample AM002. **(A)** Habitat. Samples were collected from the trapped twigs (indicated by arrowhead). **(B–F)** Surface of the ascomata. SCIF occurring on the stroma in F (indicated by an arrowhead). **(G)** Ascomata in longitudinal section. **(H)** Melanized hyphae occurring on the peridium (indicated by an arrowhead). **(I–Q)** Field and microscopic observation of the sample AM003. **(I)** Habitat. Samples were collected from the dead twigs (indicated by an arrowhead). **(J–M)** Surface of the ascomata. SCIF occurring on the stroma in M (indicated by an arrowhead). **(N)** Ascomata in longitudinal section. **(O)** Melanized hyphae occurring on the peridium (indicated by arrowhead). **(P)** Sympodial conidiogenous cell occurring on the peridium. **(Q)** Unidentified conidium. **(R–W)** Field and microscopic observation of the sample AM007. **(R)** Habitat. Samples were collected from the fallen dead twigs (indicated by an arrowhead). **(S–W)** Surface of the ascomata. SCIF occurring on the stroma in V and W (indicated by an arrowhead). **(X–AA)** Microscopic observation of the sample WS34. **(X–Y)** Surface of the ascomata. **(Z)** Basidiospore of Ustilaginomycotina on the peridium. **(AA)** *Acrodontium*-type conidia on the peridium. **(AB–AD)** Microscopic observation of the sample WS35. Surface of the ascomata. SCIF occurring on the ascomata in AC and AD (indicated by the arrowhead). **(AE–AG)** Microscopic observation of the sample WS36. Surface of the ascomata. SCIF occurring on the ascomata in AF and AG (indicated by an arrowhead)

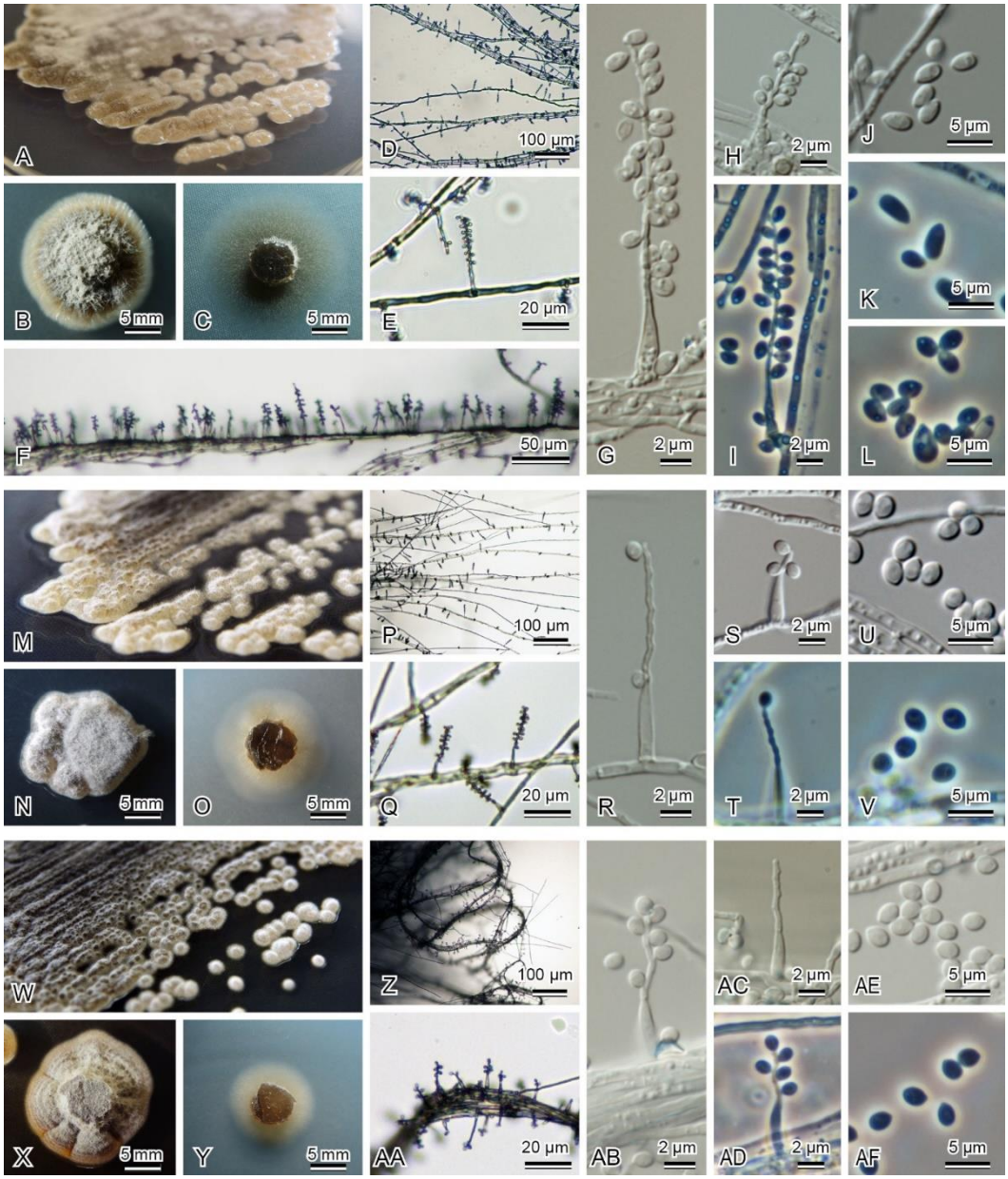

Figure 2. *Acrodontium* species. (**A**–**L**) *Acrodontium luzulae* (**A**–**C**,**F**,**G**,**H**,**I**,**L** from JCM 39231; **D**,**E**,**J**,**K** from JCM 39234). (**A**,**B**) Colonies on PDA after 8 days at 20 °C. (**C**) Colonies on MEA after 8 days at 20 °C. (**D**–**F**) Conidiogenous cells on hyphae. (**G**–**I**) Conidiogenous cells. (**J**–**L**) Conidia. (**M**–**V**) *Acrodontium* sp. 1 aff. *crateriforme* (JCM 39224). (**M**,**N**) Colonies on PDA after 8 days at 20 °C. (**O**) Colonies on MEA after 8 days at 20 °C. (**P**,**Q**) Conidiogenous cells on hyphae. (**R**–**T**) Conidiogenous cells. (**U**,**V**) Conidia. (**W**–**AF**) *Acrodontium* sp. 2 aff. *crateriforme* (JCM 39232). (**W**,**X**) Colonies on PDA after 8 days at 20 °C. (**Y**) Colonies on MEA after 8 days at 20 °C. (**Z,AA**) Conidiogenous cells on hyphae. (**AB**–**AD**) Conidiogenous cells. (**AE**,**AF**) Conidia.

Table 3. Identification of the isolated fungi in this study using BLAST.

Sample no.	Isolate no. (JCM no.)	OTU name	Closest match from GenBank using megablast	GenBank no.	
				ITS	LSU
AM002	1_1_B_As_1000	*Moesziomyces antarcticus*	*Moesziomyces antarcticus* (MH873351) 822/822 (100.00%) in LSU	LC631649	LC631614
	1_1_A_St_1000	Herpotrichiellaceae sp.	"*Capronia*" *pilosella* (DQ826737) 535/542 (98.70%) in ITS	LC631650	LC631615
	1_2_B_As_1000	*Acrodontium* sp. 1 aff. *crateriforme*	*Acrodontium crateriforme* (NR_152320) 457/457 (100%) in ITS	LC631651	LC631616
	1_2_A_St_1000	*Tilletiopsis washingtonensis*	*Tilletiopsis washingtonensis* (MH868275) 867/869 (99.77%) in LSU	-	LC631617
	1_8_A_St_1000	*Robbauera albescens*	*Robbauera albescens* (MH873380) 809/810 (99.77%) in LSU	-	LC631618
AM003	1_1_A_As_10000	*Acremonium* sp.	*Acremonium charticola* (MH859034) 554/587 (94.38%) including 9 gaps in ITS	LC631652	LC631619
	1_1_B_As_1000	*Acremonium* sp.	*Acremonium charticola* (MH859034) 545/578(94.29%) including 9 gaps in ITS	LC631653	LC631620
	1_2_A_As_1000	*Acremonium* sp.	*Acremonium charticola* (MH859034) 557/590 (94.40%) including 9 gaps in ITS	LC631654	LC631621
AM007	2_1_A_As_1000	*Dactylospora* sp.	*Dactylospora parasitica* (KY661666) 766/788(97.20%) including 1 gap in LSU	-	LC631622
	2_3_A_As_1000	*Cladosporium* sp. aff. *cladosporioides*	*Cladosporium cladosporioides* complex *sensu* Bensch et al. (2012)	LC631655	LC631623
	2_4_A_As_1000	*Acrodontium* sp. 3 aff. crateriforme	*Acrodontium crateriforme* (NR_152320) 441/442 (99.77%) in ITS	LC631656	LC631624
	2_5_A_As_1000	*Skeletocutis odora*	*Skeletocutis odora* (KY948893) 805/805 (100%) in LSU	LC631657	LC631625
WS34	1_2_A_As_10000	*Burgella* sp.	*Burgella flavoparmeliae* (KC336075) 708/710 (99.72%) in LSU	LC631658	LC631626
	2_1_A_As_1000	*Acrodontium* cf. *salmoneum*	*Acrodontium salmoneum* (MH860376) 563/565 (99.65%) including 2 gaps in ITS	LC631659	LC631627
	2_1_A_As_10000	*Penicillium* sp. 1	*Penicillium citreosulfuratum* (NR_153252) 576/578 (99.65%) in ITS	LC631660	LC631628

(Table 3. Continue)

Sample no.	Isolate no. (JCM no.)	OTU name	Closest match from GenBank using megablast	GenBank no.	
				ITS	LSU
	2_1_B_As_1000	*Sistotrema* sp. aff. *brinkmanni*	*Sistotrema brinkmannii-oblongisporum* group sense Moncalvo et al. (2006)	LC631661	LC631629
	2_1_B_As_10000	*Acaromyces ingoldii*	*Acaromyces ingoldii* (NG_058540) 445/446 (99%) in LSU	LC631662	LC631630
	2_2_A_As_10000	*Simplicillium* sp. 1 aff. *sympodiophorum*	*Simplicillium sympodiophorum* (NR_111027) 554/555 (99.82%) in ITS	LC631663	LC631631
	2_2_A_As_1000	*Neoantrodiella gypsea*	*Neoantrodiella gypsea* (KT203312) 773/774 (99.87% in LSU	-	LC631632
	2_2_B_As_1000	*Penicillium* sp. 2	*Penicillium mallochii* (NR_111674) 533/534 (99.81%) including 1 gap in ITS	LC631664	-
	2_2_B_As_10000	*Phlebia livida*	*Phlebia livid*a subsp. tuberculate (MW732462) 781/781(100%) in LSU	LC631665	LC631633
	2_3_A_As_10000	*Lenzites betulinus*	*Lenzites betulinus* (MT644927) 785/785(100%) in LSU	LC631666	LC631634
	2_4_A_As_10000	*Microporus* sp. aff. *xanthopus*	*Microporus xanthopus* (KX880659) 813/813(100%) in LSU	LC631667	LC631635
	2_5_A_As_10000	*Acrodontium luzulae*	*Acrodontium luzulae* (NR_154720) 526/526(100%) in ITS	LC631668	LC631636
	3_1_A_As_10000	*Robbauera albescens*	*Robbauera albescens* (NR_138401) 618/618(100%) in ITS	LC631669	-
WS35	1_1_A_As_10000	*Robbauera albescens*	*Robbauera albescens* (MH873380) 488/488(100%) in LSU	LC631670	LC631637
	1_2_A_As_10000	*Neoantrodiella gypsea*	*Neoantrodiella gypsea* (KT203312) 750/750(100%) in LSU	LC631671	LC631638
	1_3_A_As_10000	*Incrustoporia chrysella*	*Incrustoporia chrysella* (KP135286) 760/762(99.74%) in LSU	LC631672	LC631639
	1_5_B_As_10000	*Acrodontium* sp. 2 aff. *crateriforme*	*Acrodontium neolitseae* (NR_168148) 532/536 (99.25%) in ITS	LC631673	LC631640
	2_1_B_As_1000	*Penicillium* sp. 3	*Penicillium steckii* (NR_111488) 373/373(100%) in ITS	LC631674	-
	2_1_A_As_10000	*Trametes* sp. aff. *versicolor*	*Trametes versicolor* (KC176306) 607/611(99.35%) in ITS	LC631675	-
	2_2_B_As_10000	*Neoantrodiella gypsea*	*Neoantrodiella gypsea* (KT203312) 670/671(99.85%) in LSU	LC631676	LC631641
	2_3_A_As_10000	*Neoantrodiella gypsea*	*Neoantrodiella gypsea* (KT203291) 519/521 (99.62%) including 1 gap in ITS	LC631677	-

(Table 3. Continue)

