Graduate School of Bioresources
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Ph. D. Thesis

Systematic studies of plant pathogenic Coelomycetes

（植物病原性分生子果不完全菌類の分類学的研究）

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Content

Abstract

Coelomycetes encompass asexual fungi with conidia formation within a cavity lined by fungal or fungal and host tissue. This grouping reflects the pre-molecular reliance on morphological characteristics for fungal classification. Phylogenetic relationships have transformed fungal identification, leading to a shift in traditional fungal taxonomy towards a more objective and reliable fungal systematics. This prompted the change in Fungal Nomenclature from a dual naming system to "One fungus, one name". Following the current taxonomic criteria, this study re-examines plant pathogenic genera under Coelomycetes, aiming to elucidate diversity and update the Japanese mycoflora through multi-locus phylogenetic analysis, morphological, and culture characteristics.

The first part of the study focuses on the genus *Elsinoë* (Elsinoaceae, Myriangiales), known for causing scab, spotted anthracnose, and morphological distortions on various plants, including crops and ornamental plants. The asexual morph of *Elsinoë* is characterized by an acervular coelomycetous fungus with polyphialidic conidiogenous cells, known as *Sphaceloma*. Twenty-three Japanese collections of *Sphaceloma* and *Elsinoë* were re-examined, resulting in the proposal of three novel species: *E. hydrangeae*, *E. tanashiensis*, and *E. sumire*, as well as the establishment of a new combination for *E. akebiae*. Additionally, taxonomic treatment based on herbarium species proposed new combinations for *E. catalpae*, *E. japonicum*, *E. paderiae*, *E. peucedani*, and *E. zelkovae*.

The second part of the study examines the genus *Sphaerulina* (Mycosphaerellaceae, Mycosphaerellales), which causes leaf spot on arboreous plants and occasionally on herbaceous plants, presenting similar symptoms as *Septoria*. Historically, some *Sphaerulina* fungi were classified as *Septoria* based on generic morphological criteria. However, multi-locus phylogeny has delineated *Sphaerulina* from *Septoria* into independent genera. A total of twenty-seven Japanese isolates previously identified as *Septoria* were re-examined, leading to the proposal of seven novel species (*Sph. farfugii, Sph. hydrangeicola, Sph. idesiae, Sph. lapsanastri, Sph.*

miurae, Sph. styracis, and *Sph. viburnicola*), and the transfer of two species (*Sph. duchesnea* & *Sph. nambuana*) from the genus *Septoria*.

The third part discusses the genus *Septoria* (Mycosphaerellaceae, Mycosphaerellales), a widely distributed fungal pathogen linked to leaf spot disease. Despite exhibiting a *Mycosphaerella*-like sexual stage, the name *Mycosphaerella* does not apply to this genus. *Septoria* was traditionally distinguished by the host plant it infects. However, molecular data indicate that multiple species can infect the same host, and some *Septoria* species have a broad host range. A total of twenty-three isolates were examined, and the results show that *Septoria* species isolated from hemp are different from *Septoria cannabis* found on the same host plant in America, leading to the proposal of a novel species, *Septoria cannabicola*.

The last part investigates the genus *Diaporthe* (Diaporthaceae, Diaporthales) from asymptomatic Sakura trees in Japan. Traditionally, traits such as host specificity, disease symptoms, ascomata, and spore shape are used for the identification of new species. However, multi-locus analysis revealed homoplasy in *Diaporthe*. In this part of the study, a total of 31 isolates were examined from *Prunus* s.l. in Japan. Apart from multi-locus analysis, this chapter also uses the coalescent method to assist in species delimitation. This resulted in the identification of two novel species, *D. endoprunicola* and *D. pseudoamygdali*, along with three known species of *Diaporthe* and another three species within *Diaporthe* s.l.

This study demonstrates that an integrated approach comprising phylogenetic analysis, morphology, habitat, and host information has enhanced the understanding of fungal delimitation at the taxonomic level. While molecular analysis is crucial for delineating fungal species, morphological observations continue to be essential for species identification.

要旨

分生子果不完全菌類は、真菌、または真菌と宿主組織で覆われた空洞内に分生子を形成する無性真菌類からなる。このグループ分けは、菌類の大規模分子系統関係が明らかになる以前の形態学的特徴よる分類体系を反映している。菌類の分子系統関係は、真菌の分類を一変させ、これまでの菌類分類学をより客観的で信頼性の高い真菌系統学へと移行させた。これを機に、菌類命名法も有生世代と無性世代にべつべつに種名を認める二重命名法から、1 種の真菌に 1 つの名前を適用する方法、すなわち“One fungus, one name”に変更された。本研究では、現在の分類基準に従い、分生子果不完全菌類のうち植物病原性を持つ属を再調査した。多遺伝子座系統解析、形態学的特徴、培養特性を統合的に用い、多様性の解明と日本の菌類相に関する情報を更新することを目的とする。

第 1 章では、作物や観葉植物などの様々な植物にそうか病や葉の奇形などを引き起こす *Elsinoë* 属（*Elsinoaceae, Myriangiales*）について概観する。*Elsinoë* 属菌の無性時代は、*Sphaceloma* として知られるポリフィアライド型の分生子形成細胞を持つ、分生子層状の分生子形成様式を持つ分生子果不完全菌類である。23 株の日本の *Sphaceloma* と *Elsinoë* 属菌が再調査され、その結果、*E. hydrangeae*、*E. tanashiensis*、*E. sumire* の 3 新種と、*E. akebiae* の新組み合わせが提案された。さらに、標本に基づく分類学的処理により、*E. catalpa*、*E. japonicum*、*E. paderiae*、*E. peucedani*、および *E. zelkovae* の新組み合わせが提案された。

第 2 章では、木本植物やときに草本植物の葉に斑点症状を引き起こす *Sphaerulina* 属（*Mycosphaerellaceae*, *Mycosphaerellales*）について調査した。かつて一部の *Sphaerulina* 属の種は形態学的基準により、*Septoria* 属として分類されていた。しかし、近年になって多遺伝子座分子系統解析により、*Sphaerulina* 属は *Septoria* 属から独立した属に分類された。そこで *Septoria* 属として記録されている計 27 株の日本分離株を再調査し、7 新種（*Sph. Farfugii*、*Sph. hydrangeicola*、*Sph. idesiae*、*Sph. lapsanastri*、*Sph. miurae*、*Sph. styracis*、および

Sph. viburnicola）を提案し、2 種（*Sph. duchesnea* および *Sph. manbuana*）の新組み合わせを提案した。

第 3 章では、*Septoria* 属（*Mycosphaerellaceae*, *Mycosphaerellales*）について述べる。*Septoria* 属は、葉の斑点病に関連して広く分布している病原菌類で、有性時代は mycosphaerella-like である。*Septoria* 属は従来、感染した宿主植物によって区別されていた。しかし、分子系統解析で構築された系統樹のデータによると、複数の種が同じ宿主に感染する可能性があり、実際 *Septoria* 属菌の中には広い宿主範囲を持つものもある。結果、アサから分離された *Septoria* 属菌はアメリカ産の同じ宿主植物から分離された *Septoria cannabis* とは異なることが示され、新種 *Septoria cannabicola* が提案された。

また、日本の無病徴のサクラの樹から分離した *Diaporthe* 属を調査した。従来、新種の同定には、宿主特異性、病徴、子嚢果、胞子形状などの形質が用いられてきたが、多遺伝子座分子系統解析により、*Diaporthe* 属の類別が困難な同質性が明らかとなった。この研究では、日本広義サクラ類から分離された計 31 株を調査した。多遺伝子座分子系統解析とは別に、この章では種の区分を補強するため、合意系統樹を用いた種仮定を用いた。その結果、2 つの新種 *D. endoprunicolo* と *D. pseudoamygdali* が同定され、既知の 3 種の *Diaporthe* 属と 3 種の広義 *Diaporthe* 属菌が同定された。

分生子果不完全菌類は、子嚢菌門の無性時代の分類群の一つである。この研究は、系統解析、形態学的特徴、分布、宿主といった異なる形質からなる総合的な基準により、分類学的の種境界への理解が向上した

Chapter 1 – General Introduction

Coelomycetes is a general term encompassing the asexual stage of both Ascomycota and Basidiomycota. It is characterized by conidia formation within a cavity lined by fungal tissue, host tissue, or both (Grove, 1919; Sutton, 1980; Wijayawardene et al., 2012). This group comprises more than 1000 genera, with over 500 synonyms and 7000 species.

Saccardo (1884) initially used the terms Sphaeropsidearum and Melanconiearum to classify fungi based on their conidiomata. Coelomycetes was described with the following key feature: "Conidia in pycnidia, or room-like locules, pertaining to Sphaeropsidales, the conidia produced from a layer so that the 'conidium bearer' are 'free-standing,' the Melanoconiales and Hypomycetes where the conidia are borne on conidiophore, single or at the most grouped in coremia." Grove (1919) used the term "coelomycetes" and discussed the accommodation of *Phomopsis*, *Phelospora*, and *Phyllosticta*. As fungi imperfecti gained attention from plant pathologists and mycologists for their role as plant pathogens and their ecological significance, the genera within Coelomycetes expanded, and Hypomycetes were rejected from Coelomycetes as they bore conidia on the exterior surface and were not enclosed by fungal or host tissue (Sutton, 1980).

The term Coelomycetes was rejected as a formal taxonomic rank but is still used as a collective term. This is mainly due to DNA sequence-based phylogenetic studies, where DNA sequences were able to link the sexual state to the asexual state of Coelomycetes (Li et al., 2020). This also influenced the dual-naming system of the anamorph and teleomorph of fungi, leading to the discontinuation of the dual-naming system and the establishment of the "One fungus, one name" system.

Brief History and Issue Presence

Traditionally, Coelomycetes occupy various ecological niches, and many plant pathogenic fungi fall under this group. Kendrick (2017) considered it a convenient term, while Taylor (1995) rregarded it as an artificial class of fungi. This artificial class is divided into three orders: Sphaeropsidales with pycnidial conidiomata, Melanconiales with acervular conidiomata, and Pycnothyriales with pycnothyrial conidiomata. These classification systems reflect the grouping prior to the molecular biology era, where fungal morphological characteristics are used for classification.

Prior to the development of molecular methods in the identification of fungi, morphological classification was the principal method of assessment and classification (Wijayawardene et al., 2012). Literature such as Nag Raj's 'The Coleomycetes and Coelomycetous Anamorphs with Appendage Bearing Conidia' (1993) provides examples based on morphological classification. Although morphological identification remains a crucial part of the preliminary identification of fungi, it is partly subjective and subject to human bias (Guarro et al., 1999). Additionally, micromorphological features such as conidiogenous cells, conidia, and conidiophores may depend on nutrient uptake, leading to differences in environmental conditions and artificial media, which indirectly result in difficulties in identification. Currently, the group Coelomycetes includes the families Dothediomycetes, Leotiomycetes, and Sordariomycetes.

In the traditional sense, of taxonomy and nomenclature, the binomial system developed by Carl Linnaeus is used to convey information regarding the characteristics of organisms. In 1981, the International Association of Mycologists (IMA) made some changes and simplified the naming system of fungi, leading to familiar terminology today such as anamorph, teleomorph, and holomorph (Hawksworth, 2011). The usage of molecular phylogenetics has pushed for the abandonment of the dual naming system (Seifert & Rossman, 2010), prompting the transition from the dual naming system to the 'One fungus, one name' policy, officially discontinuing the dual nomenclature of fungi. These changes and the usage of polyphasic approaches in identification lead to the need

to reassess Coelomycetes, as a single genus of Coelomycetes is often associated with multiple teleomorphic genera (Wijayawardene et al., 2012).

A considerable amount of study was conducted to understand the plant pathogenic coelomycetes, especially by using multi-locus analysis along with morphological characteristics, leading to species delimitation and establishment of independent species (Quaedvlieg et al., 2013; Verkley et al., 2013). Apart from that, the poly- and paraphyletic relationship of a genus were also observed and recognized in molecular phylogeny (Gao et al., 2017).

Research Gap, Scope, and Limitation of Study

This study focuses on the plant pathogenic collection of coelomycetes in Japan, which were stored in various herbariums in Japan under the original name, bearing either the anamorphic or teleomorphic form, as most of the specimens used in this study predate the molecular era. Identification was conducted solely based on observations of the host plant, characteristics in artificial media, and microscopic features in their natural habitat.

There is a necessity to update the collection of Japanese fungal collection in herbaria and to assess the current position in phylogeny. Some collections were catalogued under the anamorphic name, while others could be under their teleomorphic name. Accurate naming of a fungus is necessary to prevent the spread of the plant disease as well as for plant quarantine purposes.

In this study, four different genera of coelomycetes fungi are being re-examined for their position in the phylogenetic tree as well as their morphological characteristics. The genus *Elsinoë* is the asexual morph of the genus Sphaceloma, and both names are known to be linked to each other. The genera *Sphaerulina* and Septoria were also included in this study as they are fungi that cause devastating leaf-spot diseases in Japan. Recent

advancements in molecular phylogeny have demarcated *Sphaerulina* from Septoria, leading to the discovery that several *Sphaerulina* species in Japan were catalogued as *Septoria* based on morphological observation. As for the genus *Diaporthe*, which was also recorded as *Phomopsis*, it is still under extensive study as both morphological characteristics and molecular phylogeny are unable to fully resolve the species yet.

Objectives and Outline of Study

Although the molecular phylogeny offers different insight in analytical method that changes the traditional identification method, the molecular technique itself is not sufficient in accurate identification and hence is still used to complement the identification of species.

This study aims to provide an update on the Japanese mycoflora by employing a polyphasic approach, which includes using multi-locus phylogenetic analysis, morphological observation of fungi in natural environments, growth in artificial media, host families, and geographical distribution of fungi.

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Chapter 2 – *Elsinoë*

Summary

Elsinoë are plant pathogenic fungi that causes scabs, spotted anthracnose and some morphological distortions on various plants including woody plant, economically important crops, and ornamental plant. This chapter re-evaluates Japanese *Elsinoë* isolates using morphological and molecular-phylogenetic analyses of the internal transcriber spacer region (ITS), large subunit gene (LSU), RNA polymerase II subunit (rpb2), and Translation elongation factor 1-alpha (tef). The Japanese isolates formed four clades, revealing three novel species: *Elsinoë hydrangeae*, *E. sumire*, and *E. tanashiensis*. Furthermore, *Sphaceloma akebiae* was transferred to the genus *Elsinoë* based on molecular and morphological feature. This study addresses the lack of contemporary taxonomic scrutiny for *Elsinoë* species in Japan.

Keywords: new combination, new species, phylogeny, taxonomy, Sphaceloma

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INTRODUCTION

The genus *Elsinoë* (Myriangiales, Ascomycota) encompasses of plant pathogenic fungi that causes disease on many plants, including crops, ornamental plants, and even woody plants. The symptoms of the disease caused by *Elsinoë* species can be seen by scabs that are often exhibited as raised, cork-like necrotic lesions on leaves and stems (Fan et al., 2017; Marin-Felix et al., 2019). This genus was established by Raciborski (Raciborski, 1900) with the type species *Elsinoë canavaliae* Racib. Under the current code, the International Code of Nomenclature for Algae, Fungi, and Plants (Turland et al., 2018), the holomorphic name was decided to propose the generic name *Elsinoë* Racib. as a protection name over the generic name used for asexual morphs, *Sphaceloma* de Bary (Crous et al., 2021; Rossman et al., 2015, 2016; Wijayawardene et al., 2014) .

Scab is widely used as a disease name on leaves. Jenkins (Jenkins, 1947) recommended the alternative term “spot anthracnose” to refer to disease names caused by *Elsinoë* and *Sphaceloma* species instead of anthracnose, more broadly used by disease caused by *Colletotrichum* species (Marin-Felix et al., 2019). However, the symptoms caused by *Elsinoë* species is not limited to only necrotic lesion on leaves and stem. In some hosts, it causes distortion in infected organs, such as twisting of infected stems of *Ipomea batatas* (L.) Lam. (Goto, 1937) and *Bidens* spp. (Guatimosim et al., 2015), and elongation of stem in *Manihot esculenta* Crantz (Rademacher & Graebe, 1979). Although there was a lot of description of *Elsinoë* species causing diseases in crops, the impact of diseases caused by this fungus is more on the appearance of the harvested product rather than the crop productivity itself (Swart et al., 2001). However, there are records of *Elsinoë* causing economically important diseases such as avocado scab by *Elsinoë perseae* (Jenkins) Rossman & W.C. Allen, citrus scab by *Elsinoë fawcetti* Bitanc. & Jenkins (Fig. 2.1a, b) and *Elsinoë australis* Bitanc. & Jenkins, and grape spot anthracnose by *Elsinoë ampelina* (de Bary) Shear (Fig. 2.1c).

In the revision of *Elsinoë* taxonomy, a total of 79 species was accepted in the genus *Elsinoë* including new combinations transferred from the genus *Sphaceloma* (Fan

et al., 2017; Jayawardena et al., 2014; Marin-Felix et al., 2019). In previous phylogenetic studies with multi genetic-loci, *Elsinoë* species appear to be host specific fungus, as 77 out of 81 species confined to only one host species or genus (Marin-Felix et al., 2019). The identification of *Elsinoë* species is often challenging as there is overlapping morphological characteristics, such as small conidia, similar conidiogenous cells, continuously expanding wide acervuli, and absence of fertile structure in nature (Fan et al., 2017). Additionally, the establishment of pure culture was challenging due to the slow growth of the isolates, making it susceptible to contaminations by other fungi (Fan et al., 2017). Conversely, scab symptom is considered a significant characteristic of *Elsinoë* infection. The isolates with similar cultural characteristics obtained from the typical symptoms can often be helpful for species identification (Fan et al., 2017).

Figure 2.1: *Elsinoë* scabs causing citrus scab and anthracnose grape spot in Japan.

In Japan, reports of *Elsinoë* and *Sphaceloma* infecting plants have been recorded infecting crops such as citrus, soybean, and grapevines. The Japanese isolates of *Elsinoë* species recorded are based on morphological characteristics and limited phylogenetic information obtained only from Internal Transcriber Spacer (ITS) region of rDNA. As the advancement in molecular technique involving the usage of polyphasic approach in species recognition by Consolidated Species Concept (Quaedvlieg et al., 2014), a comprehensive study needs to be conducted to update the databased of the Japanese isolates of *Elsinoë*. In this study, the isolates that were stored as *Elsinoë* and *Sphaceloma* in culture collection of different research institute in Japan were re-examined for their taxonomical position by using multi-locus phylogenetic analysis by using the regions, Internal Transcriber Spacer (ITS) and nuclear large subunit ribosomal (LSU) of the rDNA,

RNA polymerase II subunit (*rpb2*) and Translation elongation factor 1-alpha (*tef*). Morphological characteristics were examined on host plants and media as well. This study aims to contribute to the phylogenetic backbone of Japanese isolates of *Elsinoë* species.

MATERIALS AND METHOD

Sample Collection and Morphological Study

Specimens and culture were obtained from the National Agriculture and Food Research, Organization (NARO), NITE Biological Resource Centre (NRBC), Herbarium of Forest Mycology and Pathology, Forestry and Forest Products Research Institute, Tsukuba, Japan (TFM: FPH), Agriculture Promotion Office, Tokyo Metropolitan Government (Tachikawa, Tokyo, Japan), National Museum of Nature and Science (TNS), and Culture Collection of Laboratory of Phytopathology, Mie University (Tsu, Mie, Japan) and examined in the framework of morphological and molecular-phylogeny approaches. The isolates were obtained from symptomatic plant of various host (Table 2.1). The isolates were cultured on malt agar (MA), oatmeal agar (OMA) (Difco, Becton Dickinson, Franklin, NJ), and potato dextrose agar (PDA) (Nissui Pharmaceutical Co., LTD., Tokyo, Japan) at 22 ℃ for 21 days. Colony colours were described after 3 weeks according to the colour chart by Rayner (Rayner & others, 1970) Observation of specimens' collections (Table 2.2) were conducted by using shear solution as mounting medium.

DNA Isolation, Amplification, and Sequencing

Genomic DNA was extracted from mycelia growing on MA by using DNeasy Ultra Clean Microbial Kit (Qaigen, Hilden, Germany) according to manufacturer instruction. ITS and LSU region of the rDNA as well as TEF-1α (*tef*) and RPB2 (*rpb2*) were amplified via polymerase chain reaction using the T100 thermal cycler (Bio-Rad, Tokyo, Japan). PCR mixture of final volume of 12.5 µL for all reaction were prepared as followed; 1–10 ng of genomic DNA, 0.1 uL of 0.25 U Taq DNA polymerase (Bioline, London, UK), 2.5 mM of $MgCl_2$, 1.25 µL of 10× NH_4 reaction buffer (Bioline), 40 µM dNTPs (Bioline), and 0.2 µM of each primer.

Table 2.1. Isolates of Japanese *Elsinoë* isolates used in this study.

Previous identification	Region	Host Species	Herbarium No.	Isolate No.	Fungal species
Sphaceloma akebiae	Tokyo	*Akebia trifoliata*	TSU-MUMH 11977	MUCC 2982 (MAFF 243582)	*Elsinoë akebiae*
Elsinoë bidentis	Gunma	*Bidens pilosa*		MUCC 2983 (MAFF 243588)	*E. bidentis*
Sphaceloma glycine	Akita	*Glycine max*		MUCC 2984 (MAFF 305214)	*E. glycine*
Sphaceloma glycine	Miyagi	*Glycine max*		MUCC 2985 (MAFF 305611)	*E. glycine*
Sphaceloma kurozawarnum	Gunma	*Amphicarpaea edgeworthii*		MUCC 2986 (MAFF 243587)	*Elsinoë* sp.
Sphaceloma sp.	Tokyo	*Hyderangea serrata*	TSU-MUMH 11976	MUCC 2988 (MAFF 243315)	*E. hydrangeae*
Sphaceloma sp.	Tokyo	*Tilia playtphyllos*		MUCC 2989 (MAFF 243584)	*E. tilliae*
Sphaceloma sp.	Tokyo	*Tilia europaea*		MUCC 2990 (MAFF 243585)	*E. tilliae*
Sphaceloma tsujii	Ibaraki	*Paulownia tomentosa*		MUCC 2991 (MAFF 410486)	*Sphaceloma tsujii*
Sphaceloma violae	Tokyo	*Viola sp.*	TSU-MUMH 11975	MUCC 2992 (MAFF 243579)	*Elsinoë sumire*
Elsinoë fawcettii	Shizuoka	*Citrus unshiu*		MUCC 2993 (MAFF 675004)	*E. fawcettii*
Elsinoë fawcettii	Miyazaki	*Citrus hassaku*		MUCC 2994 (MAFF 675008)	*E. fawcettii*
Elsinoë fawcettii	Wakayama	*Citrus unshiu*		MUCC 2999 (MAFF 675005)	*E. fawcettii*
Elsinoë fawcettii	Kochi	*Citrus unshiu*		MUCC 3000 (MAFF 675006)	*E. fawcettii*
Elsinoë ampelina	-	*Vitis sp.*		MUCC 2995 (MAFF 243580)	*E. ampelina*
Elsinoë ampelina	Yamanashi	*Vitis vinifera*		MUCC 2996 (MAFF 244135)	*E. ampelina*
-	-	*Vitis vinifera*		MUCC 2362	*E. ampelina*
Elsinoë araliae	Gunma	*Aralia elata*		MUCC 2997 (MAFF 243589)	*E. araliae*
Elsinoë araliae	Ibaraki	*Aralia cordata*		MUCC 3490 (NBRC 6166)	*E. araliae*
Elsinoë corni	Tokyo	*Cornus florida*		MUCC 2998 (MAFF 243590)	*E. corni*
Sphaceloma sp.		*Juglans sp.*		MUCC3463 (MAFF 410340)	*E. tanashiemsis*
Sphaceloma populi	Tokyo	*Populus sp.*	TFM 01697	MUCC3466 (MAFF 410485)	*E. tanashiensis*

All PCR condition used in this study follows the previous study, where the PCR condition was as follows. For ITS and LSU (Fan et al., 2017): Initial denaturation (95 ℃, 2 minutes), 35 cycle of amplification (denaturation at 95 ℃, 30 seconds; annealing at 48 ℃, 1 minute; extension at 72 ℃, I minute), and final extension at 72℃ for 8 minutes; for *tef* (Hyun et al., 2009): Initial denaturation (95 ℃, 2 minutes), 35 cycle of amplification (denaturation at 94 ℃, 30 seconds; annealing at 60 ℃, 1 minute; extension at 72 ℃, 2 minute), and final extension at 72 ℃ for 10 minutes; for *rpb2*: Initial denaturation (95 ℃, 5 minutes), touch-down amplification which consist of 5 cycle of 95 ℃ for 45 seconds, 56 ℃ for 45 seconds and 72 ℃ for 2 minutes, followed by 5 cycle of 95 ℃ for 45 seconds, 53 ℃ for 45 seconds and 72 ℃ for 2 minutes, and 30 cycles of 95 ℃ for 45 seconds, 50 ℃ for 45 seconds and 72 ℃ for 2 minutes, and final elongation of 72 ℃ for 8 minutes. Amplicons were sequenced in both directions by using BigDye Terminator version 3.1 cycle Sequencing Kit (Applied Biosystem, Foster City, CA) at Mie University Advance Science Research Promotion Centre (Tsu, Mie, Japan). Primer sets used in this study are summarized in Table 2.2.

Table 2.2: List of regions and primer set used in this study.

Region /Locus	**Primer**	**Sequences**	**Reference**
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)
	ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)
LSU	LR0R	GRATCAGGTAGGRATACCCG	Crous et al. (2009)
	LR5	TCCTGAGGGAAACTTCG	Vilgalys & Hester (1990)
TEF	Elongation-1-F	AGCCCCTCCGTCTTCCTCTCCAG	Hyun et al. (2009)
	Elongation-1-R	CGGTACGGCGGTCAATCTTCTCG	Hyun et al. (2009)
RPB2	fRPB2-5F	GGGGWGAYCAGAAGAAGGC	Sung et al. (2007)
	fRPB2-7cR	CCCATRGCTTGTYYRCCCAT	Liu et al. (1999)

Phylogenetic Analyses

The resulting sequences were assembled and aligned with 81 sequences of *Elsinoë* retrieved from previous study by Marin-Felix et al. (Marin-Felix et al., 2019)and Fan et al. (Fan et al., 2017) on MEGA X software package (Kumar et al., 2018) (Table 2.3). This matrix was aligned by using MAFFT online version (Katoh et al., 2018a) and edited

manually using AliView (Larsson, 2014). Maximum-likelihood (ML) and Bayesian inference (BI) analyses was used in this study to estimate the phylogenetic relationship of the samples. ModelTest-NG (Darriba et al., 2020) were used to estimate the best substitution model for each gene for ML and BI analysis, and ML analysis were performed by using RAxML-NG (Kozlov et al., 2019). Tree strength were tested by bootstrap analysis of 1000 replication (Felsenstein, 1985). BI analysis was performed by using MrBayes 3.2 (Ronquist et al., 2012) to estimate the posterior probability (PP) of the tree topologies based on Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) algorithm of four chain which run from random tree for 150,000,000 generation with evolutional model set as GTR model for ITS and LSU and HKY model for *tef* and *rpb2*. Trees were sampled and saved for each 1000 generation. First 25% of the tree were discarded as burn-in phase of analysis and the PP were determined by remaining tree. *Myriangium hispanicum* (CBS 327.33) was selected as outgroup in all analysis and tree were viewed by using FigTree v. 1.4.2 (Institute of Evolutionary Biology, University of Edinburgh, http://tree.bio.ed.ac.uk/software/figtree). The alignments and respective phylogenetic trees were deposited in TreeBASE, study number S30162.

Table 2.3: Representative sequence used in this study. Isolates used in this study mark in boldface.

Fungal species	Isolate number	Host	Locality	Accession number			
				ITS	LSU	RPB2	TEF-1a
Elsinoë abutilonis	CBS 510.40	*Callianthe striata* (syn. *Abultilion striaum*)	Brazil	KX887185	KX886949	KX887068	KX886831
E. akebiae	**MUCC 2982**	***Akebia trifoliata***	**Japan**	**OQ504591**	**OQ504615**	**OQ472906**	**OQ472926**
E. ampelina	CBS 208.25	*Vitis vinifera*	Brazil	KX887186	KX886950	KX887069	KX886832
E. ampelina	**MUCC 2995**	***Vitis vinifera***	**Japan**	**OQ504587**	**OQ504611**	**OQ472902**	**OQ472922**
E. ampelina	**MUCC 2996**	***Vitis vinifera***	**Japan**	**OQ504588**	**OQ504612**	**OQ472903**	**OQ472923**
E. ampelina	**MUCC 2362**	***Vitis* sp.**	**Japan**	**OQ504589**	**OQ504613**	**OQ472904**	**OQ472924**
E. anacardia	CBS 470.62	*Anacardium occidentale*	India	KX887189	KX886953	KX887072	KX886835
E. annonae	CBS 228.64	*Annona* sp.	USA	KX887190	KX886954	KX887073	KX886836
E. arachidis	CBS 511.50	*Arachis hypogaea*	Brazil	KX887191	KX886955	KX887074	KX886837
E. araliae	**MUCC 2997**	***Aralia elata***	**Japan**	**OQ504590**	**OQ504614**	**OQ472905**	**OQ472925**
E. araliae	**MUCC 3490**	***Aralia cordata***	**Japan**	**OQ504586**	**OQ504610**	**OQ472901**	**OQ472921**
E. arrudai	CBS 220.50	*Tournefortia breviflora*	Brazil	KX887194	KX886958	KX887077	KX886840
E. asclepiadea	CPC 18544	*Asclepias mellodora* (syn. *A. curassavica*)	Brazil	KX887195	KX886959	KX887078	KX886841
E. australis	CBS 314.32	*Citrus aurantium*	Brazil	KX887198	KX886962	KX887081	KX886844
E. banksiicola	CBS 113734	*Banksia prionote*	Australia	KX887199	KX886963	KX887082	KX886845
E. barleriicola	CBS 471.62	*Barleria gibsonii*	India	KX887200	KX886964	KX887083	KX886846
E. bidentis	CBS 512.20	*Bidens pilosa*	Brazil	KX887201	KX886965	KX887084	KX886847

E. bidentis	**MUCC 2983**	***Bidens pilosa***	**Japan**	**OQ504595**	**OQ504619**	**OQ472910**	**OQ472930**
E. brasiliensis	CPC 18528	*Chamaesyce hyssopifolia*	Brazil	KX887204	N/A	KX887087	KX886850
E. caleae	CBS 221.50	*Calea pinnatifida*	Brazil	KX887205	KX886968	KX887088	KX886851
E. centrolobii	CBS 222.50	*Centrolobium robustum*	Brazil	KX887206	KX886969	KX887089	KX886852
E. citricola	CPC 18535 = RWB 1175	*Citrus limonia*	Brazil	KX887207	KX886970	KX887090	KX886853
E. corni	**MUCC 2998**	***Cornus florida***	**Japan**	**OQ504596**	**OQ504620**	**OQ472911**	**OQ472931**
E. coryli	CBS 275.76	*Corylus avellana*	France	KX887209	KX886972	KX887092	KX886855
E. diospyri	CBS 223.50	*Diospyros kaki*	Brazil	KX887210	KX886973	KX887093	KX886856
E. embeliae	CBS 472.62	*Embelia ribes*	India	KX887211	KX886974	N/A	KX886857
E. erythinae	CPC 18542	*Erythrina* sp.	Brazil	KX887214	KX886977	KX887096	KX886860
E. eucalypticola	CBS 124765	*Eucalyptus* sp.	Australia	KX887215	KX886978	KX887097	KX886861
E. eucalyptorum	CBS 120084	*Eucalyptus propinqua*	Australia	KX887216	KX886979	KX887098	KX886862
E. euphorbiae	CBS 401.63	*Euphorbia pariflora* (syn. *Euphorbia pilulifera*)	India	KX887217	KX886980	KX887099	KX886863
E. fagarae	CBS 514.50	*Fagara riedelianum*	Brazil	KX887218	KX886981	KX887100	KX886864
E. fawcettii	CBS 139.25	*Citrus* sp.	USA	KX887219	KX886982	KX887101	KX886885
E. fawcettii	**MUCC 2993**	***Citrus unshiu***	**Japan**	**OQ504575**	**OQ504599**	**OQ472891**	**OQ472912**
E. fawcettii	**MUCC 2994**	***Citrus hassaku***	**Japan**	**OQ504577**	**OQ504601**	**OQ472893**	**OQ472914**
E. fawcettii	**MUCC 2999**	***Citrus unshiu***	**Japan**	**OQ504578**	**OQ504602**	**OQ472894**	**OQ472915**
E. fawcettii	**MUCC 3000**	***Citrus unshiu***	**Japan**	**OQ504576**	**OQ504600**	**OQ472892**	**OQ472913**
E. fici	CBS 515.50	*Ficus luschnathiana*	Brazil	KX887223	KX886986	KX887105	KX886869