Sample no.	Isolate no. (JCM no.)	OTU name	Closest match from GenBank using megablast	GenBank no.	
				ITS	LSU
	2_4_B_As_1000	*Talaromyces* sp.	*Talaromyces aurantiacus* (NR_103681) 464/474 (97.89%) including 6 gaps in ITS	LC631678	LC631642
	2_6_B_As_1000	*Beauveria bassiana*	*Beauveria bassiana* (NR_111594) 501/504 (99.40%) including 1 gap in ITS	LC631679	LC631643
	3_6_B_As_10000	*Simplicillium* sp. 2 aff. *sympodiophorum*	*Simplicillium sympodiophorum* (NR_111027) 559/561 (99.64%) including 1 gap in ITS	LC631680	-
	2_4_B_As_1000	*Talaromyces* sp.	*Talaromyces aurantiacus* (NR_103681) 464/474 (97.89%) including 6 gaps in ITS	LC631678	LC631642
	3_8_B_As_10000	*Moesziomyces antarcticus*	*Moesziomyces antarcticus* (NR_155406) 686/689 (99.56%) including 2 gaps in ITS	LC631681	-
WS36	1_1_B_As_10000	*Aspergillus* sp. aff. *versicolor*	*Aspergillus versicolor* (KU729039) 506/508 (99.60%) including 2 gaps in ITS	LC631682	-
	1_2_B_As_10000	*Cerrena zonata*	*Cerrena zonata* (MW785060) 711/712 (99.86%) including 1 gap in LSU	LC631683	LC631644
	1_4_A_As_10000	*Sistotrema* sp.	*Sistotrema coroniferum* (KF218968) 628/646 (97.21%) including 4 gaps in LSU	LC631684	LC631645
	2_3_b_As_10000	*Neoantrodiella gypsea*	*Neoantrodiella gypsea* (KT203312) 750/751 (99.87%) including 1 gap in LSU	LC631685	LC631646
	3_1_A_As_10000	*Acrodontium luzulae*	*Acrodontium luzulae* (NR_154720) 480/480 (100%) in ITS	LC631686	LC631647
	3_3_A_As_1000	*Meira* sp.	*Meira miltonrushii* (NG_060234) 470/472 (99.58%) including 1 gap in LSU	-	LC631648

***Acrodontium* sp. 2 aff. *crateriforme* (J.F.H. Beyma)** de Hoog (1972) (Figure 2 W–AF)

Strain examined: WS35_1_5_B_As_10000

Note: The conidial features of this strains are as follow: conidia in culture hyaline, thin-walled, smooth, solitary, spherical to oblong with obtuse apex, 2–3.8 × 1.6–2.2 µm (av. 2.7 × 1.8 µm, n = 30), l/w 1.7–3.3 (av. 2.4, n = 76). Although the strain is closely related to the ex-holotype of *A. crateriforme* in the ITS region (Figure 3) and only one nucleotide difference without gap observed, the strain has slightly smaller conidia than those of ex-type strain, and broader than *Acrodontium* sp. 1 aff. *crateriforme* (vs. 1.5–1.7 µm in AM002_1_2_B_As_1000). We treated this strain as *Acrodontium* sp. 2 aff. *crateriforme* in this paper.

Acrodontium* sp. 3 aff. *crateriforme (J.F.H. Beyma) de Hoog (1972)

Strains examined: AM007_2_4_A_As_1000

Note: In the phylogenetic tree, AM007_2_4_A_As_1000 clustered with the *A. crateriforme*

complex (Figure 3). Sequence differences among the complex were found to be one to three, or completely matched with one to three gaps or without a gap in the ITS. We did not observe the morphological features of AM007_2_4_A_As_1000. Therefore, we tentatively treated that the isolates were *Acrodnotinum* sp. 3 aff. *crateriforme* according to their phylogenetic affinities.

***Acremonium* sp.** (Figure 4A–H)

Strains examined: AM003_1_1_A_As_10000; AM003_1_1_B_As_1000; AM003_1_2_A_St_1000

Note: The morphological features of the above strains were as follows: conidiogenous cells arising from weakly fasciculate aerial hyphae, hyaline, thin-walled, smooth, ampulliform, tapering imperceptibly, and conidia ellipsoidal, apiculate at both ends, 3.2–5.6 × 1.4–2.4 µm (av. 4.1 × 1.7 µm, n = 30), l/w 1–1.9 (av. 1.5, n = 30), hyaline, smooth, without chlamydospores. These features concurred with *Acremonium* section *Acremonium* sensu Gams (1975).

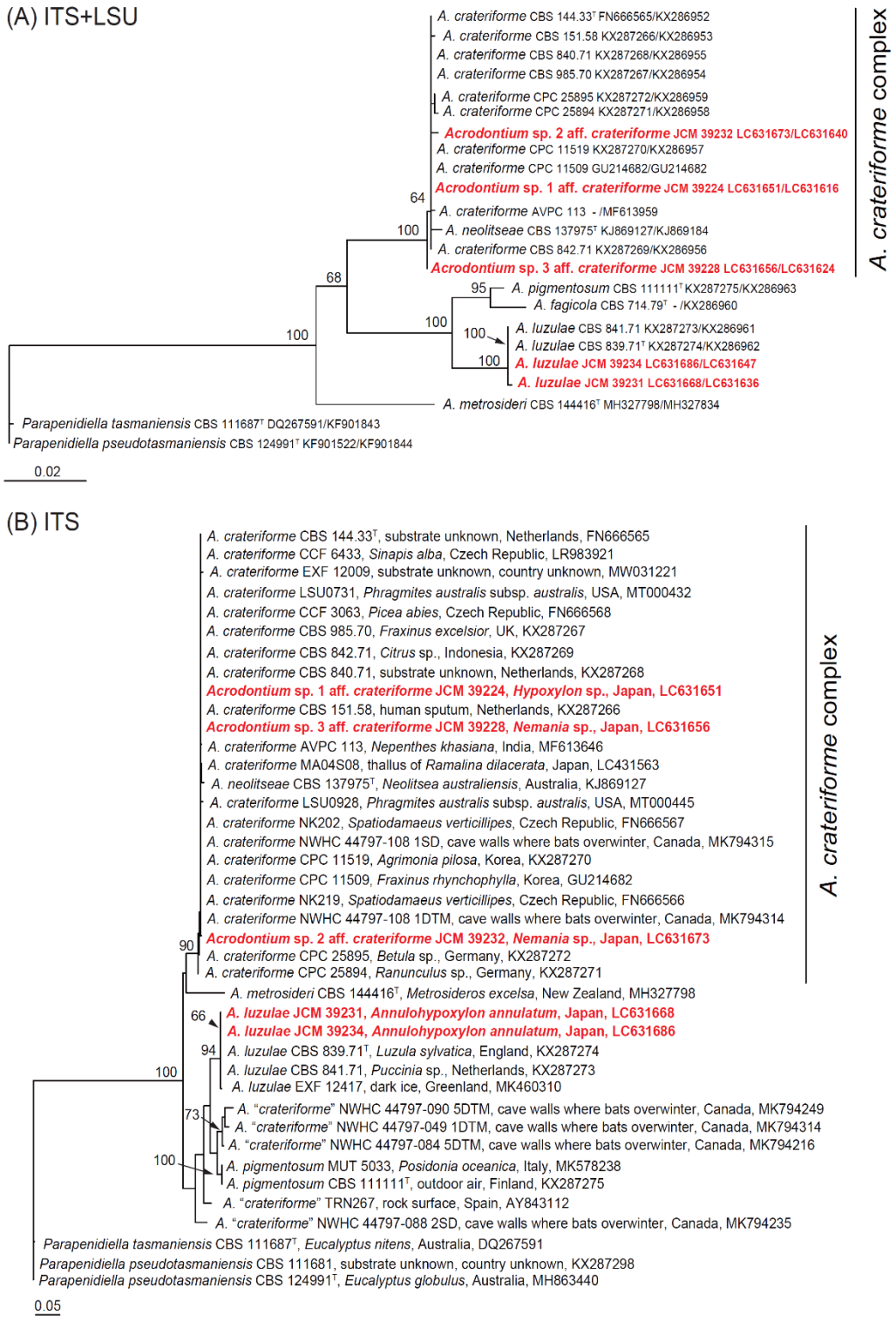

Figure 3. Maximum-likelihood (ML) tree of *Acrodontium* species. (**A**) Species relationship of *Acrodontium* by GTR+F model based on the ITS and LSU sequences. (**B**) Comparison of environmental samples with *Acrodontium* species by TIMe+R2 model based on the ITS sequences. Standard nonparametric bootstrap greater than or equal to 60% are presented at the nodes. Ex-type strains are indicated with a superscript T. The newly obtained sequences are shown in bold. The scale bar represents nucleotide substitution per site

Acremonium species are saprobic fungi that have been isolated from soil, air, and plant debris (Glenn et al., 1996; Lucheta et al., 2016; Manandhar et al., 1987). These species have also been reported as SCIF, for example, the strains isolated from *Psilocybe fasciata* (He et al., 2006), *Tuber* (Pacioni and Leonardi, 2016), and Xylariales fungi or *Puccinia* sp. (Gams, 1975). A BLAST search in the ITS region showed that our strains were closely related to *Acremonium charticola* [CBS 117.25; MH854807, 453/489 (93%, 5 gaps)]. The closest hits were five strains of unidentified fungal endophytes that were isolated from the tropical leaves of Fabaceae in Panama (Figure 4B). These strains showed identical match or 1–2 nucleotide differences with one gap. In the phylogenetic analysis, the three strains were grouped with *Acremonium sclerotigenum* clade sensu Summerbell et al. (2011) (Figure 6A). Since we do not have adequate material for detailed investigation and we lack reproducible isolation/detection, we treated this fungus as *Acremonium* sp. in this paper.

Acrodontium* cf. *salmoneum de Hoog (1972) (Figure 4 I–P)

Strain examined: WS 34_2_1_A_As_1000

Note: The morphological features of the above strain were as follows: conidiogenous cells hyaline, thin-walled, cylindrical, straight to flexuous, proliferating sympodially and forming a rachis in the upper part, 36–58 µm long, and 0.6–0.9 µm wide at apex, 1.6–3.2 µm at base, with multiple conidiogenous loci slightly thickened but not darkened and conidia hyaline, thin-walled, smooth, solitary, spherical to oblong with obtuse apex, 2.4–3.1 × 1.5–1.7 µm (av. 2.6 × 1.6 µm, n = 30), l/w 1.4–1.8 (av. 1.6, n = 30). This feature closely matched the description of *Acrodontium salmoneum* (de Hoog, 1972).

Acrodontium salmoneum and allied strains were isolated from various substrates. de Hoog (1972) reported that the ex-holotype culture was isolated from human sputum (CBS 847.71; de Hoog, 1972) and three ex-paratype cultures originating from culture contaminants (CBS 580.67, CBS 846.71, NRRL Online Catalog; https://nrrl.ncaur.usda.gov/, accessed 18 October 2021) and

soil of beech forest (CBS 848.71). The fungus has also been recorded in caves, mites, crab shells, and decayed wood in previous studies (de Hoog, 1972; Kozlova and Mazina, 2020; Steiman et al., 1995; Stejskal et al., 2017). Phylogenetic analysis showed that our strain grouped with the ex-paratypes (CBS 848.71 and CBS 580.67) and unidentified endophytic fungus (strain ZLY-2010 M29) from *Abies beshanzuensis* reported by Yuan et al. (2011). Although DNA sequence data suggested the potential for cryptic species for these strains (3–19 nucleotide differences in ITS), no sequence data are currently available for the ex-holotype of this species. The conidia in our strains were somewhat smaller than those of the original description [vs. (3.5–) 4.5–5.5(–7) × (1.5–)2–3(–3.5) µm in de Hoog (1972)]. Based on the phylogenetic and morphological differences, we treated this fungus as *Acrodontium* cf. *salmoneum* in this paper.