E. fici-cariae	CBS 473.62	*Ficus caria*	India	KX887224	KX886987	KX887106	KX886870
E. flacourtiae	CBS 474.62	*Flacourtia sepiaria*	India	KX887225	KX886988	KX887107	KX886871
E. freyliniae	CBS 128204	*Freylinia lanceolata*	South Africa	KX887226	KX886989	KX887108	KX886872
E. genipae	CBS 342.39	*Genipa americana*	Brazil	KX887227	KX886990	KX887109	KX886873
E. genipae-americanae	CBS 516.50	*Genipa americana*	Brazil	KX887228	KX886991	KX887110	KX886874
E. glycines	CBS 389.64	*Glycine soja*	Japan	KX887229	KX886992	KX887111	KX886875
E. glycines	**MUCC 2984**	***Glycine soja***	**Japan**	**OQ504593**	**OQ504617**	**OQ472908**	**OQ472928**
E. glycines	**MUCC 2985**	***Glycine soja***	**Japan**	**OQ504594**	**OQ504618**	**OQ472909**	**OQ472929**
E. hederae	CBS 517.50	*Herdera helix*	Brazil	KX887231	KX886994	KX887113	KX886877
E. hydrangeae	**MUCC 2988**	***Hydrangea serrata***	**Japan**	**OQ504583**	**OQ504607**	**OQ472898**	N/A
E. ichnocarpi	CBS 475.62	*Ichnocarpus frustescencs*	India	KX887232	KX886995	KX887114	KX886878
E. jasminae	CBS 224.50	*Jasminum sambac*	Brazil	KX887233	KX886996	KX887115	KX886879
E. jasminicola	CBS 212.63	*Jasminum malabaricum*	India	KX887234	KX886997	N/A	KX886880
E. krugii	CPC 18531	*Euphorbia heterophylla*	Brazil	KX887235	KX886998	KX887116	KX886881
E. lagoa-santensis	CBS 518.50	*Brysonima coccolobifolia*	Brazil	KX887239	KX887002	KX887120	KX886885
E. ledi	CBS 167.33	*Rhododendron neoglandulosum* (syn. *Ledum glandulosum*)	USA	KX887240	KX887003	KX887121	KX886886
E. lepagei	CBS 225.50	*Manikara zapota* (syn. *Achras sapota*)	N/A	KX887241	KX887004	KX887122	N/A
E. leucospermi	CBS 111207	*Leucospermum* sp.	South Africa	KX887242	KX887005	KX887123	KX886887
E. lippiae	CBS 166.40	*Phyla lancelolata* (syn. *Lippia lanceolate*)	USA	KX887248	KX887011	KX887129	KX886893
E. mangiferae	CBS 226.50	*Mangifera foetida* (syn. *M. indica*)	Cuba	KX887249	KX887012	KX887130	KX886894

E. mattiroloanum	CBS 287.64	*Arbutus unedo*	Argentina	KX887250	KX887013	KX887131	KX886895
E. menthane	CBS 322.37	*Mentha piperita*	USA	KX887253	KX887016	KX887134	KX886898
E. mimosae	CPC 19478	*Mimosa invisa*	Brazil	KX887255	KX887018	KX887136	KX886900
E. oleae	CBS 227.59	*Olea europaea*	Italy	KX887256	KX887019	KX887137	KX886901
E. othonnae	CBS 139910	*Othonna quinquedentate*	South Africa	KR476726	N/A	MK540083	N/A
E. perseae	CBS 406.34	*Persea americana*	USA	KX887258	KX887021	KX887139	KX886903
E. phaseoli	CBS 165.31	*Phaeseokus lunatus*	Cuba	KX887263	KX887026	KX887144	KX886908
E. piri	CBS 163.29	*Pyrus communis*	N/A	KX887267	KX887030	KX887148	KX886912
E. pitangae	CBS 227.50	*Eugenia pitanga*	Brazil	KX887269	KX887032	KX887150	KX886914
E. poinsettiae	CBS 109333	*Eugenia pulcherrima*	Guatemala	KX887270	KX887033	KX887151	KX886915
E. pongamiae	CBS 402.63	*Pongamia pinnata*	India	KX887272	KX887035	KX887153	KX886917
E. populi	CBS 298.64	*Populus deltoides subsp. deltoides (syn. P. serotine)*	Argentina	KX887273	KX887036	KX887154	KX886918
E. protearum	CBS 113618	*Protea* sp.	Zimbabwe	KX887275	KX887038	KX887156	KX886920
E. punicae	CPC 19968	*Punica granatum*	South Africa	KX887276	KX887039	KX887157	KX886921
E. quercus-illcis	CBS 232.61	*Quercus ilex*	Italy	KX887277	KX887040	N/A	KX886922
E. randii	CBS 170.38	*Carya* sp.	Brazil	KX887278	KX887041	KX887158	KX886923
E. rhois	CBS 519.20	*Toxicodendron vernix* (syn. Rhus	Brazil	KX887280	KX887043	KX887160	KX886925
E. ricini	CBS 403.63 = ATCC15030	*Ricinus communis*	India	KX887281	KX887044	KX887161	KX886926
E. rosarum	CBS 212.33	*Rosa* sp.	USA	KX887283	KX887046	KX887163	KX886928
E. salicina	CPC 17824	*Salix* sp.	USA	KX887286	KX887049	KX887166	KX886931

E. samecarpi	CBS 477.62	*Melanichyla caesia* (syn.	India	KX887287	KX887050	KX887167	KX886932
E. sesseae	CPC 18549	*Ceatrum laevigatum?*	Brazil	KX887288	KX887051	KX887168	KX886933
E. sicula	CBS 398.59	*Prunus amygdalus*	Italy	KX887289	KX887052	KX887169	KX886934
E. soludagnis	CBS 191.37	*Solidago fistulosa*	USA	KX887290	KX887053	KX887170	KX886935
E. sumire	**MUCC 2992**	***Viola* sp.**	**Japan**	**OQ504585**	**OQ504609**	**OQ472900**	**OQ472920**
***Elsinoë* sp.**	**MUCC 2986**	***Amphicarpaea edgeworthii***	**Japan**	**OQ504592**	**OQ504616**	**OQ472907**	**OQ472927**
E. tanashiensis	**MUCC 3463**	***Juglans* sp.**	**Japan**	**OQ504581**	**OQ504605**	N/A	N/A
E. tanashiensis	**MUCC 3466**	***Populus* sp.**	**Japan**	**OQ504582**	**OQ504606**	**OQ472897**	**OQ472918**
E. tectificae	CBS 124777 = CPC 14594		Australia	KX887292	KX887055	KX887172	KX886937
E. terminaliae	CBS 343.39	*Terminalia catappa*	Brazil	KX887293	KX887056	KX887173	N/A
E. theae	CBS 228.50	*Cemellia sinensis* (syn. *Thea sinensis*)	Brazil	KX887295	KX887058	KX887175	KX886939
E. tiliae	CBS 350.73 = ATCC 24510	*Tilia cordata*	New Zealand	KX887296	KX887059	KX887176	KX886940
E. tiliae	**MUCC 2989**	***Tilia playtphyllos***	**Japan**	**OQ504579**	**OQ504603**	**OQ472895**	**OQ472916**
E. tiliae	**MUCC 2990**	***Tilia europaea***	**Japan**	**OQ504580**	**OQ504604**	**OQ472896**	**OQ472917**
E. veneta	CBS 164.29 = ATCC 1833	*Rubus* sp.	N/A	KX887297	KX887060	KX887177	KX886941
E. verbenae	CPC 18561 = RWB 1232	*Verbena bonariensis*	Brazil	KX887298	KX887061	KX887178	KX886942
E. violae	CBS 336.35	*Viola* sp.	USA	KX887302	KX887065	KX887182	KX886946

E. zizyphi	CBS 378.62 = STCC 14656	*Zizyphus rugisa*	India	KX887303	KX887066	KX887183	N/A
Sphaceloma tsujii	**MUCC 2991**	***Paulownia tomentosa***	**Japan**	**OQ504584**	**OQ504608**	**OQ472899**	**OQ472919**
Myriangium hispanicum	CBS 247.33	*Acer monspessulanum*	N/A	KX887304	KX887067	KX887184	KX886948

RESULT

Phylogeny

The sequencing results of all regions were combined and aligned in a data matrix of 104 OTU belonging to the genus *Elsinoë*. The final alignment contained a total of 2470 characters consisting of four regional sequences, ITS: 605 sites, LSU: 740 sites, *rpb2*: 747 sites, and *tef*: 371 sites, including alignment gaps. ML tree is shown in Figure 2.2, where the topologies of the generated tree from ML and BI analyses were congruent. Japanese isolates analysed in this study formed four major clades with hitherto known or newly recognized species.

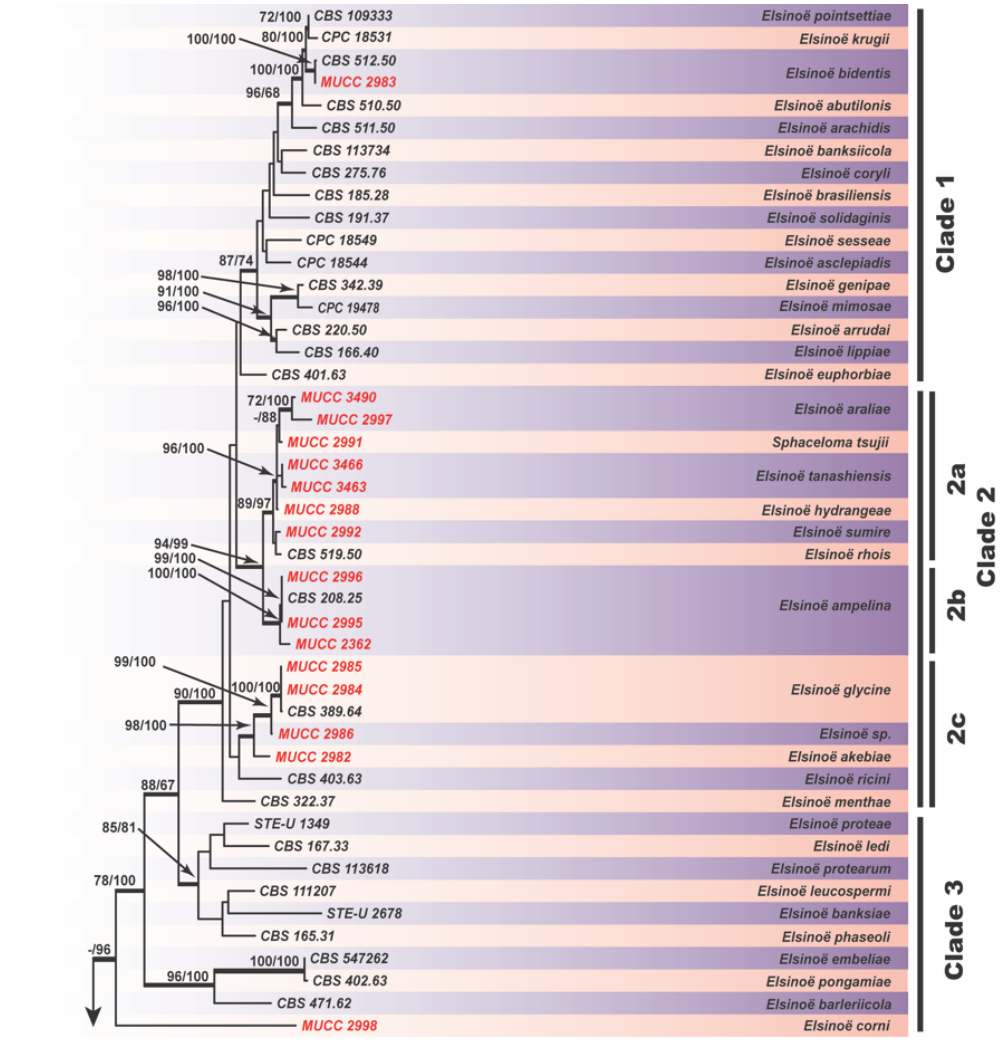

Figure 2.2: Phylogenetic tree of *Elsinoë* spp. constructed by ML using combined ITS, LSU, *rpb*2, and *tef* gene sequence dataset. ML bootstrap values and Bayesian PPs are given near branches (ML/PP).

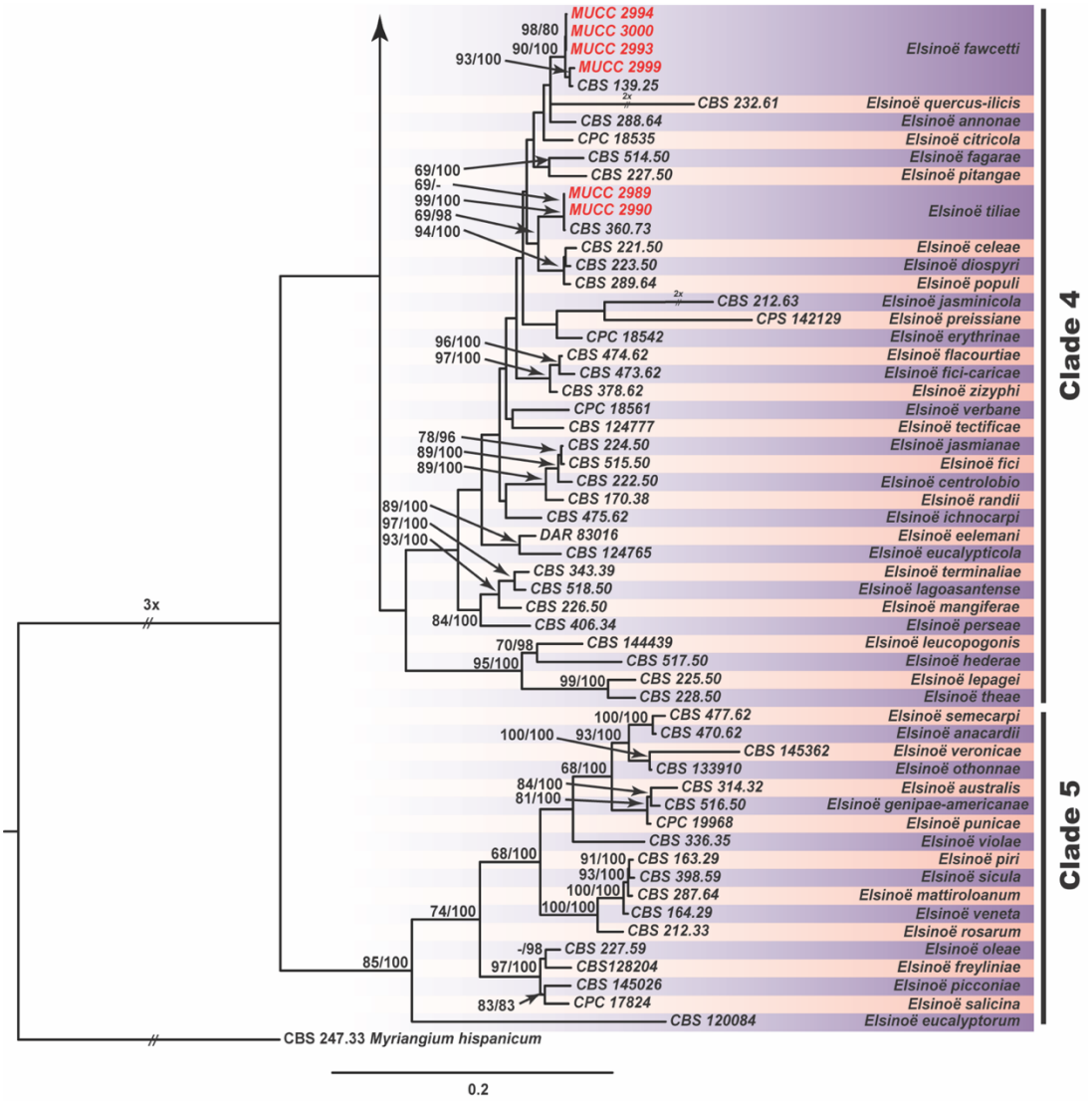

Figure 2.2: continued.

Clade 1 including *Elsinoë bidentis* (MUCC2983), clade 2 composed of most Japanese isolates including *E. ampelina,* clade 3 consisting Japanese isolates of *E. glycine* (MUCC2985, MUCC2984), *E. akebiae* (MUCC2982), *Elsinoë* sp. from *Amphicarpeae edgeworthii* (MUCC2986), and *E. corni* (MUCC2998), and clade 4 including cosmopolitan species such as *E. fawcetti* and *E. tiliae*.

For LSU, six *Elsinoë* species from different hosts isolated in Japan have identical sequences, this includes *Elsinoë* species isolated from *Amphicarpha edgeworthii* (MUCC2986), *E. hydrangea* (MUCC2988), *E. tanashiensis* (MUCC3466), *E. sumirensis* (MUCC2992), *E. araliae* (MUCC2997, MUCC3490), and *Sphaceloma tsujii* (MUCC2991). In the same analysis of LSU gene, it was also found that *E. asclepiadis* (CPC 18544) from

Brazil have identical sequences as *E. akebiae* (MUCC2982) and *E. bidentis* (MUCC2983) isolated from Japan. *E. citricola* (CPC 18535) from Brazil have identical sequences as *E. ampelina* of Japanese isolates (MUCC2362) and *E. fawcetti* of Japanese isolates (MUCC2993, MUCC2994, MUCC3000).

However, in our study, it shows that LSU gene were able to distinguish the USA isolates *Elsinoë violae* (CBS 336.35) from *E. sumire* (MUCC2992) as well as *E. populi* (CBS 298.64) from Argentina with *E. tanashiensis* (MUCC3463, MUCC3466) that share a same host plant of *Viola* sp. and *Populus* sp. Based on this analysis, it could be said that although most of the Japanese isolates have identical LSU gene nonetheless, LSU can give good resolution based on geographical distribution.

Taxonomy

Elsinoë akebiae (Kuros. & Katsuki) A.H. Ujat & C. Nakash., **comb. nov.** Figure 2.3. MycoBank no: MB847771

≡ *Sphaceloma akebiae* Kuros. & Katsuki, Botanical Magazine Tokyo 70: 131, 1957.

Spots on the leaves scattered and aggregated near leaves veins, usually amphigenous, rounded or near circular, 0.2–2 mm in diam., often coalescing and extending, flat or slightly depressed in the middle, first brown, then gray-white or grayish brown, with black-brown or purplish-brown margins. In stems, presented as more elongated, 3–5 mm in long side, depressed in the center. *Acervuli* dark brown of 2–3mm, often coalescing. *Conidiogenus cell* subcuticular, subsequently erupting with piles of compact conidiophores 9–20 µm thick, hyaline, 5–7 × 2.6–4 µm, *conidia* hyaline, elliptical, 3.9–8 × 2–3.3 µm. (Kurosawa & Katsuki, 1957). On *MA*: colony cerebriform, straw to yellow, folded towards center of colony; reverse, pale luteous with folding towards center is observable. On *PDA*: surface; covered with white short dense mycelia, pale luteous margin that turns salmon over time; Reverse, pale luteous that turns olivaceous buff over time. On *OMA*: surface; olivaceous with short arial mycelia with folding towards the center, agar color around the colony changed to olivaceous buff; Reverse, rust around the center with citrine growth margin with folding towards the center.

Holotypus: on *Akebia trifoliata* (Thunb.) Koidz., Japan, Gunma, Tanigawa, 19 July 1936, by E. Kurosawa and S. Katsuki (TNS-F-99470 = SK 1498).

Herbarium specimens examined: on *A. quinate* (Thunb. ex Houtt.) Decne., Mt. Tsukuba, 15 August 1935, collected by E. Kurosawa (TNS-F-99468 = SK 1495) ; on *A. trifoliata*, see holotypus; Japan, Tokyo, Machida, July 2008, collected by T. Ono (**epitype designated here**, TSU-MUMH11977; ex-epitype culture MUCC2982); on *Stauntonia hexaphylla* (Thunb.) Decne., Japan, Kagoshima, Kagoshima University Botanical Garden, 26 October 1949, collected by S. Katsuki (TNS-F-99471 = SK1510, SK Herbarium collection housed in TNS).

Note: Present species was described by Kurosawa and Katsuki (Kurosawa & Katsuki, 1957). The morphological characteristics of holotype specimen was confirmed as the anamorphic state of the genus *Elsinoë*, the genus *Sphaceloma*. For the phylogenetic study, the epitype specimen was selected from the currently collected specimen. The nucleotide sequence of LSU gene is not enough to deliminate species of *E. akebiae* (MUCC2982), *E. bidentis* (MUCC2983) and *E. asclepiadis* (CPC 18544). The new combination *E. akebiae* is proposed in this study. It is inhabiting on *Akebia* sp. and *Stauntonia hexophylla*, which are of the family *Lardizabalaceae* whereby these plants are native to Far East Asia.

Elsinoë hydrangeae T. Ono, A. H. Ujat & C. Nakashima, **sp. nov.**, Figure 2.4. MycoBank no: MB847772

Etymology: Named after the genus of the host plant (*Hydrangea*) from which the ex-type strain was obtained.

Leaf spot pale yellow to purple slate with brick margin, circular, 2 mm in diameter on the leaves, along the leaves vein, expanded towards adaxial surface and coalescent in a chain-like pattern, becoming greyish white in the middle, petiole and stems crinkled, often observed on newly developed leaves. Brown lesion with pale yellow scab can be observed on the abaxial surface of the leaves when the spots coalescent. *Mycelia* dense on the irregular to globose acervuli. *Asexual morph acervuli* dark brown, often coalescing, 7–27 µm in height, 1.4–6.8 mm in diam., subcuticular, subsequently erupting with compact conidiogenous cells. *Conidiogenous cells* hyaline, cylindrical monophialidic, sporulating enteroblastically, determinate, 15–27 × 3.2–4.5 µm. *Conidia* hyaline, elliptical to spindle-shaped, aseptate, 5–7.5 × 2–3.3 µm. *On MA*: surface raised, cerebriform, erumpent, salmon to sienna; reverse, red with white margin. *On PDA*: surface; erumpent and folded towards the center, yellow to salmon, sparse white aerial mycelium, reverse: dark red and folded in towards the center. *On OMA*: surface irregular, folded, yellow to red, dense short aerial mycelia, mucilaginous drops observable centrally. Reverse, flesh to rust with white margins and folded on the margins.

Holotypus: on *Hydrangea serrata* (Thunb.) Ser., Japan, Tokyo, Tachikawa, 25 August 2008, collected by T. Ono (TSU-MUMH11976, ex-type culture MUCC2988).

Host: *Hydrangea serrata*, *H. serrata* (Thunb.) Ser. var. *yesoensis* (Koidz.) H. Ohba, *H. hirta* (Thunb.) Siebold et Zucc..

Herbarium specimen examined: see holotype.

Notes: This study also proposes *Elsinoë hydrangeae* as novel species of *Elsinoë* infecting *Hydrangea* species. Ono et al., (Ono et al., 2010)described this as *Sphaceloma* sp. on *Hydrangea* where it was first observed in *Hydrangea serrata* in the open field in Tachikawa and Hino in August 2008. The collected sample was brought back for pathogenicity test on other *Hydrangea* species to determine the host specificity. Based on previous study, *E. hydrangeae* isolated from *H. serrata* shows a range of different pathogenicity on different *Hydrangea* species. While *E. hydrangeae* shows no pathogenicity on *H. quercifolia* W.Bartram, weak pathogenicity was observed on *H. macrophylla* (Thunb.) Ser. and *H. arborescence* L. Furthermore, it shows the same pathogenicity level as *H. serrata* and *H. hirta* (Thunb.) Siebold & Zucc. and *H*. *serrata* var. *yesoensis* (Koidz.) H.Ohba. This result shows that *Elsinoë* species is highly related to the specific host plant and its pathogenicity are differentiated within a host plant genus. Initial molecular analysis conducted by Ono et al., (Ono et al., 2010) by using rDNA ITS region shows that *E. hydrangeae* was closely related to *E. araliae*. As shown in Figure 2.2, although *E. hydrangeae* and *E. araliae* were placed in the same clade, the clade composed of species on various host plants that are native to East Asia. Moreover, the morphology of isolates of *E. araliae* and *E. hydrangeae* grown on malt agar, oatmeal agar and potato dextrose agar are very distinct from each other.

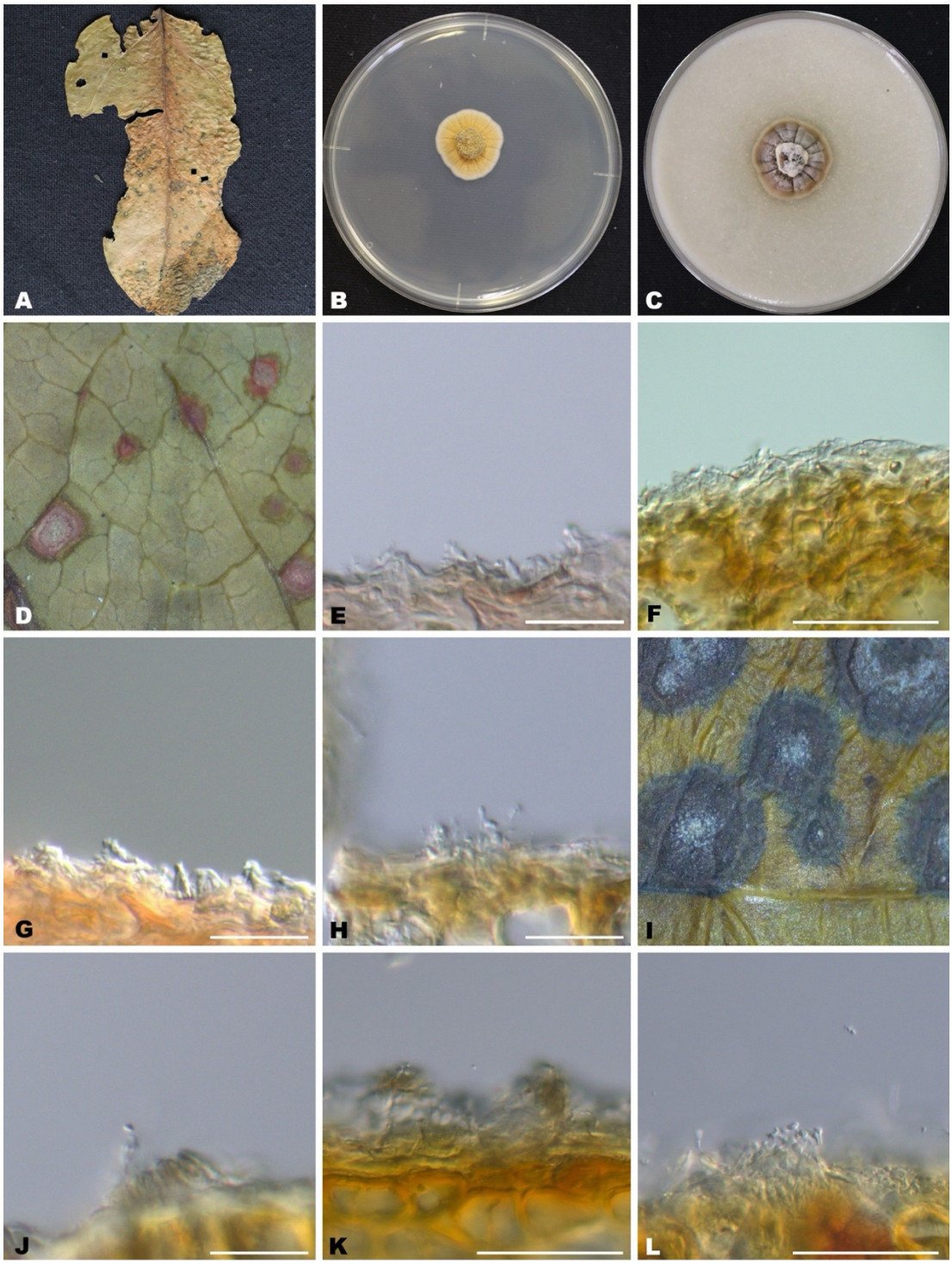

Figure 2.3: Morphological feature of *Elsinoë akebiae* [A, D, F-H: TNS-F-99468; B-C, E: TSU-MUMH11977 (MUCC2982); I-L: TNS-F-99471]. (A) Specimen TNS-F-99468. (B-C) Isolate MUCC2982 on MA (B) and OMA (C). (D) Symptom of scab forming on the leaf of *Akebia trifoliata*. (E-G) Acervuli. (H) Conidia. (I) Symptom of scab forming on leaf of *Stauntonia hexaphylla*. (J-K) Acervuli. (L) Conidia. Scale bars, 25 µm (F-H) and 50 µm (J-L)

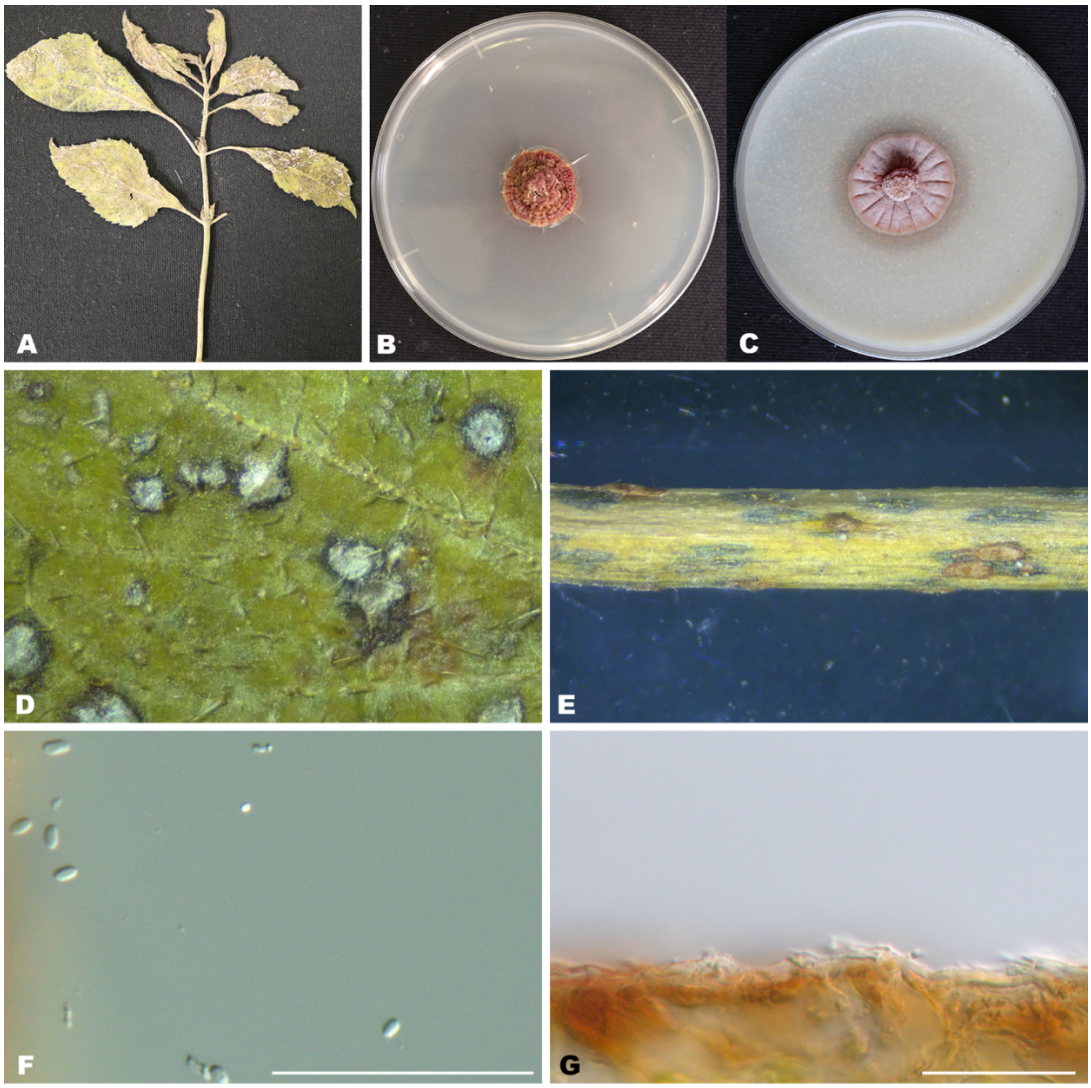

Figure 2.4: Morphological feature of *Elsinoë hydrangeae* [A-G: TSU-MUMH11976 (MUCC2988)]. (A, D-E) Specimen TSU-MUMH11976. (B-C) Isolate MUCC2988 on MA (B) and OMA (C). (F) Conidia. (G) Acervuli. Scale bars, 50 µm.