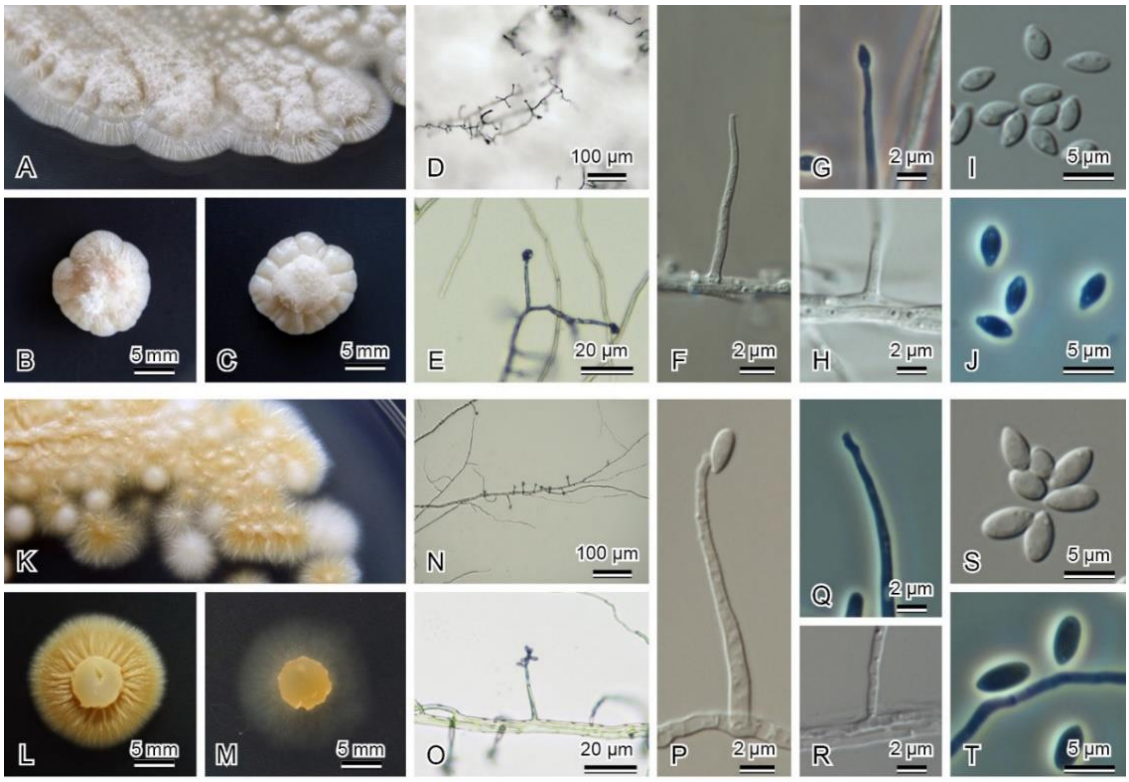

Figure 4. *Acremoniunm* sp. and "*Acrodontium*" cf. *salmoneum*. **(A–J)** *Acremonium* sp. (A, B from the culture AM003_1_1_B_As_1000, C from the culture AM003_1_2_A_As_1000, D–J from the culture AM003_1_1_A_As_10000). **(A–C)** Colonies on PDA after 10 days at 28 °C. **(D, E)** Conidiogenous cells on hyphae. **(F–H)** Conidiogenous cells. **(I, J)** Conidia. **(K–T)** "*Acrodontium*" cf. *salmoneum* (K–T from the culture WS34_2_1_A_As_1000). **(K, L)** Colonies on PDA after 8 days at 20 °C. **(L)** Colonies on MEA after 8 days at 20 °C. **(N, O)** Conidiogenous cells on hyphae. **(P–R)** Conidiogenous cells. **(S, T)** Conidia.

Videira et al. (2016) suggested that *A. salmoneum* should be excluded from the genus *Acrodontium* because this species was phylogenetically separated from the type species of the genus based on the LSU sequence. However, its phylogenetic position has not been resolved in their study.

The results of our phylogenetic analyses using LSU sequences suggested a close relationship of the "*A. salmoneum*" clade with Sarocladiacee (Figure 4A), but its generic placement for *Acrodontium*

remains unresolved. Thus, we tentatively treated this species as "*Acrodontium*" *salmoneum*. Further isolates and sequences of the ex-holotype strain of *A. salmoneum* are needed to clarify the ecological aspects and generic position of the species.

***Simplicillium sympodiophorum* complex**

Several species of *Simplicillium* have been reported as SCIF in a previous study (Zare and Gams, 2001). *Simplicillium sympodiophorum* was originally isolated from soil under *Asplenium antiquum* at Aogashima, Izu Islands in Japan (Nonaka et al., 2013). The species was isolated from the fruit of *Prunus avium* (Serradilla et al., 2013) and from the arthropod *Armadillidium vulgare* (Jaber et al., 2016). Unfortunately, most strains were not available for morphological comparison, but genetic variations were found in ITS sequences (one to two nucleotide differences and with or without gaps). Therefore, it is unclear whether these genetic variations implicate population or species differences.

Simplicillium* sp. 1 aff. *sympodiophorum Nonaka, Kaifuchi & Masuma (2013) (Figure 6 A–K)

Strain examined: WS34_2_2_A_As_10000

Note: The morphological features of our strain were as follows: conidiogenous cells solitary or in whorls of 2–4, simple and slender, tapering toward the tip, 12–26.5 µm long, 0.4–0.8(–1.6) µm wide at apex, 0.8–1.6 µm at base, and conidia 2.4–4.0 × 1.5–2.0 µm (av. 3.0 × 1.7 µm, n = 30), l/w 1.4–2.5 (av. 1.8, n = 30). These features almost agree with the description of *S. sympodiophorum*, although the length of conidiogenous cells was slightly shorter than that of the original description. The colony was slow-growing, reaching 13–18 mm diam. in 7 days at 25 °C on PDA with salmon pinkish pigment [vs. 20–34(–47) µm long in conidiogenous cells; 21–22 mm diam. colony with yellowish-white, without pigment in Nonaka et al. (2013)]. The ITS sequences of our strain showed only one nucleotide difference to that of *S. sympodiophorum* (NR_111027) obtained from the ex-holotype strain (JCM 18184) of this species, and we treated this strain as *Simplicillium* sp. 1 aff.

sympodiophorum.

Simplicillium* sp. 2 aff. *sympodiophorum Nonaka, Kaifuchi & Masuma (2013) (Figure 6 L–V)

Strain examined: WS35_3_6 B_10000

Note: The morphological features of the above isolate were as follows. Conidiogenous cells solitary or in whorls of 2–4, simple and slender, tapering toward the tip, 17.5–28 µm long, 0.2–0.8 µm wide at apex, 0. 8–1.7 µm at base; conidia oval to ellipsoidal, 2.4–3.0 × 1.6–2.0 µm (av. 2.6 × 1.8 µm, *n* = 15), l/w 1.2–1.9 (av. 1.5, *n* = 15), smooth-walled, and one-celled. The morphological features were similar to those of *S. sympodiophorum*, but this isolate had slightly shorter and broader conidia than those of *S. sympodiophorum* (vs. 2.2–3.5 × 1.0–2.0 µm in Nonaka et al. [61]). *Simplicillium* sp. 2 aff. *sympodiophorum* is similar to *Simplicillium* sp. 1 aff. *sympodiophorum* in conidial size, but could be distinguished by slightly slender conidia *(vs.* 2.4–4.0 × 1.5–2.0 µm*)*. This fungus produced yellowish pigment on PDA (Figure 6M), while *Simplicillium* sp. 1 aff. *sympodiophorum*

produced pinkish one (Figure 6B). In addition, one nucleotide difference with one gap in the ITS region was found between *Simplicillium* sp. 2 aff. *sympodiophorum* and *Simplicillium* sp. 1 aff. *sympodiophorum* (JCM 39230). We treated this fungus (JCM 39233) as *Simplicillium* sp. 2 aff. *sympodiophorum* in this paper.

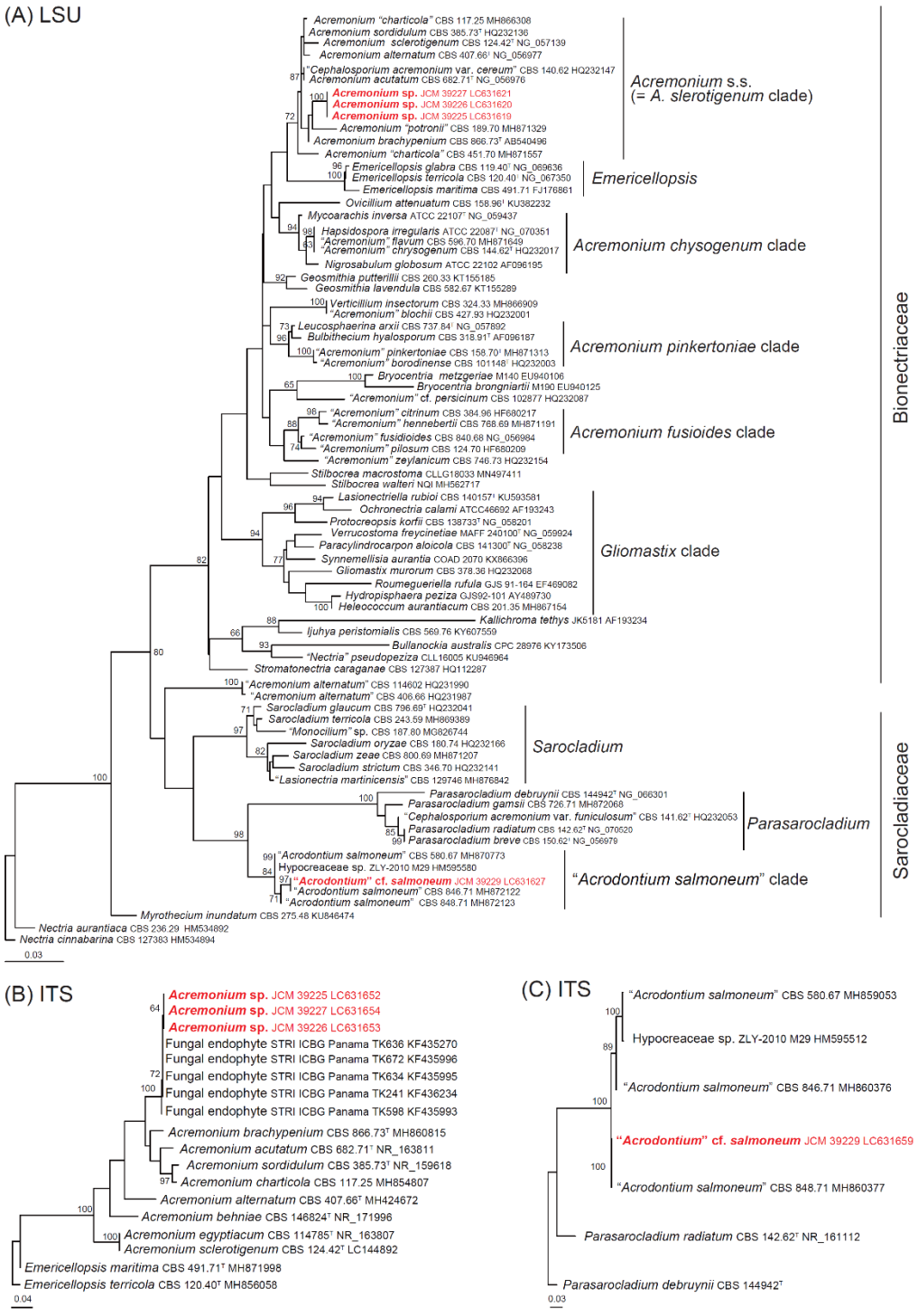

Figure 5. Maximum-likelihood (ML) tree of Bionectriaceae and Sarocladiaceae. (**A**) Species relationship of Bionectriaceae and Sarocladiaceae by TIM3e+I+G4 model based on the LSU sequences. (**B**) Species relationship of *Acremonium* spp. by TIM2e+G4 model based on the ITS sequences. (**C**) Comparison of environmental samples with "*Acrodontium*" *salmoneum* complex by TNe model based on the ITS sequences. Standard nonparametric bootstrap greater than or equal to 60% are presented at the nodes. Ex-type strains are indicated with a superscript T. The sequences in this study are shown in bold. The scale bar represents nucleotide substitution per site.