Elsinoë sumire T. Ono, A. H. Ujat & C. Nakashima, **sp. nov.**, Figure 2.5. MycoBank no: MB847783

Etymology: Name derived from the *Viola sp.* generic name in Japan

Lesions white to straw irregular, circular to angular, surrounded with citrine green margin, scabbed in numerous on adaxial and abaxial surface of the leaves, scattered along the vein, enlarged, overlapped, up to 3 mm eventually perforated. Asexual morph. *Acervuli* pale brown, globose and oblate, enlarged and confluent eventually, solitary on

the stems, up to 1 mm in width. *Conidiogenous cells* hyaline, cylindrical to ampulliform, monophialidic, sporulating enteroblastically, integrated, 0.1–0.2 ×1.0–1.4 µm. Conidia hyaline, aseptate, globular, ellipsoid to irregular, 0.1–0.15 × 0.1–1.0 µm. *On MA*: colony surface slightly raised, smooth, salmon with white margin; aerial mycelia short and dense; reverse, red with white margin, folded. *On PDA*: surface; folded and covered with white dense aerial mycelia sparse on the edges, salmon; Reverse, bay to umber with white margin and folded towards the center. *On OMA*: surface; smooth, bay to rust and covered by white arial mycelia; reverse, red and folded with cracks forming towards the center.

Holotypus: Japan, Tokyo, Itabashi, on *Viola* sp., 11 June 2008, collected by T. Ono (TSU-MUMH11975; ex-type culture: MAFF 243579 = MUCC2992).

Host: *Viola sp.*

Herbarium specimens examined: see holotype; on *Viola odorata* L., Japan, Matsudo, Chiba, 3 October 1939, collected by E. Kurosawa (TNS-F-185408)

Notes: The type specimen had been identified as "*Sphaceloma violae*" and its isolate was deposited in the culture collection, the Research Center of Genetic Resources, NARO, Tsukuba, Ibaraki, Japan (MAFF 243579 = MUCC2992). However, it is genetically and morphologically distinguishable from the ex-type strain of *E. violae* (CBS 336.35). The original description provided by Massey and Jenkins, (Massey & Jenkins, 1935) stated that the scab formed will enlarge into scabby, circular spot, often vinaceous buff although it may be ashen or white. While on *E. sumire* scab formed is epileptic and will overlap on each other when enlarged, often white to straw with citrine green margin.

Elsinoë sumire, which is the second *Elsinoë* species infecting *Viola* spp., was proposed as a new species in this study. Massey and Jenkins (1935) described another *Elsinoë* species, *E. violae,* from the scab disease of *Viola* spp. in the United States. According to the Fungal Databases, U.S. National Fungus Collections, ARS, USDA (Farr & Rossman, 2022), the species habitats were mainly from North America and Europe, and on more than 15 *Viola* species. On the other hand, Kurosawa and Katsuki (Kurosawa & Katsuki, 1957) reported the distribution of *E. violae* in Japan without the morphological

descriptions of several voucher specimens. Two herbarium specimens of them were examined in this study. Those morphological characters and symptoms on the host plants were distinguishable, as mentioned above. In the phylogenetic tree (Figure 2.2), *E. sumire* and *E. violae* are placed in two different clades. While the host plant of *E. violae* and *E. sumire* are of the same genus, the symptoms on the host plant and the micromorphology varies greatly.

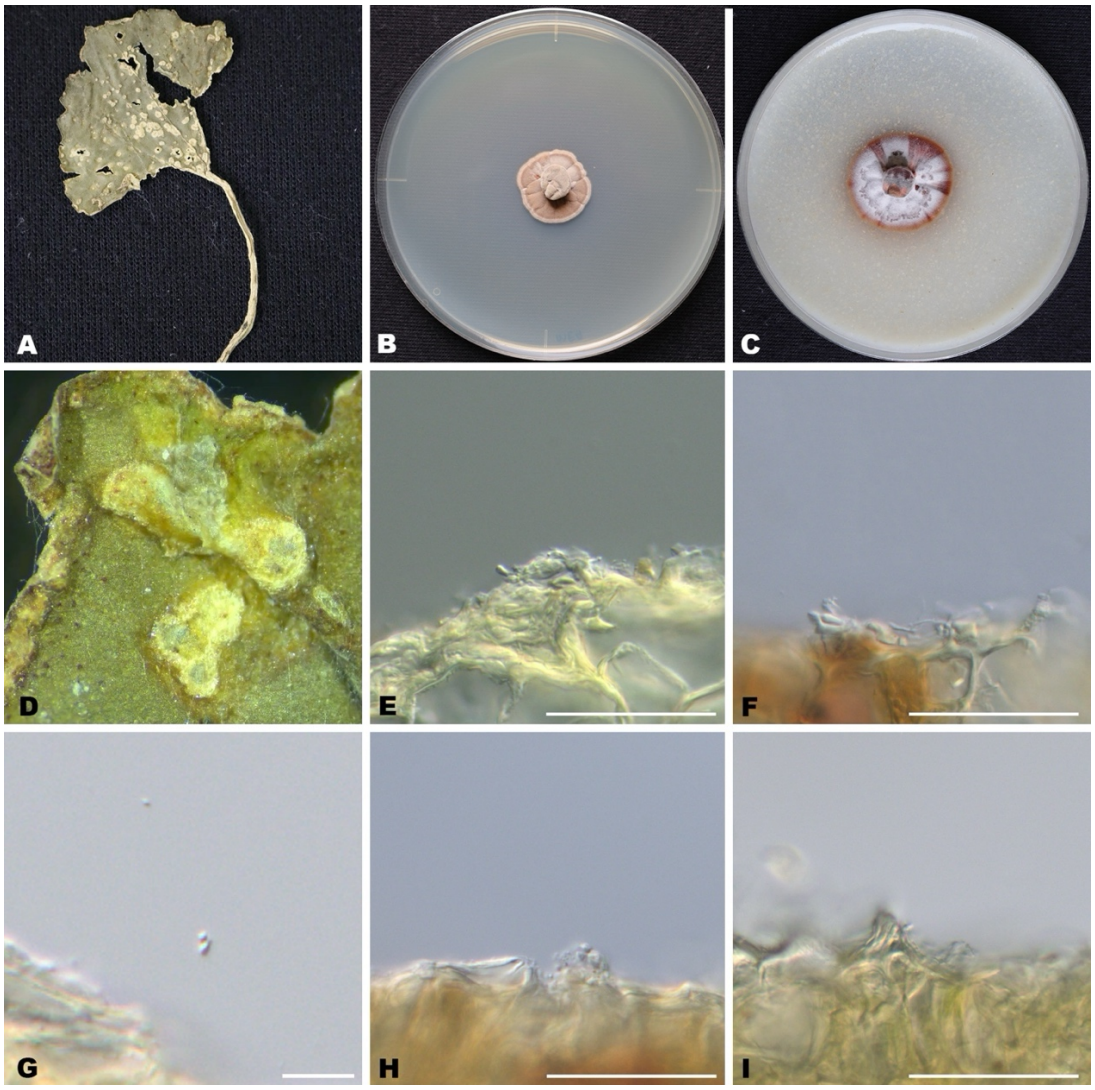

Figure 2.5: Morphological feature of *Elsinoë sumire* [A-I: TSU-MUMH11977 (MUCC2992)]. (A, D) Specimen TSU-MUMH11976. (B-C) Isolate MUCC2992 on MA (B) and OMA (C). (E-F) Conidiogenous cell. (G) Conidia. (H-I) Acervuli. Scale bars, 100 µm (E-F, H-I) and 10 µm (G).

Elsinoë tanashiensis A.H. Ujat & C. Nakashima, **sp. nov.**, Figure 2.6. MycoBank no: MB847784

Etymology: The name reflects the locality of holotype collected, Tanashi, Tokyo

On leaves surface, lesions are observed as both black and white spots. Black spots, scattered, circular, depressed, and turned into greyish brown at the centre, or developed as white spot and raised, scabbed in numerous on adaxial and abaxial surface of the leaves, black spotted scab turned int light brown with no necrosis, on the abaxial surface, 1–2 mm in diameter. Light brown to yellow mucilaginous masses observed on the lesion with greyish brown scab under humid condition, pale orange to brown mucilaginous masses observed on white scab. *Asexual morph*: *Acervuli* pale brown, globose and oblate, enlarged and confluent eventually, solitary on the stems, up to 1 mm in width. *Conidiogenous cells* hyaline, erumpent, cylindrical to ampulliform, monophialidic, sporulating enteroblastically, integrated, 70–200mm. *Conidia* hyaline, aseptate, globular, ellipsoid to irregular, 4–5.5 × 1.5 µm. *On MA*: colony surface with short dense aerial mycelia, salmon, folded towards center of colony; reverse, bay to flesh with rings. *On PDA*: surface; slightly raised and folded, covered with white aerial mycelia, saffron with pale luteous margin; reverse; pale luteous with rings. *On OMA:* surface; bay, covered by white arial mycelia with rings, agar around the colony changed to amber; Reverse, amber to umber with folding towards the center.

Holotypus: Japan, Tokyo, Tanashi, on *Populus deltoides*, October 1956 by O. Chiba and T. Kobayashi (TFM:FPH-01697, Ex-type culture: MUCC3466 = MAFF 410485 = S3-7)

Host: *Populus deltoides* Marshall, *P. nigra* × *P. trichocarpa*, *P. deltoides* var. *misouriensis*, *P. charkowiensis* × *P. candina*, *P euramericana* I-154, *P. euramericana* × *P. serotina*, *P. gelrica* × *P. robusta*, *P. "Leipzig"*, *P. eucalyptus* (Chiba & Kobayashi, 1957)

Herbarium specimens examined: see holotype; on *Juglans* L., Japan, Akita, Funaoka, Kawabe, 26 June 1951, collected by K. Ito and O. Chiba, (TFM:FPH-0330); on *Juglans regia* L., Japan, Saitama, Hatogaya, 21 August 1938, collected by E. Kurosawa (TNS-F-185379).

Note: The isolate MCUU3466 was firstly described as "*Sphaceloma* sp." on Populus (Chiba & Kobayashi, 1957) as the morphological description is different from *E. populi* described by Jenkins (Jenkins, 1932). ITS region of MUCC3466 is identical with MUCC3463.

Elsinoë tanashiensis, isolated from *Populus deltoides in Japan,* is closely related to *Elsinoë* isolated from *Juglans spp. B*ased on phylogenetic analysis, the ITS region is identical, while the LSU gene is highly similar with 99% (1/1185) similarity. RPB2 and tef could not differentiate these two species as there are no sequences obtained from *Elsinoë* isolated from *Juglans sp.* As a pure culture of *Elsinoë* isolated from *Juglans* could not be established*,* morphological differences between isolates from *Populus sp.* and *Juglans sp.* could not be observed. However, the morphology of the conidiogenous cell of these two *Elsinoë* species on herbarium specimens is identical, whereby the size and shape of the conidiogenous cell are also the same. Due to the similarity of the conidiogenous cell and conidia size, these two species could not be determined as different species even though they infect different host plants.

In this study, a novel species of *E. tanashiensis* is also proposed as the species infecting *Populus* sp. in Japan. Although there is already an established species of *Elsinoë populi* known to infect *Populus* species in South America (Fan et al., 2017) and Europe, (Jenkins, 1932), the first record of occurrence in Japan was only recorded as *'Sphaceloma* sp.' on *Populus* (Chiba & Kobayashi, 1957). It was mentioned in a previous study by Chiba and Kobayashi (1957) that this fungus could not be identified as Sphaceloma populi due to differences in the morphological description with the protologue. Although *Populus deltoides*, the host plant of *Elsinoë tanashiensis*, is not native to Japan, it is widely planted in Japan. Similarly, for *Elsinoë* sp. infecting *Juglans* sp. in Japan, this study identifies the isolates as *E. tanashiensis* as the phylogenetic analysis and observation from specimens show high similarity with specimens from *Populus* sp. Although there are established species of *Elsinoë randii* known to infect Carya pecan in Brazil (Jenkins & Bitancourt, 1938) and *Juglans* sp. in North and South America (Jenkins & Bitancourt, 1965) and on *Juglans regia* L. and *Juglans mandshurica* Maxim. (Kurosawa & Katsuki, 1956b), this study shows that *E. randii* is placed in a different clade compared to *E. tanashiensis*.

Elsinoë sp. on *Amphicarpha edgeworthii***, Figure 2.7.**

On MA: colony surface cerebriform, ranging from blood colour to bay to pale luteous. Older mycelia turn herbage green, folded towards center of colony; reverse, scarlet to pale luteous, folded towards center, raised. *On PDA*: surface; folded towards center, saffron to peach on the outer part, herbage green to yellowish green on the center.; reverse; raised, crack towards center, blood colour to bay. *On OMA:* surface; conidiomata olivaceous, mycelia grow into straw and turn citrine overtime; Reverse, buff and turn agar into translucent colour overtime.

Asexual morph: *Acervuli* hyaline, coalescing, compact, up to 100 µm wide. *Conidiogenous cells* hyaline, erumpent, cylindrical to spindle-shape, monophialidic, sporulating enteroblastically, integrated, 15–20mm. *Conidia* hyaline, aseptate, globular to ellipsoid, 1.0–2.3 × 1.0 µm.

Host: *Amphicarpea edgeworthii* Benth.

Herbarium specimen examined: on *Amphicarpea edgeworthii* (syn. *Falcata japonica* (Oliv.) Kom.), Japan, Tokyo, Saginomiya, 17 October 1937, collected by E. Kurosawa (TNS-F-185385).

Note: *Sphaceloma kurozawarnum* was first proposed as a new species infecting *Amphicarpea edgeworthii* (syn. *Falcata japonica*) by Kurata (Kurata, 1957). Although the host plant was of the same family *Fabaceae* as *Glycine* sp., the author noted that it was different from *Elsinoë glycine*. This species was treated as *nomen nudem* due to no description of morphological characteristics. In this study, the isolate used for phylogenetic analysis (MUCC2986) and the specimens observed (TNS-F-185385) are not linked. Both bootstrap value of ML analysis and PP value of Bayesian analysis shows strong support of independent cladding, hence this isolate was treated as *Elsinoë* sp. on *Amphicarpea edgeworthii*.

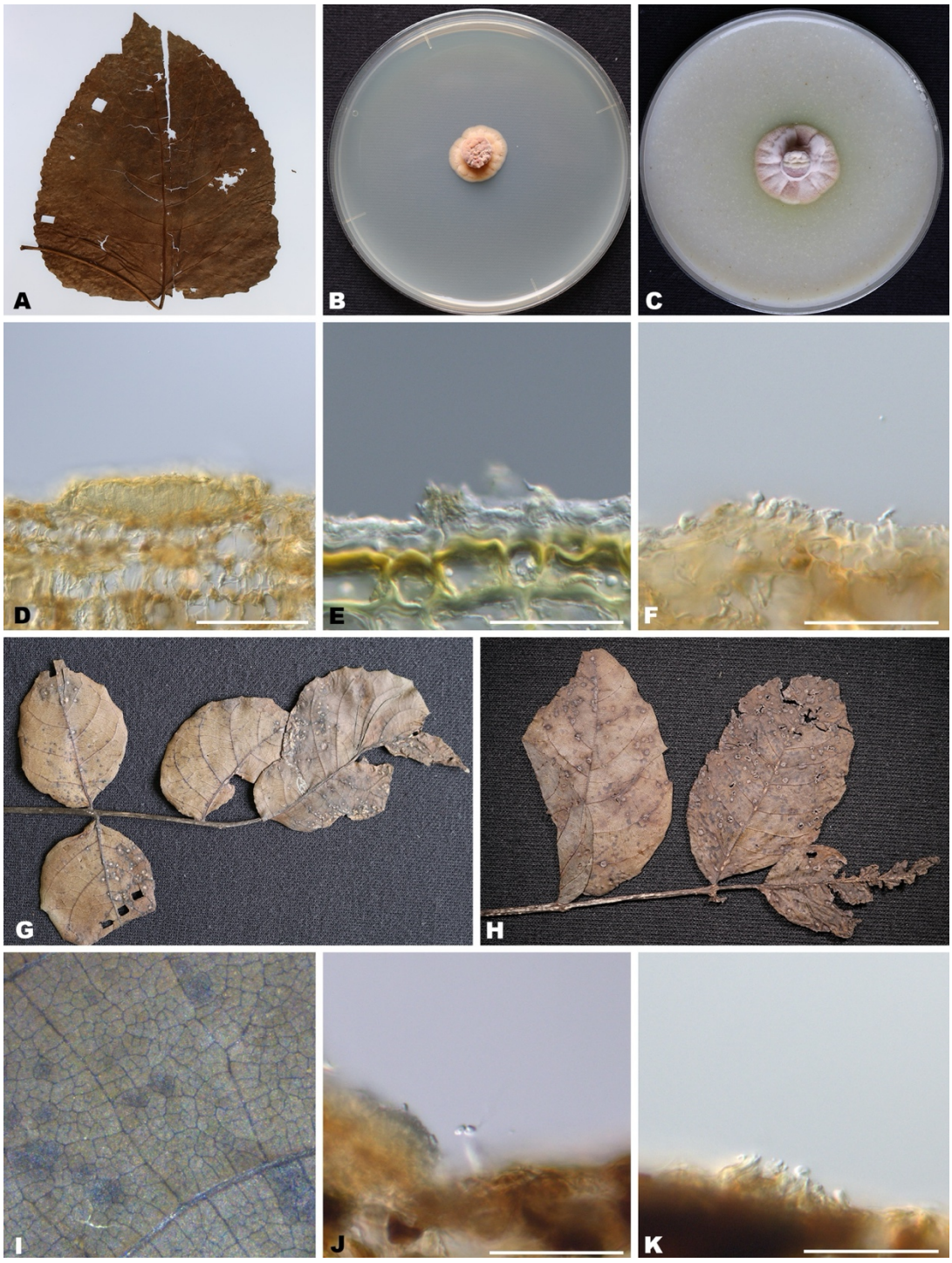

Figure 2.6: Morphological feature of *Elsinoë tanashiensis* on *Populus deltoides* [A-F: TFM:FPH-01697 (MUCC3466)] and *Juglans* sp. [G, J-K: TFM:FPH-0330; H-I: TNS-F-185379]. (A) Specimen TFM:FPH-01697. (B-C) Isolate MUCC3466 on MA (B) and OMA (C). (D-E) Acervuli. (F) Conidiogenous cell. (G) Specimen TFM:FPH-0330. (H) Specimen TNS-F-185379 (I) conidiomata on *Juglans* sp. leaf (TNS-F-185379). (H) Conidia. (I) Acervuli. Scale bars, 100 µm (D) and 50 µm (E-F, J-K).

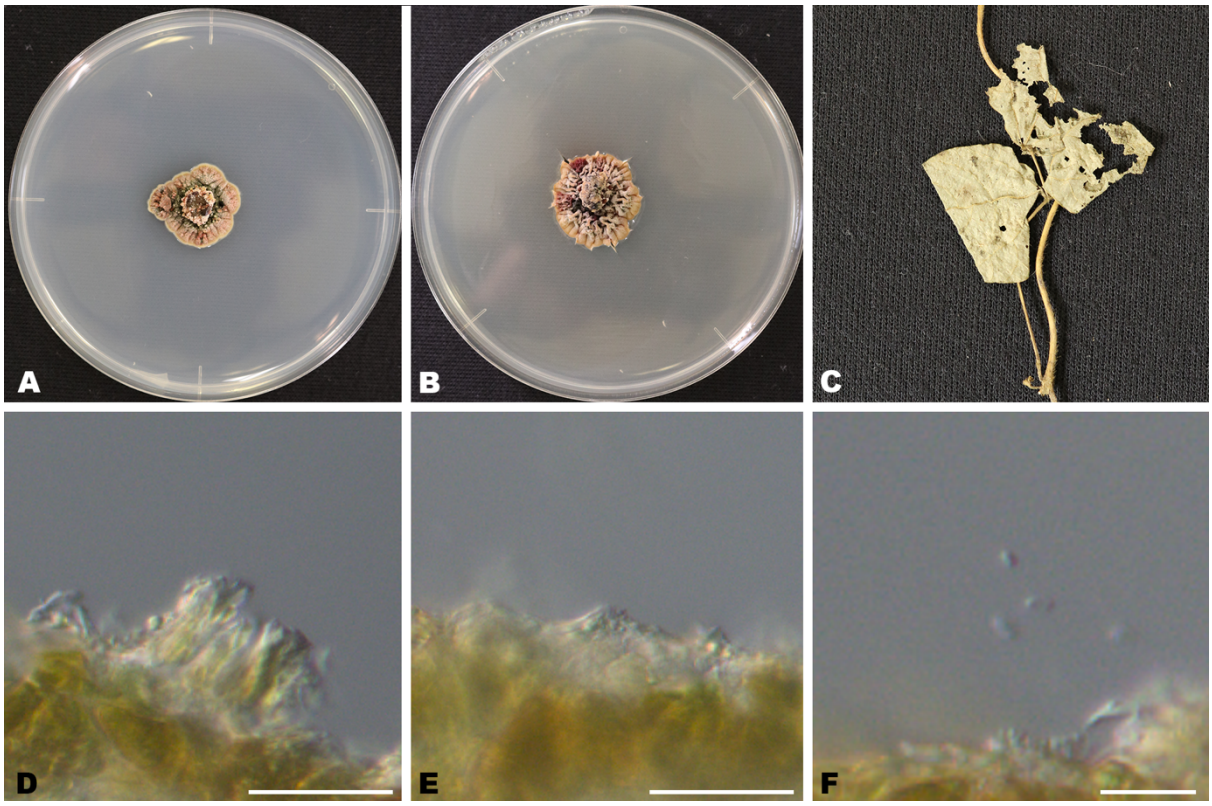

Figure 2.7: Morphological feature of *Elsinoë sp.* on *Amphicarpaea edgeworthii* [A-B: MUCC2986; C-F: TNS-F-185385] (A-B) Isolate MUCC2986 on MA (A) and PDA (B). (C) Specimen TNS-F-185385. (D) Conidiogenous cell. (E) Acervuli. (F) Conidia. (I) Acervuli. Scale bars, 50 µm (D-E) and 10 µm (F).

Sphaceloma tsujii Hara, **Figure 2.8.**

Paulownia leaves, buds, petioles, and stems are first observed with small, slightly amber spots on the leaf surface, which enlarged in round or conical shape, with dark brown margins of 0.8–1.2 mm in diameter, the center symptoms greyish red, slightly concave, eventually perforated. The abaxial symptoms observed as reddish brown with light brown edges. On the petiole, veins, and shoots, the spots may be round or elliptical and reddish brown in colour, but later turn grey and fall into a blotch.

Asexual morph: Mycelium branched with septate, colourless, 3–4 um width, acervuli scattered, subcuticular subsequently erumpent, 30–60 µm in diameter. Conidiogenous cell cylindrical, hyaline, monophialidic, 5–13.2 µm (Hara, 1927). Cultural characteristic: On MA: colony surface cerebriform, salmon; reverse, bay to flesh. On PDA: surface;

cerebriform, folded towards center, rust with buff margin; reverse; brick to cinnamon, folded towards center. On OMA: surface; amber, covered by white short dense arial mycelia folded towards center; Reverse, straw with folding towards the center.

Host: *Paulownia tomentosa* Steud.

Herbarium specimen examined: on *Paulownia tomentosa* Steud., Japan, Tokyo, Forest Experimental Station Meguro, 6 July 1959, collected by K. Ito, (TFM:FPH-0448).

Note: ITS region could not differentiate *Elsinoë tsujii* (MUCC2991) and *Elsinoë rhois* (CBS 519.50), *rpb2* region could not differentiate *Elsinoë tsujii* (MUCC2991) and *Elsinoë tanashiensis* (MUCC3466). Initially described as *Gloeosporium sp.* on *Paulownia sp*. (Tsujii, 1926), *Sphaceloma tsujii* was proposed as a new species by Hara (Hara, 1927) as a pathogen of *Paulownia* sp., however, in the protologue, there was no record of the specimens and isolates kept as holotypus. The isolate used in this study is not linked to any specimens available in records.

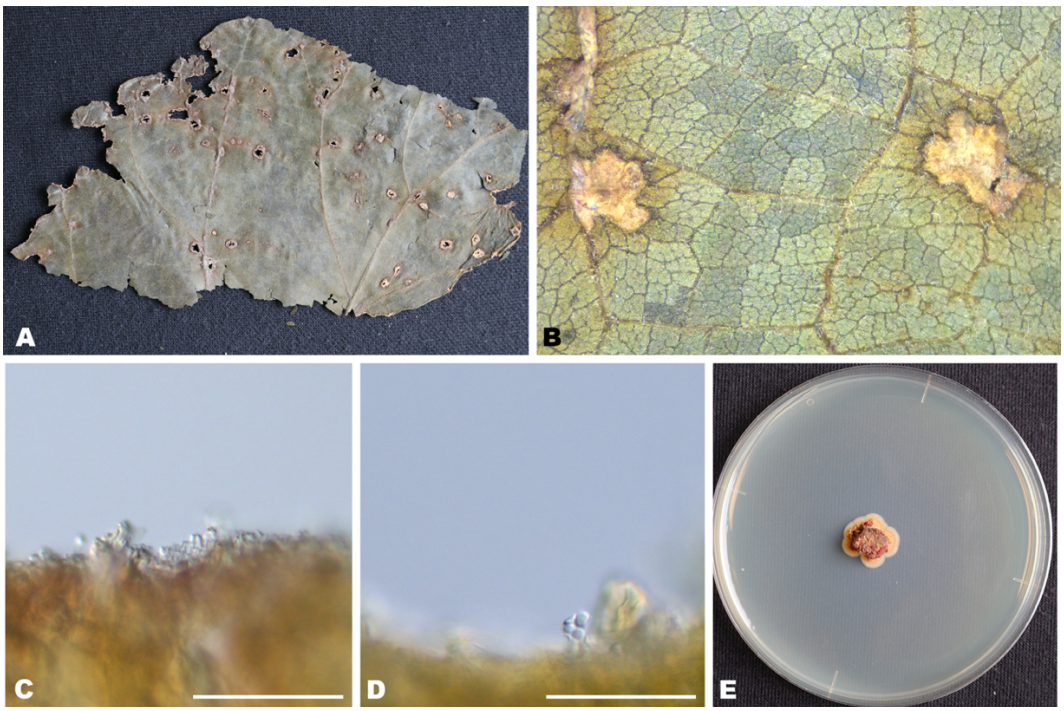

Figure 2.8: Morphological feature of *Sphaceloma tsujii* on *Paulownia tomentosa* [A-E: TFM:FPH-0448. (A) Specimen TFM:FPH-01697. (B) Conidiomata on leaf of *Paulownia tomentosa.* (D) Acervuli. (E) Conidia. (F) Isolate MUCC2991 on MA. Scale bars, 50 µm.

Taxonomical Treatment Based on the Herbarium Specimens

Elsinoë catalpae (Kuros. & Katsuki) A.H. Ujat & C. Nakash. **comb. nov.**

≡ Sphaceloma *catalpae* Kuros. & Katsuki, Botanical Magazine Tokyo 69: 316, 1956.

Holotype: Japan, Tokyo, Hinodai, on *Catalpa speciosa* E.Y. Teas, 2 September 1951, collected by E. Kurosawa (SK 1062).

Herbarium specimen examined: Japan, Chiba, Matsudo, on *Catalpa speciosa* E.Y. Teas, 12 September 1938, by E. Kurosawa (TNS-F-185400 = SK 1061).

Notes: The examined specimen was described in the protologue by Kurosawa and Katsuki (Kurosawa & Katsuki, 1956b).

Elsinoë japonicum (Kuros. & Katsuki) A.H. Ujat & C. Nakash. **comb. nov.**

≡ Sphaceloma *japonicum* Kuros. & Katsuki, Botanical Magazine Tokyo 70: 132, 1956.

Holotypus: Japan, Saitama, Hatogaya, on *Ilex serrata var. sieboldii* (Miq.) Rehder [= *Ilex serrata* Thunb.], 5 September 1938, by E. Kurosawa (SK 1484).

Herbarium specimen examined: Japan, Saitama, Hatogaya, on *Ilex serrata var. sieboldii* (Miq.) Rehder [= *Ilex serrata* Thunb.], 27 September 1938, collected by E. Kurosawa (TSN-F-185384 = SK 1486).

Notes: The examined specimen was described in the protologue by Kurosawa and Katsuki (Kurosawa & Katsuki, 1957).

Elsinoë paederiae (Kuros. & Katsuki) A.H. Ujat & C. Nakash. **comb. nov.**

≡ *Sphaceloma paederiae* Kuros. & Katsuki, Annals of the Phytopathological Society of Japan 21: 15, 1956.

Holotype: Japan, Tokyo, Gotanda, on *Paederia scandens* (Lour.) Merr. [= *P. foetida* L.], 24 July 1938, collected by E. Kurosawa (TNS F-185381 = SK 1380).

Herbarium specimen examined: See holotype.

Notes: The examined specimen was described in the protologue by Kurosawa and Katsuki (Kurosawa & Katsuki, 1956a).

Elsinoë peucedani (Kuros. & Katsuki) A.H. Ujat & C. Nakash. **comb. nov.**

≡ *Sphaceloma peucedani* Kuros. & Katsuki, Botanical Magazine Tokyo 70: 134, 1956.

Holotype: Japan, Kanagawa, Mt. Ohkusu-yama, on *Peucedanum decursivum* (Miq.) Maxim. [= *Angelica decursiva* (Miq.) Franch. et Sav.], 15 September 1940, collected by E. Kurosawa (TNS F-185382 = SK 1512).

Herbarium specimen examined: Japan, Kanagawa, Mt. Ohkusu-yama, on *Peucedanum decursivum* (Miq.) Maxim. [= *Angelica decursiva* (Miq.) Franch. et Sav.], 15 September 1940, collected by E. Kurosawa (holotype TNS-F-185382 = SK 1512; TNS-F-185404).

Notes: The examined specimen was described in the protologue by Kurosawa and Katsuki (Kurosawa & Katsuki, 1956b).

Elsinoë zelkovae (Kuros. & Katsuki) A.H. Ujat & C. Nakash. **comb. nov.**

≡ *Sphaceloma zelkovae* Kuros. & Katsuki, Botanical Magazine Tokyo 69: 318, 1956.

Holotype: Japan, Saitama, Yorii, on *Zelkova serrata* (Thunb.) Makino, 30 July 1936, by E. Kurosawa (TNS-F-185380 = SK 1470).

Herbarium specimens examined: Japan, Saitama, Yorii, on *Zelkova serrata* (Thunb.) Makino, 30 July 1936, collected by E. Kurosawa (TNS-F-185380 = SK 1470); Mie, Tsu, on *Z. serrata*, 11 October 2022, collected by A.H. Ujat & C. Nakashima (TSU-MUMH11970).