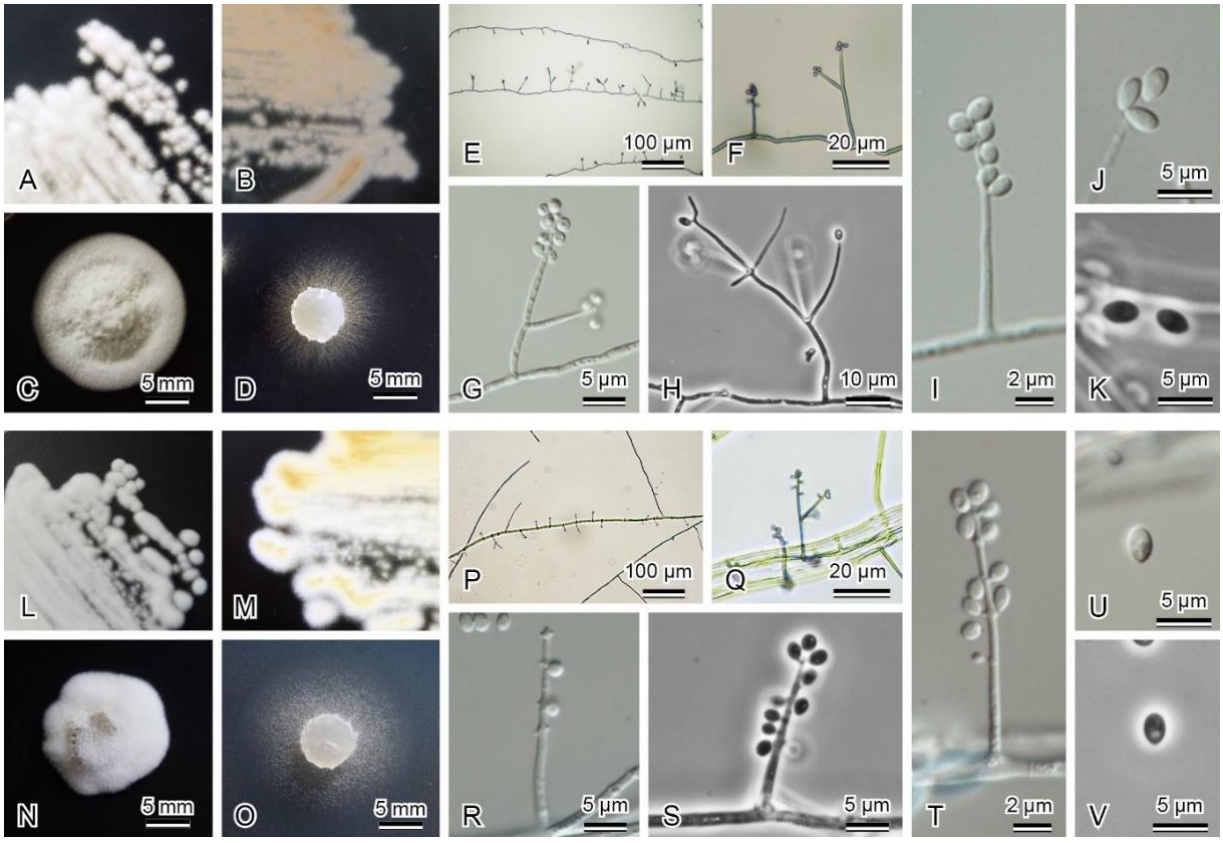

Figure 6. *Simpllicillium* species. **(A–K)** *Simpllicillium* sp. 1 aff. *sympodiophorum* (A–K from WS34_2_2_A_As_10000). **(A–C)** Colonies on PDA after 7 days at 25 °C. **(D)** Colonies on MEA after 7 days at 25 °C. **(E–I)** Conidiogenous cells. **(J, K)** Conidia. **(L–V)** *Simpllicillium* sp. 2 aff. *sympodiophorum* (L–V from WS34_3_6_B_As_10000). **(L–N)** Colonies on PDA after 7 days at 25 °C. **(O)** Colonies on MEA after 7 days at 25 °C. **(P–T)** Conidiogenous cells. **(U, V)** Conidia.

2.4 Discussion

Previous studies of SCIF have focused on species diversity, host preference, physiological features, and isolates from mushrooms or sequestrate fungi (Buzzini et al., 2005; Perlińska-Lenart et al., 2020; Pacioni et al., 2007; Maurice et al., 2021). Our study presented new information on the

mycobiota of the ascomata of xylarialean fungi using direct observation and dilution plate methods. Several noteworthy microfungi were identified by focusing on their genetic, morphological, and ecological diversity. *Acremonium*, *Acrodontium*, and *Simplicillium* species and basidiomycetous yeasts were found (Table 3) and they were previously reported as SCIF by Gams (Gams et al., 2004). Some of these hypocrealean species have been reported as dominant SCIF groups on *Tuber* sp. (Pacioni et al., 2007; Perlińska-Lenart et al., 2021; Pacioni et al., 2007) and they are known as occurring on the old stroma of xylarialean fungi (Læssoe and Peterson, 2019; Becker et al., 2020; Dennis, 1981). In contrast, helotialean or *Trichoderma* species were not isolated in this study. It may be due to an operational problem, such as isolating medium or seasonal and microenvironmental differences. Other species, such as polypores or corticioid mushrooms, were isolated probably from spores. *Cerrena*, *Incrustoporia*, *Lenzites*, *Microporus*, *Neoantrodiella*, *Phlebia*, *Skeletocutis,* and *Trametes* species are common decomposers of woody materials in forest ecosystems (Hattori, 2005; Justo et al., 2017). *Phlebia livida* and the *Sistotrema brinkmanni* complex are known to be

distributed worldwide, including in Asia, Europe, North and South America, and Antarctica (Gresblin and Rajchenberg, 2003; Maekawa, 1993; Hao et al., 2010). These species are known as wood-decaying mushrooms (Maekawa, 1993), although they are also assumed to be important decomposers under extremely low nutrient conditions (Hao et al., 2010). These species seem to exist on the fungal surface as dispersed spores.

Fungal isolates occasionally could not be identified based on ITS sequences and morphological features, although species diversity of *Acremonium*, *Acrodontium*, *Aspergillus*, *Penicillium*, and *Simplicillium* have been well studied in previous studies (Videira et al., 2016; Summerbell et al., 2011; Nonaka et al., 2013; Gräfenhan et al., 2011; Samson et al., 2014). Noteworthy species in the present study were identified as *Acrodontium* sp. 1, sp. 2, and sp. 3 aff. *crateriforme*, *Acrodontium luzulae,* "*Acrodontium*" cf. *salmoneum, Acremonium* sp.*,* and *Simplicillium* sp. 1 and sp. 2 aff. *sympodiphorum*. We did not focus on *Aspergillus* and *Penicillium* species because these saprotrophic fungi are easily found on other substrates as well as decaying

sporocarps.

Our investigation focused on enumerating SCIF using direct observations and dilution plate method. Common species, such as *Acrodontium* spp., *Robbauera albescens*, and *Moesziomyces antarcticus*, were isolated from an urban moist forest in the Sakuragawa area and urban dry forest in the Tsukuba area, but the overall species composition differed (Table 3). The species composition, in particular, was similar among the Tsukuba area but clearly differed among the Sakuragawa areas. Soil-borne fungi, such as *Aspergillus*, *Beauveria*, *Penicillium*, and *Talaromyces*, or basidiomycetes (mushrooms), were detected in the Tsukuba area compared to those in the Sakuragawa area. The xerophile decomposer fungi, such as Aspergillaceae, might predominantly colonize fungal tissue in the urban dry forest area. These geographical or environmental factors may also be associated with the species diversity of SCIF. Collado et al. (Collado et al., 1999) reported that environmental factors may affect the composition of fungal communities in endophytic fungi. To clarify the mycobiota of SCIF in detail, further studies are required to elucidate the effects of temperature and humidity.

This exploration of SCIF on Xylariales ascocarps revealed the mycobiota and its hidden diversity with the existence of several interesting species of microfungi. In particular, the existence of species complexes or different populations were implicated in *Acrodontium* and *Simplicillium*. From these genera, a potential antifungal substance such as acrodontiolamide (from "*Acrodontium* *salmoneum* (de Gusmáo et al., 2003)) and simplicilliumtide (from *Simplicillium obclavatum* (Liang et al., 2017)) were reported. Identification and enumeration of SCIF should be investigated properly to elucidate the dynamics of the life cycle of fungicolous fungi, and this may also help in the discovery of novel bioactive substances.

CHAPTER 3.
MOLECULAR ANALYSIS OF *BISPORELLA DISCEDENS* (PEZIZELLACEAE, HELOTIALES) IN JAPAN

3.1 Introduction

Calycella used to use for generic name of common yellow species (Korf and Carpenter, 1974). Subsequently, Korf and Carpenter proposed *Bisporella* and transferred four species of *Calycella* to *Bisporella*. Nineteen species are accepted to be member of *Bisporella* (Kirk, 2008). One of fungicolous fungi *Bisporella* was found on xylarialean fungi. This fungus is *Bisporella disceden.* is described for the first time in 1975 (Carpenter, 1975). *Bloxamia truncate* was reported as anamorph of *Bi. discedens*, it was found on xylarialean fungi association with *Bi. discedens* (Johnston, 1988). This species has three types of life cycles, asexual, sexual, and synasexual. As shown in (Figure 9), phialide appears in peridium side. This synasexual morph is "*Chalara*-like".

Historically, *Bi. discedens* was proposed to be synonym of *Bi. claroflava* by Lizon and Korf (1995). Hence, the name of *B. claroflava* should be designated to use for later study. In 2013, Baral

proposed some members of *Bisporella* to be member of *Calycina*, such as *Calycina claroflava*, *C. drosodes*, *C. lacteal*. *C. languida*, and *C. scolochloae*. Subsequently, *Bi. claroflava* name were treated to be *C. claroflava*. The reason is because *C. claroflava* is grouped with *Calycina* type species *C. herbarum* (Baral et al., 2013). However, anamorph of *C. claroflava* was not described in this study. Furthermore, some researchers treated *Bi. claroflava* and *C. claroflava* to be distinct species in the phylogenetic tree without any reason (Ekanayakana et al., 2019; Zhuang et al., 2017).

The sequences of *Bi. discedens* is limited in GenBank. Evolutionary, the relationship between *Bi. discedens* and its allies need to be solved. Thus, the aim of this study is to re-evaluate the relationship between *B. discedens* and its allies.

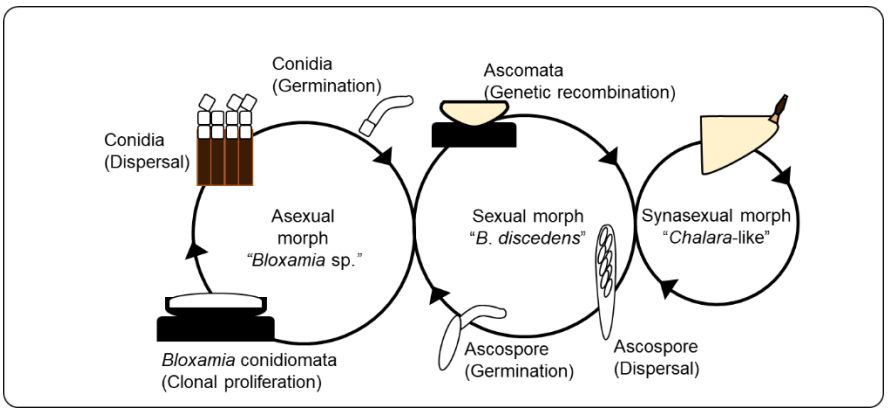

Figure 9. Life cycles of *Bi. discedens*

3.2 Materials and Methods

3.2.1 Isolating *Bi. discedens* and *Bloxamia* from Xylariales fungi.

Six strains of *Bi. discedens* were collected in Fukushima, Ibaraki, Kanagawa, Tokyo, and Tochigi from black dead twig that expected to be xylarialean fungi. All strains were maintained on Potato Dextrose Agar (PDA; Becton, Dickinson and Company). Each strain were preserved in slant agar containing PDA and in freeze stock that contained DMSO and Glycerol in the laboratory.

3.2.2 Morphological observation

We prepared rice straw agar to induce asexual morph, then we can use it for morphological observation. Observation of asexual morph are including conidiophore, conidiogenous cell, and conidia. Tissue layers were sectioned with a freezing microtome and were measured. Photographs were taken using an OPTIPHOT2 microscope with a differential interference contrast device and a DS-L2 digital camera (Nikon).

3.2.3 Molecular phylogeny and phylogenetic analysis

DNA was extracted from culture using DNA extraction kit and amplified using the internal transcribed spacer (ITS) genes with primer pairs ITS5/ITS4 (White et al., 1990), and large subunit ribosomal (LSU) with primer pairs LR0R/LR5 (Vilgalys and Hester, 1990). PCR was performed in 25 µl volume containing 5 µl extracted template DNA, 3 µl miliQ, 10 µl dNTPs, 12.5 µl 5 × PCR buffer, 1 µl × 20 pM each primer, and 0.5 µl KOD FX Neo (Toyobo, Tokyo, Japan). The PCR condition were 94°C for 2 minutes followed by 35 cycles at 98°C for 10 sec, 61.5 °C for 30 sec, and 68°C for 1 minutes. The amplified product were determined by electrophoresis on 1.5% agarose gels with known standard DNA marker and then purified using the PCR clean- up kit. For sequencing, a 6.5 µl reaction volume containing 2 µl purified PCR product, 3 µl miliQ, 2 µl 5 × PCR buffer, 1 µl appropriate primer, and 0.5 µl BigDye. Sequencing was performed using thermocycler program with 25 cycle. The sequences were edited using ChromasPro version version 2.1.8 (Technelysium Pty Ltd, Helensvale, Australia). DNA sequences were compared by BLAST

search (Altschul et al., 1997) with the GenBank databases (https://www.ncbi.nlm.nih.gov). Sequences for each data set were aligned using MAFFT version 7.429 as the default setting (Katoh and Standley, 2013). Ambiguously aligned portions of the alignments were manually removed using MEGA7 (Kumar et al., 2016). A maximum-likelihood (ML) analysis was performed using IQ-TREE 2.1 (Minh et al., 2020) with 1000 bootstrap replicates. The phylogenetic relationships of the strains were decided through the maximum likelihood method consisting of 1000 ultra-fast boostraps replications (UFBS). These analyses were based on 59 sequences including isolated *Bloxamia* sequences in this study. *Amorpotheca resinae* and *Oidiodendron maius* were used as outgroup taxa.