Notes: The examined specimen was described in the protologue by Kurosawa and Katsuki (Kurosawa & Katsuki, 1956b).

DISCUSSION

This study examined the taxonomical position of 22 fungal isolates of the genus *Elsinoë* from Japan based on their morphological and molecular phylogeny. Multi-locus phylogenetic analyses showed that isolates were divided into several different clades, and three isolates were recognized as new species. These are *Elsinoë hydrangeae*, *E. tanashiensis*, and *E. sumire*. In accordance with the "one fungus, one name" concept, the anamorphic genus *Sphaceloma* is being relocated under the teleomorphic genus *Elsinoë* (Fan et al., 2017; Marin-Felix et al., 2019). In this study, one new combination of Japanese *Sphaceloma* species was proposed to transfer into *Elsinoë* based on the morphological characteristics of the type specimen and phylogenetic position of the ex-epitype isolate. Moreover, many Japanese isolates formed a clade supported by a high posterior probability value (BS/PP= 88/67) (Fig. 2.2, Clade 2). The internal clade of clade 2 (Clade 2a; BS/PP = 89/97) is composed of several species on various host plants, including herbaceous and arboreal plants. On the other hand, it includes the ex-type strains of *Elsinoë rhois* (Bitanc. & Jenkins) X.L. Fan & Crous on *Toxicodendron vernix* from Brazil and sister to a cosmopolitan species *Elsinoë ampelina* (de Bary) Shear on *Vitis vinifera* from Brazil, forms another internal clade (clade 2b) with Japanese three isolates supported with a high BS/PP (99/100). In addition, another clade (Clade 2c; BS/PP = 90/100) was composed of *E. ricini* (Jenkins & C.C. Cheo) X.L. Fan & Crous from India, *E. akebiae* from Japan, *Elsinoë sp.* on *A. edgeworthii* from Japan, and *E. glycines* (Kurata & Kurib.) X.L. Fan & Crous from Japan. These results suggest that *Elsinoë* species acquire various host plants and speciated in Japan. it could be hypothesised that Japan might be one of the centers of the speciation and diversification of the genus *Elsinoë*.

Elsinoë populi (Sacc.) X.L. Fan & Crous from Argentina (Clade 4) and *E. tanashiensis* from Japan (Clade 2a) are assign to different subclade, despite sharing a common host plant genus, *Populus*. Similar instances are observed on *Elsinoë* species on the plant genus *Viola* (*E. violae* (Massey & Jenkins) X.L. Fan & Crous (Clade 5) and *E. sumire* (Clade 2a)). It is suggested that these species independeantly acquire host plant at a different geographical location. *Elsinoë corni*, isolated from *Cornus florida* in Japan are placed in Clade 3 as an independent species. *Elsinoë corni*, isolated from *Cornus florida* in Japan, is positioned in Clade 3 as an independent species. In this study, a

comparison of morphological characteristics and phylogenetic analysis could not be conducted as there are no specimens linked to the isolate used in this study (MUCC2998), and no phylogenetic analysis was performed on the holotype material by Jenkins & Bitancourt. (Jenkins & Bitancourt, 1948).

In contrast, several species with widespread global distribution and comparable genetic sequences at specific loci, regardless of considerable geographical distances, were documented in previous studies (Fan et al., 2017; Hyun et al., 2009; Marin-Felix et al., 2019), and some were also reconfirmed in this study. These are *E. ampelina*, *E. bidentis* (Bitanc. & Jenkins) Fan & Crous, *E*. *fawcettii* Bitanc. & Jenkins and *E. tiliae* Creelman. Regarding *Elsinoë* species infecting Japanese citrus cultivars, only *E. fawcetti* was identified, not *E. australis* or *E. citricola*. Although E. australis is known as a citrus phytopathogen, its occurrence is confined to Australia, Bolivia, Brazil, and Ethiopia (Fan et al., 2017). *E. citricola* on the other hand was initially identified as *Sphaceloma fawcettii*; however, it can be distinguished based on molecular data from the rpb2 and tef regions, even though the ITS and LSU regions failed to distinguish between E. fawcetti and E. citricola. (Fan et al., 2017). This was confirmed in this study as well, where the *E. fawcetti* isolated in Japan have similar ITS and LSU region with *E. citricola* but different *rpb2* (63/745) and *tef* (2/370). The host plants of these species have been cultivated and distributed globally, suggesting the spread of specific strains of *Elsinoë* species alongside the migration of their host plants.

Generally, each *Elsinoë* species has a narrow host range, which occurs on only one host species or genus (Fan et al., 2017; Marin-Felix et al., 2019). In this study, despite having the same host plant, *Viola* sp., a new species of *Elsinoë* was proposed based on the morphological and phylogenetic analysis. Fan et al., (Fan et al., 2017) showed that *E. violae* has more than one host genus, *Viola* sp. and *Symphocarpos* sp. (*Caprifoliaceae*), from the phylogenetic analysis using a multi-locus combined matrix. In contrast, our results suggest that E. sumire is a closely related species to E. rhois (Bitanc. & Jenkins) X.L. Fan & Crous on *Rhus vernix* L. (syn. *Toxicodendron vernix* Kuntze). According to Fan et al. (Fan et al., 2017), *Elsinoë leucospermi*, *E. anacardia*, *E. violae* and *E. piri* were found to occur on more than one host genus. *Elsinoë piri* (Woron.) Jenkins on different host genera, *Pyrus* (CBS 163.29) and *Malus* (Rosaceae) (CBS 179.82), has been recognized as

one species of *Elsinoë*, having 6/370 site changes of *tef* region sequence. In this study, the criteria for species delimitation within the genus *Elsinoë* were comprehensively assessed based on the host plants, morphological characteristics, and phylogenetic relationships, without synonymizing the previously known species. Further discussion on *Elsinoë* species delimitation, based on experimental host range or new barcode region sequences within this genus, is warranted.

CONCLUSION

A total of 22 fungal isolates from Japan underwent examination to determine their taxonomical position through a polyphasic approach, combining morphological and molecular methods. The multi-locus phylogenetic assessment revealed that Japanese isolates were mostly clustered together on the phylogenetic tree of the genus *Elsinoë*. This also led to the identification of three novel species in the Japanese mycoflora. According to the code of "one fungus, one name," *Sphaceloma akebiae* position was confirmed in the *Elsinoë* phylogeny, necessitating the systematic repositioning of this species within the genus *Elsinoë* and the need for a new combination, *Elsinoë akebiae*.

From this study, it is observed that Japanese isolates of *Elsinoë* coalesce into a distinct clade with robust support. It also shows that although the same genus of fungi is found on the same genus of host plant, geographical region might play a part in the diversification of the genus *Elsinoë*. This suggests that the genus *Elsinoë* within Japan is endemic, and diversification happens on the island of Far East Asia. However, there are also several species that display a worldwide geographical distribution regardless of the geographical distance. This shows the adaptability of cosmopolitan species of *Elsinoë*, and providing some insight into the migration pattern of the fungi following the host plant.

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Chapter 3 – *Sphaerulina*

Summary

Sphaerulina species are plant pathogenic fungi that cause leaf spot diseases of various plants, including arboreous and herbaceous plants. Those morphological characteristics of the anamorphic state and leaf spot symptoms are the same as those caused by *Septoria* spp., known as a synanamorph of *Sphaerulina* under the previous code. The recent revision to the taxonomy at the genus level based on morphological characteristics and phylogenetic relationships resulted in the differentiation of these two genera into independent genera of *Sphaerulina* and *Septoria* under the new code. This study contributes to revealing the species diversity of the genus *Sphaerulina* from Japan and those phylogenetic relationships by multi-loci phylogenetic relationships. Moreover, from the results of phylogenetic analysis, seven novel species (*Sph. farfugii, Sph. hydrangeicola, Sph. idesiae, Sph. lapsanastri, Sph. miurae, Sph. styracis,* and *Sph. viburnicola)* were proposed, and two species (*Sph. duchesnea* & *Sph. nambuana*) were transferred from the genus *Septoria*.

Keywords: Multi-locus phylogenies, *Sphaerulina*, Systematics, Taxonomy

INTRODUCTION

The genus *Sphaerulina, Mycosphaerellaceae,* was established by Saccardo (1878) and is mainly known as foliar pathogens of arboreous and herbaceous plants. It has sphere-shaped perithecia and forms cylindrical or oblong, 3-pluriseptate, and hyaline ascospores. Its type species is *Sphaerulina myridea*, occurring on the plant family *Fagaceae*.

Based on the ascospore septation, this genus was traditionally distinguished from the genus *Mycosphaerella*, which has 1-septate ascospore, shares common synanamorphs, and is known as plant parasitic fungi. However, the number of septations in ascospores was an unstable criterion for distinguishing the ascomycetous genera by phylogenetic analyses (Crous et al., 2003, 2011). On the other hand, the morphology of the anamorphic state of *Sphaerulina* is recognised as *Septoria* s*ensu lato* (s.l.) or *Septoria*-like belonging to *Mycosphaerellaceae* (Quaedvlieg et al., 2013; Verkley et al., 2013). From a comprehensive taxonomical study of *Mycosphaerellaceae* with multi-loci phylogenetic analyses and morphology (Videira et al., 2017), each genus of *Septoria* complex (Quaedvlieg et al., 2013) formed independent clades recognised as a genus. In a recent study (Videira et al., 2017), *Sphaerulina* species formed a well-supported clade with *Miuraea* species, which bear cylindrical to obclavate conidia in pale, and it was separated from *Septoria sensu stricto* (s.s.) clade even though these species are morphologically similar in morphology having numerous cylindrical to acicular conidia in a pycnidium.

As of 2023, 217 species of *Sphaerulina*, including synonyms, were recorded in the Mycobank Database (https://www.mycobank.org). In Japanthe listed species of *Sphaerulina* are exclusively based on the teleomorphic state, which follows the dual-naming system in accordance with the International Code of Botanical Nomenclature that the current code, the International Code of Nomenclature for Algae, Fungi, and Plants had replaced. Hence, this implies the true diversity of Japanese *Sphaerulina* species is still unclear as it currently includes synamorphs belonging to *Mycosphaerllaceae*, such as *Cercospora* species. The aim of this study was thus to reveal the species diversity of the genus *Sphaerulina* in Japan based on the

current taxonomical criteria, using multi-loci phylogenetic analyses, morphology, and cultural characteristics.

In the context of a taxonomic and systematic study, it's crucial to emphasize the significance of accurately identifying and classifying species within the genus *Sphaerulina*. By delving into the taxonomic intricacies of *Sphaerulina* species, this study aims to contribute to our broader understanding of fungal diversity and evolution. Accurate species identification is essential not only for academic purposes but also for practical applications such as disease identification and management in agricultural and natural ecosystems. Furthermore, elucidating the phylogenetic relationships within *Sphaerulina* can provide insights into their evolutionary history and ecological adaptations. By employing multi-loci phylogenetic analyses, morphology assessments, and cultural characteristics, this study seeks to refine our taxonomic understanding of *Sphaerulina* species in Japan.

MATERIAL AND METHOD

Sample collection and morphological study

Specimens and culture kept at the Herbarium (TSU-MUMH) and its Culture Collection (MUCC), the Laboratory of Phytopathology, Mie University, Mie, Japan, and the Herbarium, the Laboratory of Forest Pathology, Forestry and Forest Products Research Institute (TFM: FPH), Tsukuba, Ibaraki, Japan, were examined for morphological and molecular phylogenetic analyses, along with the isolates obtained from the NARO Genebank Project of the National Agriculture and Food Research Organization, Tsukuba, Japan (MAFF). Newly collected symptomatic specimens and those isolates are shown in Table 3.1. Cultural characteristics of isolates were observed on Oatmeal agar (OMA; Becton Dickinson, MD, USA), Potato Dextrose Agar (PDA; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), and 2% Malt Extract Agar (MEA; Becton Dickinson) for 25 days and maintained on Malt Agar (MA; Becton Dickinson). The hand section of the herbarium specimens was prepared to observe the morphological characteristics of fungal bodies on the host plant and observed under a compound microscope Axio Imager A1 with the Shear's solution (Chupp, 1940a) as mounting medium.

DNA extraction, amplification, and sequencing

The genomic DNA was extracted using DNeasy Ultra Clean Microbial Kit (Qiagen, Hilden, Germany) from mycelia of growing culture according to the manufacturer's instructions. Seven genomic loci of the DNA regions, including ITS, LSU, BTUB, RPB2, TEF-1a, CAL, and ACT, were amplified via polymerase chain reaction using the T100 thermal cycler. 12.5 µL of PCR final mixture was prepared as follows: 1–10 ng of genomic DNA, 0.1 µL of 0.25 U Taq DNA polymerase (Bioline, London, UK), 2.5 mM of $MgCl_2$, 1.25 µL of 10× NH_4 reaction buffer (Bioline), 40 µM dNTPs (Bioline) and 0.2 µM of each primer. For RPB2, 12.5 µL PCR final mixture was prepared as follows: 20-25 ng of genomic DNA, 0.07 µL of TaKaRa Taq hot start version (TaKaRa Bio, Shiga, Japan), 1.25 µL of Buffer $MgCl_2$ mixture (TaKaRa Bio), 1 µL of dNTPs (TaKaRa Bio) and 0.25 µL of each primer. All PCR conditions and primers used in this study were as follows with different annealing temperatures as listed in Table 3.2: Initial denaturation at 94°C for 5 minutes, followed by denaturation

Table 3.1. Host plant, Isolate number, voucher number and locality.

Isolate name	Recorded name	Isolate number	Voucher number	Locality	Host Plant
Sphaerulina abeliceae	*Septoria abeliceae*	MUCC 1568, MAFF 237886		Kanagawa	*Zelkova serrata*
Sph. abeliceae	*Sep. abeliceae*	MUCC 3542	TSU - MUMH 11970	Tsu, Mie	*Z. serrata*
Sph. abeliceae	*Sep. abeliceae*	MUCC 3616	TSU - MUMH 11992	Tsu, Mie,	*Z. serrata*
Sph. azaleae	*Sep. azaleae*	MUCC 2868	TSU - MUMH 11887	Tsu, Mie	*Rhododendron sp.*
Sph. azaleae	*Sep. azaleae*	MUCC 3546	TSU - MUMH 11974	Tsu, Mie	*Rhododendron sp.*
Sph. azaleae	*Sep. azaleae*	MUCC 637, MAFF 240608	TSU - MUMH 10583	Nishinomote, Kagoshima	*Rhododendron sp.*
Sph. azaleae	*Sep. azaleae*	MUCC 639, MAFF 240610	TSU - MUMH 10586	Nishinomote, Kagoshima	*Rhododendron scabrum*
Sph. chaenomelis	*Pseudocercosporella chaenomelis*	MUCC 1510		Tsu, Mie	*Chaenomeles sinensis*
Sph. duchesneae	*Septoria duchesneae*	MUCC 1589, MAFF 238130	TSU - MUMH 11999	Ohara, Kyoto	*Duchesnea chrysantha*
Sph. farfugii	*Sep. tussilaginis*	MUCC 1576, MAFF 237889		Matsuida, Gunma	*Farfungium japonicum*
Sph. farfugii	*Sep. tussilaginis*	MUCC 1579, MAFF 238285		Tsukuba, Ibaraki	*F. japonicum*
Sph. farfugii	*Sep. tussilaginis*	MUCC 1605, MAFF 237233		Kanoya, Kagoshima	*F. japonicum*
Sph. farfugii	*Sphaerulina* sp.	MUCC 3622	TSU - MUMH 11998	Tsu, Mie	*F. japonicum*
Sph. hydrangeicola	*Septoria* sp.	MUCC 694	TSU - MUMH 10651	Sugadira, Nagano	*Hydrangea paniculata*
Sph. idesiae	*Septoria* sp.	MUCC 813, MAFF 240634	TSU - MUMH 10917	Kunigami, Okinawa	*Idesia polycarpa*
Sph. juglandis	*Pseudocercosporella juglandis*	MUCC 1478, MAFF 23762		Itoigawa, Toyama	*Juglans sieboldiana*

Sph. lapsanastri	*Septoria* sp.	MUCC 1582, MAFF 237801	TSU - MUMH 12001	Tokyo	*Lapsanastrum humile*
Sph. miurae	*Sep. astericola*	MUCC 731	TSU - MUMH 10857	Hidakagawa, Wakayama	*Aster tataricus*
Sph. nambuana	*Sep. nambuana*	MUCC 1586, MAFF 237810	TSU - MUMH 12000	Nikko, Tochigi	*Lysimachia fortunei*
Sphaerulina sp.	*Sep. alni*	MUCC 1573, MAFF 410471		Meguro, Tokyo	*Alnus pendula*
Sphaerulina sp.	*Sep. alni*	MUCC 3526, MAFF 410476		Koma, Iwate	*Alnus hirsuta*
Sph. styracis	*Septoria* sp.	MUCC 1603, MAFF 240599	TSU - MUMH 10537	Aira, Kagoshima	*Styrax obassia*
Sph. viburnicola	*Septoria* sp.	MUCC 349	TSU - MUMH 10249	Chikusa, Aichi	*Viburnum wrightii*

at 94°C for 5 minutes, annealing at designated temperature for 30 seconds, elongation at 72°C for 45 seconds and final extension at 72°C for 7 minutes. Amplicons were sequenced in both directions using the respective PCR primers and BigDye terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an Applied Biosystems 3730xl DNA analyser installed at the Mie University Advanced Science Research Promotion Center (Tsu, Mie, Japan).