3.3 Results

Six species of *Bloxamia* were isolated from xylarialean fungi from Kanagawa, Fukushima, Gunma, and Tokyo. Five specimens were obtained from xylarialean fungi and one strains were

collected from driftwood (Table 4). We induced the asexual state of each strain to get information about asexsual and synasexual for morphological observation and extracted for molecular analysis. Four speciement were examined to get morphological feature.

Table 4. Sample list and their sampling site

Isolate no.	Species	Host/substrate	Sampling site
WS18	*Bloxamia* sp.	Xylarialean	JAPAN, Kanagawa, Manazuru, Mt. Tomyo
WS29t	*Bloxamia* sp.	Xylarialean	JAPAN, Tokyo, Hachiouji, Uratakao, near Mt. Kagenobuyama
WS30	*Bloxamia* sp.	Xylarialean	JAPAN, Tokyo, Hachiouji, Uratakao, near Mt. Kagenobuyama
WS31	*Bloxamia* sp.	Xylarialean	JAPAN, Tokyo, Hachiouji, Uratakao, near Mt. Kagenobuyama
AH1643	*Bloxamia* sp.	Driftwood	JAPAN, Fuushima, Nakoso, Nakoso coast
AH1468	*Bloxamia* sp	Xylarialean	JAPAN, Gunma, Aduma, Nagano, Kawaharayu, around Gunma prefacture rode route 375

3.3.1 Morphological descriptions of isolates

Ther results from taxonomical examination are described based on asexual and synasexual morph.

Bloxamia sp. -1

Strain examines: WS18

Diagnosis. Asexual: Characterized by peridium thickness 12.5–25.5 µm, 7–9-layer, basal layer thickness 12.5–24 µm, 5–9-layer, hymenium thickness 32.5–45.5 µm, conidiophores cells 7–22 × 1.5–2.5 µm, conidiogenous cells 24–33 × 1.5–2 µm, conidia 2.5–4.1 × 1.5 –1.8 µm. Synasexual: conidiophores 3.5–7 µm, 1–2 cells, conidiogenous cells 13–18.5(–22.5) µm length 3–4 µm width at base, 2–2.5 µm width at apex, conidia 2.5–3.5 × 1.5–2 µm.

Bloxamia sp. -2

Strain examines: WS29

Diagnosis. Asexual: Characterized by peridium thickness 8–10.5 µm, 3–4-layer, basal layer thickness 6.5–9 µm, 4–5-layer, hymenium thickness 30.5–36 µm, conidiophores cell 6.5–14 × 2–2.5 µm, conidiogenous cells 23–30.5 × 2–3 µm, conidia 3–4 × 1.6–1.7 µm.

Bloxamia sp. -3

Strain examines: WS30

Diagnosis. Asexual: Characterized by Peridium thickness 9.5–20 µm, 5–6-layer, basal layer thickness 11–18.5 µm, 7–8-layer, hymenium thickness 29–37.5 µm, conidiophores cells 8.5–16.5 × 2–2.5 µm, conidiogenous cells 22.5–25.5 × 2–2.5 µm, conidia 2.5–3.2 × 1.4 –1.7 µm. Synasexual: conidiophores 5 µm, 1–2 cells, conidiogenous cells 10.5–16 µm length, 3–4.8 µm width at base 2–2.5 µm width at apex, conidia 2.5–4 × 1.5–2 µm

Bloxamia sp. -4

Strain examines: WS31

Diagnosis. Asexual: Characterized by peridium thickness 8–12.5 µm, 3–6-layer, basal layer thickness 9–14.5 µm, 3–5-layer, hymenium thickness 20–32.5 µm, conidiophores cells 7–9.6 × 2–

3 µm, conidiogenous cells 17.5–26 × 1.5–2.5 µm, conidia 2.5–4 × 1.5 –1.7 µm. Synasexsual: conidiophores 8–13.5 µm, 3–4 cells, conidiogenous cells 9–16 × 2.5–4 µm. conidia 2.5–4 × 1.5–2 µm

Asexual conida observation showed strain WS29 and WS31 shared the same feature. Whereas WS30 and WS18 have varieties size. The size of conidia somehow was smaller or bigger. Anamorph of *Bi. discedens* were also observed in previous study (Hosoya and Zhao, 2016), with conidiogenous cells (14–)18–23×2 µm and Conidia 2–3×2–2.5 µm.

Synasexual morph was observed from WS18, WS30, and WS31, and it was lack synasexual morph in WS29. Hence, we could only compare WS18, WS30, and WS31 with previous study (Hosoya and Zhao, 2011), characterized by Conidiophores 1–2 cells, Conidiogenous cells, 3–4 µm width at base, 2 µm width at apex

3.3.2 Phylogenetic analyses

BLAST results using ITS showed each strains were identified as *Bloxamia* and *B. discedens* with 99-100% similiarity (Table 5). All strains were related to *Bloxamia* strain obtained from Japan (TNS: F24589 and F37025).

Table 5. Identification of the *Bloxamia* and its allies using BLAST.

Sample no.	Species	Closest match from GenBank
WS18	*Bloxamia* sp.	*Bloxamia* sp. (TNS: F24589, LC169493) 486/489(99%)
WS29t	*Bloxamia* sp.	*Bisporella discedens* (TNS: F37025, LC169492) 546/546(100%)
WS30	*Bloxamia* sp.	*Bloxamia* sp. (TNS: F24589, LC169493) 439/445(99%)
WS31	*Bloxamia* sp.	*Bloxamia* sp. (TNS: F24589, LC169493) 545/548(99%)
AH1468	*Bloxamia* sp.	*Bloxamia* sp. (TNS: F24589, LC169493) 496/501(99%)
AH1643	*Bloxamia* sp.	*Bloxamia* sp. (TNS: F24589, LC169493) 529/534(99%)

Phylogenetic tree was shown in Figure 10. All isolated strains were grouped with *Bloxamia* type species *Bl. truncate*. Strong supported clade was also shown in the large *Calycina sensu* Baral (96). In addition, *C. claroflava* were closely related to *Bloxamia* clade.

3.4 Discussion

This study has provided genetic information of the new six strains of *B. discedens* and *Bl. truncate* from xylarialean fungi, using fungal barcode ITS. The previous study treated *Bi. discedens* and *C. claroflava* were treated to be distinct species (Ekanayaka et al., 2019; Guatimosin et al., 2016). The present study showed a relationship among *Bi. discedens, Bl. truncate,* and *Calycina claroflava* are strongly supported in large *sensu* Baral.

The five fungi grow on xylarialean fungi and only one fungus grows on driftwood. These fungi were collected from Fukushima, Ibaraki, Kanagawa, Tokyo, and Gunma. Morphologically, the characteristic of isolated *Bloxamia* sp. in this study was similar with type species of *Bl. truncate.* However, there were some varieties on conidial size measurement in asexual morph, because the conidial size of WS30 was smaller than others. In this study, some strains of synasexual morph were also observed, except for WS29. In the previous study, this morph was sometimes present or detected in the morphological observation (Johnston, 1988; Hosoya and Zhao, 2011; Ekanayaka et

al., 2019), while this structure was not mentioned in another study (Baral et al., 2013). Thus, we could just compare our strains with reported strains in Japan. Our synasexual strains were similar to the characteristics of synasexsual strain in Japan (Hosoya and Zhao, 2011), only conidia of WS30 was smaller than the entire strains. The phylogenetic analysis resulted strongly supported clade among our six strains. This clade was grouped with *Bl. truncate* type species of *Bloxamia*. We consider the anamorph of *B. discedens* is identic with *Bl. truncate* as shown in Figure 10.

The phylogenetic tree also showed a large clade of *Calycina sensu* Baral that consists of *Bloxamia truncate/B. discedens* clade. However, anamorph of *Calycina claroflava* has not been discussed in previous study (Baral et al., 2013). Although, *Bisporella discedens* have transferred in genus *Calycina*, some researchers still have sustained *B. discedens* or *B. claroflava* and treated these species differently until now (Zhuang et al., 2017; Ekanayaka et al., 2019). Thus, in this study, we only concluded and suggested that *Bl. truncate* are closely related to *Bi. claroflava* and *Calycina* clade in large *sensu* of Baral (2013).

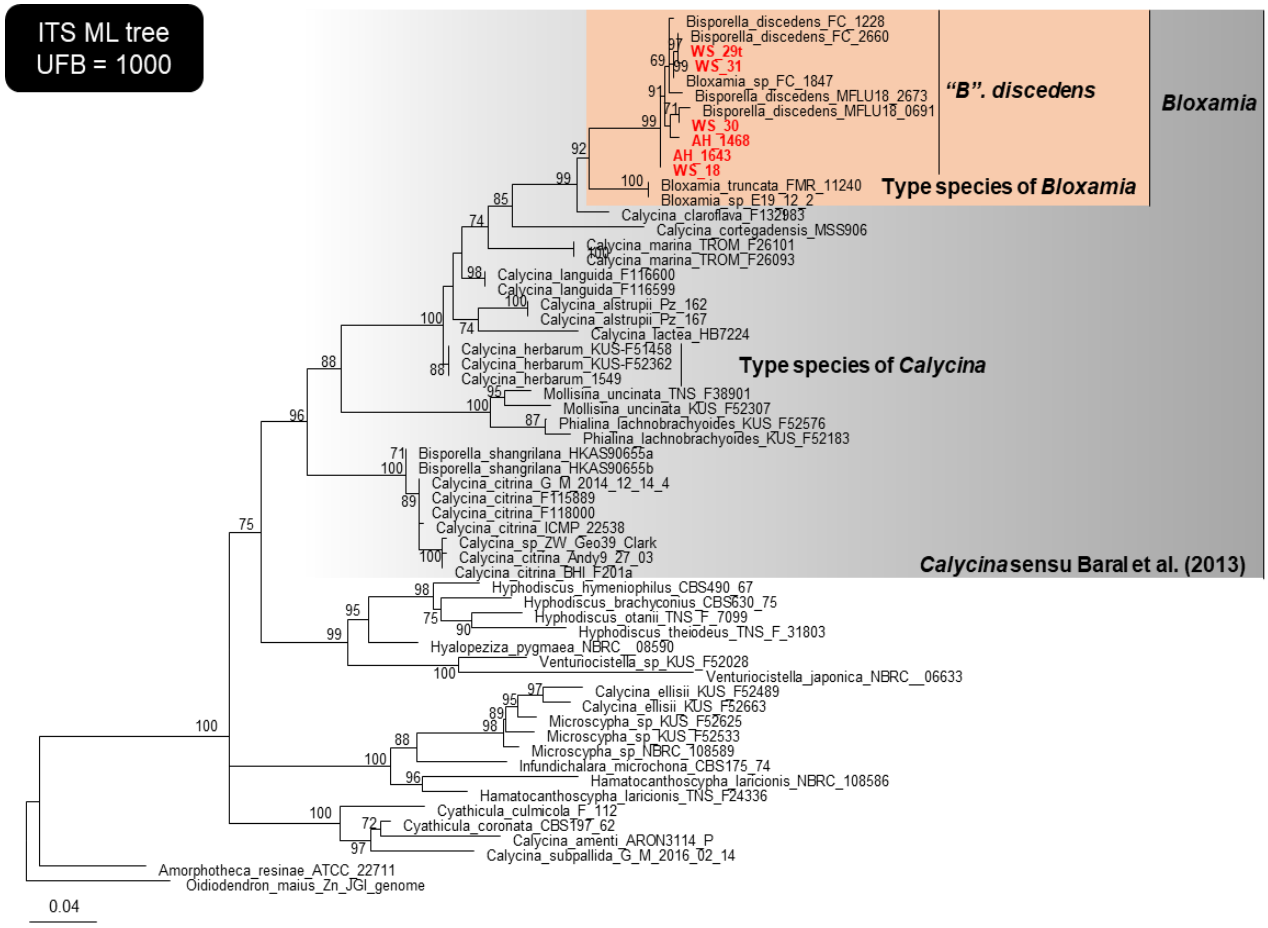

Figure 10. Maximum-likelihood (ML) tree of *Bloxamia* and its allies. Species relationship of *Bloxamia, B. discedens,* and *Calycina*

CHAPTER 4.
GENERAL DISCUSSION

This study proved that sporocarp is one of the habitats of many fungi. The results of this study answered the hypothesis of this study. We enumerated forty-four representative species belonging to Ascomycota and Basidiomycota. Fungal communities that are more diverse have been shown compared with previous studies that performed the same methods (Buzzini et al., 2005; Pacioni et al., 2007; Perlinska-lenart et al., 2020; Dimkpa and Orikoha, 2021) and increased the record of fungicolous fungi, especially in Japan. This fungal community in sporocarp of xylarialean was dominated by Hypocreales and Eurotiales that had previously been recorded in truffle-inhabiting fungi and mushroom-inhabiting fungi (Pacioni et al., 2007; Perlinska-lenart et al., 2020; Dimkpa and Orikoha, 2021).

In this study, some strains were identified using ITS or LSU only due to being able to amplify one of those regions. As designated to be the region of the official gene for fungal barcoding marker,

ITS sequence accomplished to identify most of the strains in this study at the species level, except for *Penicillium, Talaromyces,* and *Acremonium.* It is caused by ITS limitation for some genera such as *Penicillium* and *Talaromyces* that these genera are very useful for pharmaceutical purposes, nonetheless the ITS region does not well identify (Schoch et al., 2012; Seifert et al., 2007; Samson et al., 2014). *Acremonium* sp. has only 94% similarity with *A. charticola* with nine gaps in ITS regions. Subsequently, we consider these strains probably belong to different species.

Morphological identification alone was not enough to identify fungi for species level. In this study, the noteworthy species were identified based on morphology and molecular identification, e.g. "*Acrodontium*" *salmoneum*, *Acrodontium crateriforme*, and *Simplicillium sympodiophorum*. In the previous study, the taxonomy of the genus *Acrodontium* failed to classify members of this genus due to morphological identification alone. "*Acrodontium" salmoneum* is problematic species in the genus *Acrodontium* that is still unclassified at the genus level Here, we re-evaluated the taxonomy of *A. salmoneum* by using molecular phylogenetic analysis using ITS and LSU sequences, to present related

species of *A. salmoneum*. The result showed that *A. salmoneum* are closely related to the family Sarocladiaceae with *Sarocladium* and *Parasarocladium* as member of this family. However, we could not determine which genus *A. salmoneum* belongs to because the ex-holotype of this species is unavailable at GenBank. In this study, we consider *A. crateriforme* as cryptic species due to its genetically distinctness from other strains. This fungus has known associated with many different substrates. Another noteworthy species is *Simplicillium sympodiophorum.* They were reported to have different morphological features such as sympodial features from previous studies. Possibility happens due to a different habitat, while strains of this study were obtained from sporocarp of fungi, in the previous study the strains were obtained from soil. However, morphological identification alone could not enough to identify fungi at speciel level. Combination of molecular identification and morphological identification will perform better identification. We asserted that ITS and LSU sequences determined an important role for the whole isolated SCIF in this study and suggested using additional protein-coding genes to identify problematic strains for future study.

Molecular identification could uncover cryptic species with genetic diversity. Genetic diversity can affect the physiological characteristic of fungi from an applied microbiology perspective (Feckler et al., 2014). It encourages the applied scientist to conserve this genetic diversity. Some cryptic species such as *Simplicillium* and *Acrodontium* were reported to produce secondary metabolites in the previous study. Probably, these cryptic species in this study can produce different secondary metabolites from reported studies. Taxonomical problems related to dual nomenclature are still progressing. In this study, the relationship between *B. discedens* and *Bl. truncate* is strongly supported. We emphasize that both represent one species. Although the relationship between sexual and asexual was solved in this study.

In conclusion, the enumeration of fungi needs proper identification. We affirm ITS and LSU genes help the identification process disregarding some fungi include cryptic species. Molecular and morphological approach are strongly recommended for identification because morphological identification alone could not demonstrate well identification. This enumeration also helps to re-evaluate taxonomical problems in previous studies. These findings also leave a clue for future research

related to applied mycology. The community of SCIF from xylarialean fungi should be examined in detail and the experiment should be carried out not only for the sporocarp of xylarialean but also for other sporocarps.

ACKNOWLEDGEMENT

I wish to send my gratitude and acknowledge to my supervisor Prof. Moriya Ohkuma, Prof. Takeshi Nakayama, Prof. Shinya Takahashi, Prof. Masanobu Kawachi, Prof. Tsuyoshi Hosoya, Dr. Akira Hashimoto, and all professors belong to the T-LSI doctoral program for the big opportunity to study under their guidance. My research projects would not have been possible without their valuable comments and inspiration throughout my doctoral degree.

For financial assistance, the biggest praise to the University of Tsukuba and T-LSI for recommending me to get Tsukuba Scholarship during my second year of the doctoral degree and SGU MEXT scholarship Japanese government during my last year of the doctoral degree. I do grateful for this help and allow me to study for a doctoral degree in Japan with many experiences.

Many thanks to my father Moh. Anshori M. Ali, my mother Masrukah, my sisters Jayarani Fatimah Puteri and Dhama Surayya for unlimited biggest support and unconditional love until I reach this stage.

My friends Suni Rai, Ingyn Khin, Mikiko Hanawa, Annisa Sari, Annisa Krama and Rosalina for motivating me during my research.

REFERENCES

Altschul, S.F.; Madden, T.L.; Schaffer, A.A.; Zhang, J.; Zhang, Z.; Miller, W.; Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic. Acids Res. 25*, 3389–3402. https://doi.org/10.1093/nar/25.17.3389

Arastehfar, A., Gabaldon, T., Garcia-Rubio, R., Jenks, J. D., Hoenigl, M., Salzer, H. J. F., . . . Perlin, D. S. (2020). Drug-Resistant Fungi: An Emerging Challenge Threatening Our Limited Antifungal Armamentarium. *Antibiotics (Basel), 9*(12). doi:10.3390/antibiotics9120877

Arastehfar, A., de Almeida Junior, J. N., Perlin, D. S., Ilkit, M., Boekhout, T., & Colombo, A. L. (2021). Multidrug-resistant Trichosporon species: underestimated fungal pathogens posing imminent threats in clinical settings. *Crit Rev Microbiol, 47*(6), 679-698. doi:10.1080/1040841X.2021.1921695

Avis, T. J., and Belanger, R. R. (2002). Mechanisms and means of detection of biocontrol activity of Pseudozyma yeasts against plant-pathogenic fungi. *FEMS Yeast Research. 2.* 5-8.

Baral, H. O., and Krieglsteiner, L. (2013). Hymenoscyphus subcarneus, a little known bryicolous discomycete found in the Białowieża National Park. *Acta Mycologica, 41*(1), 11-20. doi:10.5586/am.2006.003

Barnett, H. L., & Lilly, V. G. (1962). A Destructive Mycoparasite, Gliocladium Roseum. *Mycologia, 54*(1), 72-77. doi:10.1080/00275514.1962.12024980

Becker, K., & Stadler, M. (2021). Recent progress in biodiversity research on the Xylariales and their secondary metabolism. *J Antibiot (Tokyo), 74*(1), 1-23. doi:10.1038/s41429-020-00376-0

Bensch, K.; Braun, U.; Groenewald, J.Z.; Crous, P.W. (2012). The genus *Cladosporium*. *Stud. Mycol.* *72*, 1–401. https://doi.org/10.3114/sim0003

Buzzini, P., Gasparetti, C., Turchetti, B., Cramarossa, M. R., Vaughan-Martini, A., Martini, A., . . . Forti, L. (2005). Production of volatile organic compounds (VOCs) by yeasts isolated from the

ascocarps of black (Tuber melanosporum Vitt.) and white (Tuber magnatum Pico) truffles. *Arch Microbiol, 184*(3), 187-193. doi:10.1007/s00203-005-0043-y

Chatterton, S., & Punja, Z. K. (2009). Chitinase and beta-1,3-glucanase enzyme production by the mycoparasite Clonostachys rosea f. catenulata against fungal plant pathogens. *Can J Microbiol, 55*(4), 356-367. doi:10.1139/w08-156

Chen, W. H., Liu, C., Han, Y. F., Liang, J. D., Tian, W. Y., and Liang, Z. Q. (2019). Three novel insect-associated species of Simplicillium (Cordycipitaceae, Hypocreales) from Southwest China. *MycoKeys, 58,* 83-102. doi:10.3897/mycokeys.58.37176

Cheng, J. T., Yu, J. H., Sun, C. F., Cao, F., Ying, Y. M., Zhan, Z. J., Li, W. J., Chen, X. A., Zhao, Q. W., Li, Y. Q., Gan, L. S., Mao, X. M. (2021). A Cell Factory of a Fungicolous Fungus *Calcarisporium arbuscula* for Efficient Production of Natural Products. *ACS Synth Biol. 10(4).* 698-706. doi: 10.1021/acssynbio.0c00371

Collado, J., Platas, G., Gonzalez, I., and Pelaez, F. (1999). Geographical and seasonal influences on the distribution of fungal endophytes in *Quercus ilex*. *New Phytol*. 144, 525–532. doi:10.1046/j.1469-8137.1999.00533.x

de Gusmáo, N. B., Kaouadji, M., Seigle-Murandi, F., Steiman, R., and Thomasson, F. (1993). Acrodontiolamide, a Chlorinated Fungal Metabolite from *Acrodontium salmoneum*. *Spectros. Lett.* 26, 1373–1380. doi:10.1080/00387019308011616

de Hoog, G.S. (1972). The genera *Beauveria*, *Isaria*, *Tritirachium*, and *Acrodontium* gen. nov. *Stud. Mycol*. *1*, 1–41.

Dennis, R. W. G. (1981). *British Ascomycetes*. Vaduz: J. Cramer

Dissanayake, A. J., Purahong, W., Wubet, T., Hyde, K. D., Zhang, W., Xu, H., . . . Yan, J. (2018). Direct comparison of culture-dependent and culture-independent molecular approaches reveal the diversity of fungal endophytic communities in stems of grapevine (Vitis vinifera). *Fungal Diversity, 90*(1), 85-107. doi:10.1007/s13225-018-0399-3

Ekanayaka, A. H., Hyde, K. D., Gentekaki, E., McKenzie, E. H. C., Zhao, Q., Bulgakov, T. S., and Camporesi, E. (2019). Preliminary classification of Leotiomycetes. *Mycosphere, 10*(1), 310-489. doi:10.5943/mycosphere/10/1/7

El-Ganiny, A. M., Yossef, N. E., & Kamel, H. H. (2021). Prevalence and antifungal drug resistance of nosocomial Candida species isolated from two university hospitals in Egypt. *Curr Med Mycol, 7*(1), 31-37. doi:10.18502/cmm.7.1.6181

Ellis, M.B.; Ellis, J.P. (1988). *Microfungi on Miscellaneous Substrates (Enlarged edition)*; The Richmond Publishing: England, UK.

Feckler, A., Zubrod, J. P., Thielsch, A., Schwenk, K., Schulz, R., Bundschuh, M., & Frid, C. (2014). Cryptic species diversity: an overlooked factor in environmental management? *Journal of Applied Ecology, 51*(4), 958-967. doi:10.1111/1365-2664.12246

Feng, D., Tan, L., Qiu, L., Ju, F., Kuang, Q. X., Chen, J. F., Li, X. N., Gu, Y. C., Guo, D. L., Deng, Y. (2020). Three new polyketides produced by Penicillium crustosum, a mycoparasitic fungus from Ophiocordyceps sinensis. *Phytochemistry Letters. 36.* 150-155. doi: 10.1016/j.phytol.2020.02.008

Gams, W. (1975). *Chephalosporium*-like hyphomycetes: Some tropical species. *Trans. Br. Mycol. Soc*, *64*, 389–404.

Gams, W.; Zare, R. (2001) A revision of *Verticillium* sect. *Prostrata*. III. Generic classification. *Nova Hedwigia*, *72*, 329–337. https://doi.org/10.1127/nova.hedwigia/72/2001/329

Gams, W., Diederich, P., and Poldmaa, K. (2004). Fungicolous fungi. *Academic Press*, 343-392. doi:10.1016/B978-012509551-8/50020-9.

Gams, W. (2006). Biodiversity of soil-inhabiting fungi. *Biodiversity and Conservation*, *16*(1), 69-72. doi:10.1007/s10531-006-9121-y

Gilman, J. C., and Tiffany, L. H. (1952). Fungicolous Fungi from Iowa. *Academy of Science, 59*(1), 99-110.

Giraldo, A., Gene, J., Sutton, D. A., Wiederhold, N., & Guarro, J. (2017). New acremonium-like species in the Bionectriaceae and Plectosphaerellaceae. *Mycological Progress, 16*(4), 349-368. doi:10.1007/s11557-017-1271-7

Glenn, A. E., Bacon, C. W., Price, R., and Hanlin, R. T. (1996). Molecular phylogeny of *Acremonium* and its taxonomic implications. *Mycologia.* 88. doi:10.2307/3760878

Goncalves, M. F. M., Vicente, T. F. L., Esteves, A. C., Alves, A. (2020). Novel halotolerant species of Emericellopsis and Parasarocladium associated with macroalgae in an estuarine environment. *Mycologia. 112(1).* 154-171. doi: 10.1080/00275514.2019.1677448

Grafenhan, T., Schroers, H. J., Nirenberg, H. I., and Seifert, K. A. (2011). An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora*, *Acremonium*, *Fusarium*, *Stilbella*, and *Volutella*. *Stud Mycol.* 68, 79–113. doi:10.3114/sim.2011.68.04

Greslebin, A. G., and Rajchenberg, M. (2003). Diversity of Corticiaceae sens. lat. in Patagonia, Southern Argentina. *N. Z. J. Bot*. 41, 437–446. doi:10.1080/0028825x.2003.9512861

Grum-Grzhimaylo, A. A., Georgieva, M. L., Debets, A. J., and Bilanenko, E. N. (2013). Are alkalitolerant fungi of the Emericellopsis lineage (Bionectriaceae) of marine origin? *IMA Fungus, 4*(2), 213-228. doi:10.5598/imafungus.2013.04.02.07

Hao, Y., Chen, S. Y., Blanchette, R. A., and Liu, X. Z. (2010). *Sistotrema brinkmannii*, a psychrotolerant fungus from Antarctic soil. *Mycosystema*, 15, 864–868.

Hattori, T. (2005). Diversity of wood-inhabiting polypores in temperate forests with different vegetation types in Japan. *Fungal Divers.* 18, 73–88.

Hawksworth, D. L. (1981). A survey of the fungicolous conidial fungi In G. T. Cole & B. Kendrick (Eds.). *Biology of Conidial Fungi Vol. 1. , London, UK: Academic Press*

Hawksworth, D. L., & Lucking, R. (2017). Fungal Diversity Revisited: 2.2 to 3.8 Million Species. *Microbiol Spectr, 5*(4). doi:10.1128/microbiolspec.FUNK-0052-2016

He, P., He, X., and Zhang, C. (2006). Interactions between Psilocybe fasciata and its companion fungus *Acremonium strictum*. *Ecol. Res.* 21, 387–395. doi:10.1007/s11284-005-0123-0

Helaly, S. E., Thongbai, B., & Stadler, M. (2018). Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascomycete order Xylariales. *Nat Prod Rep*, *35*(9), 992-1014. doi:10.1039/c8np00010g

Hoch, H. C., and Provvidenti, R. (1979). Mycoparasitic relationship: cytology of the *Sphaerotheca fuligena-Tilletiospis* sp. interaction. *Phytopathology, 69*, 359-362.

Hosoya, T., Han, J.G., Saito, Y., Zhao Y.J., Kakishima, M., and Shin, H. D. (2011). Enumeration of Remarkable Japanese Discomycetes (5): First Records of one Operculate and two Inoperculate Discomycetes in Japan. *Bulletin of the National Museum of Nature and Science. Series B, Botany, 37*(4).

Hosoya, T., and Zhao, Y. J. (2016). Enumeration of Remarkable Japanese Discomycetes (10): Two Helotiales and a Helotialean Anamorph New to Japan. *Bulletin of the National Museum of Nature and Science Series B (Botany), 42*, 113–119.

Iqbal, J., Siegrist, J. A., Nelson, J. A., & McCulley, R. L. (2012). Fungal endophyte infection increases carbon sequestration potential of southeastern USA tall fescue stands. *Soil Biology and Biochemistry, 44*(1), 81-92. doi:10.1016/j.soilbio.2011.09.010

Izumitsu, K., Hatoh, K., Sumita, T., Kitade, Y., Morita, A., Gafur, A.,Ohta, A., Kawai, M., Yamanaka, T., Tanaka, C. (2012). Rapid and simple preparation of mushroom DNA directly from colonies and fruiting bodies for PCR. *Mycoscience*, *53*, 396–401. https://doi.org/10.1007/s10267-012-0182-3

Jaber, S., Mercier, A., Knio, K., Brun, S., and Kambris, Z. (2016). Isolation of fungi from dead arthropods and identification of a new mosquito natural pathogen. *Parasit. Vectors.* 9, 491. doi:10.1186/s13071-016-1763-3

Jain, P., & Sharma, P. (2016). Isolation and preliminary screening of endophytic fungi of Ricinus communis for their antimicrobial potential. *Journal of Microbiology, Biotechnology and Food Sciences, 05*(03), 230-233. doi:10.15414/jmbfs.2015/16.5.3.230-233

Jaschke, D., Dugassa-Gobena, D., Karlovsky, P., Vidal, S., & Ludwig-Muller, J. (2010). Suppression of clubroot (Plasmodiophora brassicae) development in Arabidopsis thaliana by the endophytic fungus Acremonium alternatum. *Plant Pathology, 59*(1), 100-111. doi:10.1111/j.1365-3059.2009.02199.x

Jeffries, P. (1995). Biology and ecology of mycoparasitism. *Can J Bot 73*:1284–1290. doi: 10.1139/B95-389

Jones, E. E., and Stewart, A. (2000). Selection of mycoparasites of sclerotia of Sclerotinia sclerotiorum isolated from New Zealand soils. *New Zealand Journal of Crop and Horticultural Science, 28*(2), 105-114. doi:10.1080/01140671.2000.9514130

Justo, A., Miettinen, O., Floudas, D., Ortiz-Santana, B., Sjokvist, E., Lindner, D., Nakasone, K., Niemelä, T., Larsson, K. H., Ryvardeng, L., and Hibbett, D. S. (2017). A revised family-level classification of the Polyporales (Basidiomycota). *Fungal Biol.* 121, 798–824. doi:10.1016/j.funbio.2017.05.010

Katoh, K.; Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol. 30,* 772–780. https://doi.org/10.1093/molbev/mst010

Kern, F. D. (1910). Prediction of Relationships Among Some Parasitic Fungi. *American Association for the Advancement of Science*, *31*(804), 830-833.

Kiss, L. (2003). A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. *Pest Management Science. 59(4).* 475-483. doi: 10.1002/ps.689

Korf, R. P., and Carpenter, S. E. (1974). Bisporella, a generic name for Helotium citrinum and its allies, and the generic names Calycella and Calycina. *Mycotaxon, 1*, 51–62.

Koukol, O. (2010). What do we know about *Acrodontium crateriforme*? *Mykologické Listy.* 111, 12–18.

Kozlova, E. V., and Mazina, S. E. (2020). Biodiversity of Fungi in the photic and aphotic zones of Montenegro caves. *Aerobiologia*. 36, 589–604. doi:10.1007/s10453-020-09654-8

Kumar, S.; Stecher, G.; Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol. 33*, 1870–1874. https://doi.org/10.1093/molbev/msw054

Liang, X., Nong, X. H., Huang, Z. H., & Qi, S. H. (2017). Antifungal and Antiviral Cyclic Peptides from the Deep-Sea-Derived Fungus Simplicillium obclavatum EIODSF 020. *J Agric Food Chem, 65*(25), 5114-5121. doi:10.1021/acs.jafc.7b01238

Læssoe, T.; Peterson, J.H. (2019). *Fungi of Temperate Europe*; Princeton University Press: Princeton, US.

Le Calvez, T.; Burgaud, G.; Mahe, S.; Barbier, G.; Vandenkoornhuyse, P. (2020) Fungal diversity in deep-sea hydrothermal ecosystems. *Appl. Environ. Microbiol. 75*, 6415–6421. https://doi.org/10.1128/AEM.00653-09

Liang C, Yang J, Kovács GM, Szentiványi O, Li B, Xu X, et al. (2007) Genetic diversity of Ampelomyces mycoparasites isolated from different powdery mildew species in China inferred from analyses of rDNA ITS sequences. *Fungal Diversity; 24*:225–240.

Le Dang, Q., Shin, T. S., Park, M. S., Choi, Y. H., Choi, G. J., Jang, K. S., . . . Kim, J. C. (2014). Antimicrobial activities of novel mannosyl lipids isolated from the biocontrol fungus Simplicillium lamellicola BCP against phytopathogenic bacteria. *J Agric Food Chem, 62*(15), 3363-3370. doi:10.1021/jf500361e

Loeffler, J., & Stevens, D. A. (2003). Antifungal drug resistance. *Clin Infect Dis, 36*(Suppl 1), S31-41. doi:10.1086/344658

Lucheta, A. R., Cannavan Fde, S., Roesch, L. F., Tsai, S. M., and Kuramae, E. E. (2016). Fungal community assembly in the Amazonian dark earth. *Microb. Ecol.* 71, 962–973. doi:10.1007/s00248-015-0703-7

Lutwick, L. I., Galgiani, J. N., Johnson, R. H., & Stevens, D. A. (1976). Visceral fungal infections due to petriellidium boydii (allescheria boydii). *The American Journal of Medicine, 61*(5), 632-640. doi:10.1016/0002-9343(76)90141-8

Machida, K., Trifonov, L. S., Ayer, W. A., Lu, Z. X., Laroche, A., Huang, H. C., . . . Zantige, J. L. (2001). 3(2H)-Benzofuranones and chromanes from liquid cultures of the mycoparasitic fungus Coniothyrium minitans. *Phytochemistry, 58*(1), 173-177. doi:10.1016/s0031-9422(01)00129-7

Maekawa, N. (1993). Taxonomic study of Japanese Corticiaceae (Aphyllophorales) I. *Rept. Tottori. Mycol. Inst.* 31, 1–149.

Maheshwari, R., Bharadwaj, G., and Bhat, M. K. (2000). Thermophilic fungi: their physiology and enzymes. *Microbiol Mol Biol Rev, 64*(3), 461-488. doi:10.1128/MMBR.64.3.461-488.2000

Maicas, S. (2021). Advance in Wine Fermenetation. *Fermentation, 7(3),* 187-192. 10.3390/fermentation7030187

Manandhar, J. B., Thapliyal, P. N., Cavanaugh, K. J., and Sinclair, J. B. (1987). Interaction between pathogenic and saprobic fungi isolated from soybean roots and seeds. *Mycopathologia.* 98, 69–75.

Markovich, N. A., & Kononova, G. L. (2003). Lytic Enzymes of Trichoderma and Their Role in Plant Defense from Fungal Diseases: A Review. *Applied Biochemistry and Microbiology, 39*(4), 341-351. doi:10.1023/a:1024502431592

Maurice, S., Arnault, G., Norden, J., Botnen, S. S., Miettinen, O., & Kauserud, H. (2021). Fungal sporocarps house diverse and host-specific communities of fungicolous fungi. *ISME J*. doi:10.1038/s41396-020-00862-1

Migliori, G. B., Lange, C., Centis, R., Sotgiu, G., Mutterlein, R., Hoffmann, H., . . . Group, T. S. (2008). Resistance to second-line injectables and treatment outcomes in multidrug-resistant and extensively drug-resistant tuberculosis cases. *Eur Respir J, 31*(6), 1155-1159. doi:10.1183/09031936.00028708

Minh, B.Q.; Schmidt, H.A.; Chernomor, O.; Schrempf, D.; Woodhams, M.D.; von Haeseler, A.; Lanfear, R. (2020). Corrigendum to: IQ-TREE 2: New models and efficient methods for phylogenetic inference in the Genomic Era. *Mol. Biol. Evol. 37*, 1530–1534. https://doi.org/10.1093/molbev/msaa131

Montero, M., Sanz, L., Rey, M., Monte, E., and Llobell, A. (2005). BGN16.3, a novel acidic beta-1,6-

Moncalvo, J.M.; Nilsson, R.H.; Koster, B.; Dunham, S.M.; Bernauer, T.; Matheny, P.B.; Porter, T.M.; Margaritescu, S.; Weiß, M.; Garnica, S.; Danell, E.; Langer, G.; Langer, E.; Larsson, E.; Larsson, K.H.; Vilgalys, R. (2017). The cantharelloid clade: dealing with incongruent gene

trees and phylogenetic reconstruction methods. *Mycologia, 98,* 937–948. https://doi.org/10.1080/15572536.2006.11832623

Morgenstern, I.; Powlowski, J.; Ishmael, N.; Darmond, C.; Marqueteau, S.; Moisan, M.C.; Quenneville, G.; Tsang, A. (2012) A molecular phylogeny of thermophilic fungi. *Fungal Biol. 116*, 489–502. https://doi.org/10.1016/j.funbio.2012.01.010

Moricca, S., Ragazzi, A., Mitchelson, K. R., & Assante, G. (2001). Antagonism of the Two-Needle Pine Stem Rust Fungi Cronartium flaccidum and Peridermium pini by Cladosporium tenuissimum In Vitro and In Planta. *Phytopathology, 91*(5), 457-468. doi:10.1094/PHYTO.2001.91.5.457

Murakami, T., Takada, N., Harada, Y., Okuno, T., & Hashimoto, M. (2007). Stimulation of the biosynthesis of the antibiotics lambertellols by the mycoparasitic fungus Lambertella corni-maris under the acidic conditions produced by its host fungus in vitro. *Biosci Biotechnol Biochem, 71*(5), 1230-1235. doi:10.1271/bbb.60667

Nancy Keller, J. P. a. O. B. (2017). *Fungal Jewels: Secondary Metabolites*.

Nemeth, M. Z., Mizuno, Y., Kobayashi, H., Seress, D., Shishido, N., Kimura, Y., Takamatsu, S., Suzuki, T., Takikawa, Y., Kakutani, K., Matsuda, Y., Kiss, L., Nonomura, T. (2021). Ampelomyces strains isolated from diverse powdery mildew hosts in Japan: Their phylogeny and mycoparasitic activity, including timing and quantifying mycoparasitism of Pseudoidium neolycopersici on tomato. *PLos One. 16(5).* 1444-1464. doi: 10.1371/journal.pone.0251444

Nilsson, R. H., Kristiansson, E., Ryberg, M., Hallenberg, N., & Larsson, K. H. (2008). Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evol Bioinform Online, 4,* 193-201. doi:10.4137/ebo.s653

Nonaka, K., Kaifuchi, S., Ōmura, S., and Masuma, R. (2013). Five new *Simplicillium* species (Cordycipitaceae) from soils in Tokyo, Japan. *Mycoscience*. 54, 42–53. doi:10.1016/j.myc.2012.07.002

Obase, K., Douhan, G. W., Matsuda, Y., & Smith, M. E. (2014). Culturable fungal assemblages growing within Cenococcum sclerotia in forest soils. *FEMS Microbiol Ecol, 90*(3), 708-717. doi:10.1111/1574-6941.12428

Op De Beeck, M., Lievens, B., Busschaert, P., Declerck, S., Vangronsveld, J., and Colpaert, J. V. (2014). Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies. *PLoS One, 9*(6), e97629. doi:10.1371/journal.pone.0097629

Pacioni, G., Leonardi, M., Aimola, P., Ragnelli, A. M., Rubini, A., and Paolocci, F. (2007). Isolation and characterization of some mycelia inhabiting Tuber ascomata. *Mycological Research, 111*(Pt 12), 1450-1460. doi:10.1016/j.mycres.2007.08.016

Paul, B., & Masih, I. (2000). ITS1 region of the nuclear ribosomal DNA of the mycoparasite Pythium periplocum, its taxonomy, and its comparison with related species. *FEMS Microbiol Lett, 189*(1), 61-65. doi:10.1111/j.1574-6968.2000.tb09206.x

Perlinska-Lenart, U., Pilsyk, S., Gryz, E., Turlo, J., Hilszczanska, D., and Kruszewska, J. S. (2020). Identification of bacteria and fungi inhabiting fruiting bodies of Burgundy truffle (Tuber aestivum Vittad.). *Arch Microbiol, 202*(10), 2727-2738. doi:10.1007/s00203-020-02002-x

Perini, L., Gostincar, C., Anesio, A. M., Williamson, C., Tranter, M., and Gunde-Cimerman, N. (2019). Darkening of the Greenland ice sheet: Fungal Abundance and diversity are associated with algal bloom. *Front. Microbiol.* 10, 557. doi:10.3389/fmicb.2019.00557

Petrini, L.; Petrini, O. (1985). Xylariaceous fungi as endophytes. *Sydowia, 38*, 216–234

Porter, C. L. (1924). Concerning the characteristics of certain fungi as exhibited by their growth in the presence of other fungi. *American Journal of Botany, 11*(3), 168-188.

Poveda, J., Abril-Urias, P., & Escobar, C. (2020). Biological Control of Plant-Parasitic Nematodes by Filamentous Fungi Inducers of Resistance: Trichoderma, Mycorrhizal and Endophytic Fungi. *Front Microbiol, 11*, 992. doi:10.3389/fmicb.2020.00992

Prahl, R. E., Khan, S., Deo, R. C. (2021). The role of internal transcribed spacer 2 secondary structures in classifying mycoparasitic Ampelomyces. *PLoS One. 16(6).* e0253772. 10.1371/journal.pone.0253772

Raja, H. A., Miller, A. N., Pearce, C. J., & Oberlies, N. H. (2017). Fungal Identification Using Molecular Tools: A Primer for the Natural Products Research Community. *J Nat Prod, 80*(3), 756-770. doi:10.1021/acs.jnatprod.6b01085

Regoes, R. R., & Bonhoeffer, S. (2006). Emergence of drug-resistant influenza virus: population dynamical considerations. *Science, 312*(5772), 389-391. doi:10.1126/science.1122947

Sablayrolles, J. M. (2009). Control of alcoholic fermentation in winemaking: Current situation and prospect. *Food Research International. 42(4).* 418-424. doi: 0.1016/j.foodres.2008.12.016

Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S. B., Hubka, V., Klaassen, C. H., . . . Frisvad, J. C. (2014). Phylogeny, identification and nomenclature of the genus Aspergillus. *Stud Mycol, 78,* 141-173. doi:10.1016/j.simyco.2014.07.004

Seifert, K. A., Samson, R. A., Dewaard, J. R., Houbraken, J., Levesque, C. A., Moncalvo, J. M., . . . Hebert, P. D. (2007). Prospects for fungus identification using CO1 DNA barcodes, with Penicillium as a test case. *Proc Natl Acad Sci U S A, 104*(10), 3901-3906. doi:10.1073/pnas.0611691104

Seifert, K. A., & Gams, W. (2011). The genera of Hyphomycetes - 2011 update. *Persoonia, 27*, 119-129. doi:10.3767/003158511X617435

Serradilla, M. J., Villalobos, M. D., Hernandez, A., Martin, A., Lozano, M., and Cordoba, M. D. (2013). Study of microbiological quality of controlled atmosphere packaged 'Ambrunes' sweet cherries and subsequent shelf-life. *Int. J. Food Microbiol.* 166, 85–92. doi:10.1016/j.ijfoodmicro.2013.06.006

Shibata, S., Shoji, J., Ohta, A., & Watanabe, M. (1957). Metabolic products of fungi. XI. Some observation on the occurrence of skyrin and rugulosin in mold metabolites, with a reference to

structural relationship between penicilliopsin and skyrin. *Pharm Bull, 5*(4), 380-382. doi:10.1248/cpb1953.5.380

Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Mol Ecol, 21*(8), 1794-1805. doi:10.1111/j.1365-294X.2012.05538.x

Sishuba, A., Leboko, J., Ateba, C. N., & Manganyi, M. C. (2021). First Report: Diversity of Endophytic fungi Possessing Antifungal Activity Isolated from Native Kougoed (Sceletium tortuosum L.). *Mycobiology, 49*(1), 89-94. doi:10.1080/12298093.2020.1857009

Sklenář, F.; Jurjević, Z.; Zalar, P.; Frisvad, J.C.; Visagie, C.M.; Kolařík, M.; Houbraken, J.; Chen, A.J.; Yilmaz, N.; Seifert, K.A.; et al. Phylogeny of xerophilic aspergilli (subgenus *Aspergillus*) and taxonomic revision of section *Restricti*. *Stud. Mycol.* **2017**, *88*, 161–236. https://doi.org/10.1016/j.simyco.2017.09.002.

Spiteller, P. (2008). Chemical defence strategies of higher fungi. *Chemistry, 14*(30), 9100-9110. doi:10.1002/chem.200800292

Stadler, M., Læssøe, T., & Vasilyeva, L. (2017). The genusPyrenomyxaand its affinities to other cleistocarpousHypoxyloideaeas inferred from morphological and chemical traits. *Mycologia, 97*(5), 1129-1139. doi:10.1080/15572536.2006.11832761

Steiman, R., Benoit-Guyod J. L, Guiraud, P., and Seigle-Murandi, F. (1995). Evaluation of acrodontiolamide, a chlorinated compound produced by *Acrodontium salmoneum* de Hoog for cytotoxicity and antimicrobial activity. *Pharmazie.* 50, 693–695.

Stejskal, V., Hubert, J., and Kubátová, A. (2017). Associated-food-hazards: storage fungi and mites in poppy, mustard, lettuce and wheat. *Plant Prot. Sci.* 38, 673–680. doi:10.17221/10588-pps

Summerbell, R. C., Gueidan, C., Schroers, H. J., de Hoog, G. S., Starink, M., Rosete, Y. A., Guarro, J., and Scott, J. A. (2011). *Acremonium* phylogenetic overview and revision of *Gliomastix*, *Sarocladium*, and *Trichothecium*. *Stud. Mycol.* 68, 139–162. doi:10.3114/sim.2011.68.06

Sun, J.-Z., Liu, X.-Z., McKenzie, E. H. C., Jeewon, R., Liu, J.-K., Zhang, X.-L., Zhao, G., Hyde, K. D. (2019). Fungicolous fungi: terminology, diversity, distribution, evolution, and species checklist. *Fungal Diversity, 95*(1), 337-430. doi:10.1007/s13225-019-00422-9

Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). Enviromental DNA for Biodiveristy Research and Monitoring. doi:10.1093/oso/9780198767220.001.0001

Tu, J. C. (1980). *Gliocladium virens,* a destructive mycoparasite of *Sclerotinia sclerotium*. *The American Phytopathological Society, 70*(7), 670-674.

Tubaki, K. (1955). Studies on the Japanese hyphomycetes (II) fungicolous group. *Nagaoa***,** *5*, 11–40.

Turbyville, T. J., Wijeratne, E. M., Whitesell, L., Gunatilaka, A. A. (2005). The anticancer activity of the fungal metabolite terrecyclic acid A is associated with modulation of multiple cellular stress response pathways. *Mol Cancer Ther. 4(10).* 1569-1576. doi: 10.1158/1535-7163.MCT-05-0050

Vargas-Gastelum, L., and Riquelme, M. (2020). The Mycobiota of the Deep Sea: What Omics Can Offer. *Life (Basel), 10*(11). doi:10.3390/life10110292

Vasilyeva, L.N.; Rogers, J.D.; Miller, A.N. 2007. Pyrenomycetes of the Great Smoky Mountains National Park. V. *Annulohypoxylon* and *Hypoxylon* (Xylariaceae). *Fungal Divers, 27,* 231–245.

Videira, S.I.R.; Groenewald, J.Z.; Braun, U.; Shin, H.D.; Crous, P.W. (2016). All that glitters is not *Ramularia*. *Stud. Mycol*. *83*, 49–163. https://doi.org/10.1016/j.simyco.2016.06.001

Vilgalys, R.; Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from Several *Cryptococcus* species. *J. Bacteriol. 172*, 4238–4246.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Barbetti, M. J., Li, H., . . . Lorito, M. (2008). A novel role for Trichoderma secondary metabolites in the interactions with plants. *Physiological and Molecular Plant Pathology, 72*(1-3), 80-86. doi:10.1016/j.pmpp.2008.05.005

Vinale, F., Nigro, M., Sivasithamparam, K., Flematti, G., Ghisalberti, E. L., Ruocco, M., . . . Lorito, M. (2013). Harzianic acid: a novel siderophore from Trichoderma harzianum. *FEMS Microbiol Lett, 347*(2), 123-129. doi:10.1111/1574-6968.12231

Waksman, A. S. (1936). Associative and antagonistic effects of microorganisms. I. Historical review of antagonistic relationships. *Soil Sci, 43*, 51-68.

Waksman, S. A. (1947). What Is an Antibiotic or an Antibiotic Substance? *Mycologia, 39*(5). doi:10.2307/3755196

White, T.J.; Bruns, T.; Lee, S.; Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: a guide to methods and applications*; Innis, M.A, Gelfand, D.H., Sninsky J.J., Thomas J.W., Eds: Academic Press: San Diego, US, pp. 315–322.

Whipps, J. M., and Budge, S. P. (1990). Screening for sclerotial mycoparasites of Sclevotinia sclerotiovum. *Mycol. Res, 94*(5), 607-612

Yuan, Z. L., Rao, L. B., Chen, Y. C., Zhang, C. L., and Wu, Y. G. (2011). From pattern to process:

species and functional diversity in fungal endophytes of *Abies beshanzuensis*. *Fungal Biol.* 115, 197–213. doi:10.1016/j.funbio.2010.11.002

Zalar, P.; de Hoog, G.S.; Schroers, H.J.; Frank, J.M.; Gunde-Cimerman, N. 2005. Taxonomy and phylogeny of the xerophilic genus *Wallemia* (Wallemiomycetes and Wallemiales, cl. et ord. nov.). *Antonie van Leeuwenhoek*, *87*, 311–328. https://doi.org/10.1007/s10482-004-6783-x.

Zazerrini, A., and Tosi, L. (1985). Antagonistic activity of fungi isolated from sclerotia of Sclerotinia sclerotiorum. *Plant Pathology, 34,* 415-421.

Zeng, X., Ni, Z., Diao, H., Jiang, K., Hu, C., Shao, L., & Huang, W. (2021). Root Endophytic Fungal Community and Carbon and Nitrogen Stable Isotope Patterns Differ among Bletilla Species (Orchidaceae). *J Fungi (Basel), 7*(2). doi:10.3390/jof7020069

Zhuang, W., Zheng, H., and Ren, F. (2017). Taxonomy of genus Bisporella (Helotiales) in china with seven new species and four new records. *Mycosystema, 36*(4), 401-420. doi:10.13346/j.mycosystema.160193

List of Publications

Original papers

Wasiatus Sa'diyah[1, 2], Akira Hashimoto[2*], Gen Okada[2], & Moriya Ohkuma[1, 2], Notes on Some Interesting Sporocarp-Inhabiting Fungi Isolated from Xylarialean Fungi in Japan. *Diversity.* 13 (11). 574. doi: 10.3390/d13110574

Related articles

N/A