Table 3.2. Primer used in this study for amplification and sequencing.

Region /Locus	Primer	Sequences	Annealing temperature
Actin	ACT-512F	ATGTGCAAGGCCGGTTTCGC	52
	ACT2Rd	ARRTCRCGDCCRGCCATGTC	
TEF	EF1-728F	CATCGAGAAGTTCGAGAAGG	52
	EF2	GGARGTACCAGTSATCATGTT	
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	52
	ITS4	TCCTCCGCTTATTGATATGC	
β-tubulin	T1	AACATGCGTGAGATTGTAAGT	52
	β-Sandy-R	GCRCGNGGVACRTACTTGTT	
LSU	LSU1Fd	GRATCAGGTAGGRATACCCG	52
	LR5	TCCTGAGGGAAACTTCG	
RPB2	fRPB2-5F	GAYGAYMGWGATCAYTTYGG	49
	fRPB2-414	ACMANNCCCCARTGNGWRTTRTG	
Calmodulin	CAL-235F	TTCAAGGAGGCCTTCTCCCTCTT	50
	CAL2Rd	TGRTCNGCCTCDCGGATCATCTC	

Phylogenetic analysis

The newly analysed sequences were assembled with sequences retrieved from previous studies listed in Table 3.3, mainly conducted by Quaedvlieg et al. (2013) and Verkley et al. (2013) on MEGA X software package (Kumar et al., 2016) . The matrix was aligned using MAFFT 7.0 (Katoh & Standley, 2013) and edited manually using AliView (Larsson, 2014). Phylogenetic trees generated by Maximum-likelihood (ML) and Bayesian

Inference analyses were used in this study to estimate the phylogenetic relationship. The best substitution model for each region in the analysis was evaluated using ModelTest-NG (Darriba et al., 2020) and applied. ML analyses were performed using RAxML-NG (Kozlov et al., 2019) with 1000 replications of bootstrap analysis to test for tree strength. BI analysis was performed by using MrBayes (Ronquist et al., 2012). BI analysis was performed using MrBayes (Ronquist et al., 2012). To estimate the posterior probability of tree topologies, Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) searches were run for 10 million generations, with trees sampled and saved every 1000 generations. The first 25% of the tree was discarded as a burn-in phase based on the Average Standard Deviation of Split Frequencies (Below 0.01), and the PPs were determined using the remaining trees. *Caryophyllospetoria spergulae* (CBS 397.52) was used as an outgroup in this study, and the generated trees were viewed using FigTree v 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). The alignment and respective phylogenetic trees were deposited in TreeBASE (S30735).

Table 3.3: List of isolates and reference sequence used in this study.

Fungal species	Isolate number	Country	Host	Genbank Accession number						
				EF	TUB	RPB2	LSU	ITS	ACT	CAL
Sphaerulina abeliceae	**MUCC3616**	**Japan**	***Zelkova serrata***	**OR507753**	**OR507780**	**OR507727**	**OR492441**	**OR492384**	**OR507701**	**OR507807**
Sph. abeliceae	**MUCC1568**	**Japan**	***Zelkova serrata***	**OR507754**	**OR507781**	**OR507728**	**OR492442**	**OR492385**	**OR507702**	**OR507808**
Sph. abeliceae	**MUCC 3542**	**Japan**	***Zelkova serrata***	**OR507755**	**OR507782**	**OR507729**	**OR492443**	**OR492386**	**OR507703**	**OR507809**
Sph. abeliceae	CBS 128591	South Korea	*Zelkova serrata*	KF253540	n/a	KF252585	KF252097	KF251594	KF253894	KF254245
Sph. aceris	CBS 687.94	Netherlands	*Acer pseudoplatuanus*	KF253542	KF253061	KF252588	KF252100	KF251595	KF253897	KF254247
Sph. amelanchier	CPC 23106	Netherlands	*Castanea sp.*	KF253545	KF253064	KF252591	KF252103	KF251598	KF253900	KF254250
Sph. amelanchier	CPC 23105	Netherlands	*Quercus sp.*	KF253544	KF253063	KF252590	KF252102	KF251597	KF253899	KF254249
Sph. amelanchier	CBS 102063	New Zealand	*Actinidia deliciosa*	KF253581	KF253096	KF252627	KF252140	KF251635	KF253935	KF254286
Sph. amelanchier	CPC 23107	Netherlands	*Betula sp.*	KF253583	KF253098	KF252626	KF252139	KF251634	KF253937	KF254288
Sph. amelanchier	CBS 135110	Netherlands	*Amelanchier sp.*	KF253543	KF253062	KF252589	KF252101	KF251596	KF253898	KF254248
Sph. azaleae	**MUCC 3546**	**Japan**	***Rhododendron sp.***	**OR507771**	**OR507797**	**OR507744**	**OR492458**	**OR492401**	**OR507718**	**OR507825**
Sph. azaleae	**MUCC 2868**	**Japan**	***Rhododendron sp.***	**OR507772**	**OR507798**	**OR507745**	**OR492459**	**OR492402**	**OR507719**	**OR507826**
Sph. azaleae	**MUCC 637**	**Japan**	***Rhododendron sp.***	**OR507773**	**OR507799**	**OR507746**	**OR492460**	**OR492403**	**OR507720**	**OR507827**
Sph. azaleae	**MUCC 639**	**Japan**	***Rhododendron scabrum***	**OR507774**	**OR507800**	**OR507747**	**OR492461**	**OR492404**	**OR507721**	**OR507828**
Sph. azaleae	CBS 352.49	Belgium	*Rhododendron sp.*	KF253547	KF253066	KF252593	KF252105	KF251600	KF253902	KF254252
Sph. azaleae	CBS 128605	South Korea	*Rhododendron sp.*	KF253546	KF253065	KF252592	KF252104	KF251599	KF253901	KF254251
Sph. berbedis	CBS 324.52	Switzerland	*Berberis vulgaris*	KF253548	KF253067	KF252594	KF252106	KF251601	KF253903	KF254253
Sph. betulae	CBS 116724	Netherlands	*Betula pubescens*	KF253549	KF253068	KF252595	KF252107	KF251602	KF253904	KF254254
Sph. betulae	CBS 128600	South Korea	*Betula platyphylla*	KF253552	KF253071	KF252598	KF252110	KF251605	KF253907	KF254257

Sph. cercidis	CBS 128634	Argentina	*Cercis siliquastrum*	KF253554	KF253073	KF252599	KF252111	KF251606	KF253909	KF254259
Sph. cercidis	CBS 118910	France	*Eucalyptus sp.*	KF253553	KF253072	KF252602	KF252114	KF251609	KF253908	KF254258
Sph. cercidis	CBS 129151	Argentina	*Cercis siliquastrum*	KF253555	KF253074	KF252600	KF252112	KF251607	KF253910	KF254260
Sph. cercidis	CBS 501.50	Netherlands	*Cercis siliquastrum*	KF253556	KF253075	KF252601	KF252113	KF251608	KF253911	KF254261
Sph. chaenomelis	**MUCC 1510**	**Japan**	***Chaenomeles sinensis***	**OR507756**	**OR507787**	**OR507730**	**OR501404**	**JQ793663**	**JQ793664**	**OR507810**
Sph. cornicola	CBS 102324	Netherlands	*Cornus sp,*	KF253557	KF253076	KF252603	KF252115	KF251610	KF253912	KF254262
Sph. cornicola	CBS 102332	Netherlands	*Cornus sp.*	KF253558	KF253077	KF252604	KF252116	KF251611	KF253913	KF254263
Sph. duchesneae	MUCC 1589	Japan	*Duchesnea chrysantha*	OR507775	OR507788	OR507748	OR492462	OR492405	OR507722	OR507829
Sph. farfuginis	**MUCC 1605**	**Japan**	***Farfungium japonicum***	**OR507762**	**OR507792**	**OR507736**	**OR492449**	**OR492392**	**OR507709**	**OR507816**
Sph. farfuginis	**MUCC 1576**	**Japan**	***Farfungium japonicum***	**OR507763**	**OR507793**	**OR507737**	**OR492450**	**OR492393**	**OR507710**	**OR507817**
Sph. farfuginis	**MUCC 1579**	**Japan**	***Farfungium japonicum***	**OR507764**	**OR507795**	**OR507738**	**OR492451**	**OR492394**	**OR507711**	**OR507818**
Sph. farfuginis	**MUCC 3622**	**Japan**	***Farfungium japonicum***	**OR507765**	**OR507794**	**n/a**	**OR492452**	**OR492395**	**OR507712**	**OR507819**
Sph. frondicola	CBS 391.59	Germany	*Populus pyramidalis*	KF253572	n/a	KF252617	KF252130	KF251625	KF253927	KF254277
Sph. gei	CBS 128632	South Korea	*Geum urbanum*	KF253562	KF253081	KF252607	KF252120	KF251615	KF253917	KF254267
Sph. gei	CBS 102318	Netherlands	*Geum japnicum*	KF253560	KF253079	KF252605	KF252118	KF251613	KF253915	KF254265
Sph. hydrangeicola	**MUCC 694**	**Japan**	***Hydrangea paniculata***	**OR507770**	**OR507806**	**OR507743**	**OR492457**	**OR492400**	**OR507717**	**OR507824**
Sph. hyperici	CBS 102313	Netherlands	*Hypericum sp.*	KF253563	KF253082	KF252608	KF252121	KF251616	KF253918	KF254268
Sph. idesiae	**MUCC 813**	**Japan**	***Idesia polycarpa***	**OR507769**	**OR507805**	**OR507742**	**OR492456**	**OR492399**	**OR507716**	**OR507823**
Sph. juglandis	MUCC 1475	Japan	*Juglan sieboldiana*	OR637380	OR637381	OR637382	OR636057	OR636056	OR637379	OR637383
Sph. koreana	CBS 135462	South Korea	*Vicia amurensis*	GU384564	n/a	n/a	GU214683	GU269852	GU320556	n/a

Sph. koreana	CBS 131898	South Korea	*Vicia amurensis*	KF253586	KF253101	KF252631	KF252144	KF251639	KF253940	KF254291
Sph. lapsanastri	**MUCC 1582**	**Japan**	***Lapsanastrum humile***	**OR507768**	**OR507791**	**OR507741**	**OR492455**	**OR492398**	**OR507715**	**OR507822**
Sph. menispermi	CBS 128666	South Korea	*Menispermim dauricum*	KF253564	KF253083	KF252609	KF252122	KF251617	KF253919	KF254269
Sph. menispermi	CBS 128761	South Korea	*Menispermim dauricum*	KF253565	KF253084	KF252610	KF252123	KF251618	KF253920	KF254270
Sph. miurae	MUCC 731	Japan	*Aster tataricus*	OR507757	OR507796	OR507731	OR492444	OR492387	OR507704	OR507811
Sph. musiva	CBS 130569	Canada	*Populis deltoides*	KF253569	KF253086	KF252614	KF252127	KF251622	KF253924	KF254274
Sph. myriadea	CBS 124646	Japan	*Quercus dentata*	KF253201	KF252734	KF252256	KF251754	KF251251	n/a	n/a
Sph. nambuana	**MUCC 1586**	**Japan**	***Lysimachia fortunei***	**OR507766**	**OR507789**	**OR507739**	**OR492453**	**OR492396**	**OR507713**	**OR507820**
Sph. nambuana	CBS 128758	South Korea	*Lysimachia clethroides*	KF253582	KF253097	KF252628	KF252141	KF251636	KF253936	KF254287
Sph. oxyacanthae	CBS 135098	Netherlands	*Crataegus sp.*	KF253202	KF252735	KF252257	KF251755	KF251252	n/a	n/a
Sph. patriniae	CBS 128653	South Korea	*Patrinia scabiosaefolia*	KF253570	KF253087	KF252615	KF252128	KF251623	KF253925	KF254275
Sph. populicola	CBS 100042	USA	*Populius trichocarpa*	KF253573	n/a	KF252618	KF252131	KF251626	KF253928	KF254278
Sph. pseudovirgaureae	CBS 135109	Netherlands	*Solidago gigantea*	KF253203	KF252736	KF252258	KF251756	KF251253	n/a	n/a
Sph. quercicola	CBS 115016	Netherlands	*Quercus rubra*	KF253575	KF253090	KF252620	KF252133	KF251628	KF253930	KF254280
Sph. quercicola	CBS 115136	Netherlands	*Quercus rubra*	KF253576	KF253091	KF252621	KF252134	KF251629	KF253931	KF254281
Sph. quercicola	CBS 109009	Netherlands	*Quercus rubra*	KF253574	KF253089	KF252619	KF252132	KF251627	KF253929	KF254279
Sph. quercicola	CBS 663.94	Netherlands	*Quercus robur*	KF253577	KF253092	KF252622	KF252135	KF251630	KF253932	KF254282
Sph. rhabdoclinis	CBS 102195	Germany	*Pseudotsuga menziesii*	KF253578	KF253093	KF252623	KF252136	KF251631	n/a	KF254283
Sph. socia	CBS 355.58	—	*Rosa sp.*	KF253579	KF253094	KF252624	KF252137	KF251632	KF253933	KF254284
Sph. socia	CBS 357.58	Germany	*Chrysanthemum leucanthemum*	KF253580	KF253095	KF252625	KF252138	KF251633	KF253934	KF254285

***Sphaerulina* sp. on *Alnea* sp.**	**MUCC 1573**	**Japan**	***Alnus pendula***	**OR507776**	**OR507783**	**OR507749**	**OR492463**	**OR492406**	**OR507723**	**OR507830**
***Sphaerulina* sp. on *Alnea* sp.**	**MUCC 3526**	**Japan**	***Alnus hirsuta***	**OR507777**	**OR507784**	**OR507750**	**OR492464**	**OR492407**	**OR507724**	**OR507831**
Sph. styracis	**MUCC 1603**	**Japan**	***Styrax obassia***	**OR507759**	**OR507804**	**OR507733**	**OR492446**	**OR492389**	**OR507706**	**OR507813**
Sph. tirolensis	CBS 109018	Austria	*Rubus idaeus*	KF253585	KF253100	KF252630	KF252143	KF251638	KF253939	KF254290
Sph. tirolensis	CBS 109017	Austria	*Rubus idaeus*	KF253584	KF253099	KF252629	KF252142	KF251637	KF253938	KF254289
Sph. viburnicola	**MUCC 349**	**Japan**	***Viburnum wrightii***	**OR507761**	**OR507802**	**OR507735**	**OR492448**	**OR492391**	**OR507708**	**OR507815**
Sph. westendorpii	CBS 117478	Netherlands	*Rubus sp.*	KF253587	KF253102	KF252632	KF252145	KF251640	KF253941	KF254292
Caryophyllospetoria spergulae	CBS 397.52	Netherlands	*Dianthus caryophyllus*	KF253243	KF252777	KF252301	KF251799	KF251295	KF253604	KF253958

RESULT

Phylogeny

A total of 67 OTUs belonging to the genus *Sphaerulina*, including 23 OTUs obtained in this study, were aligned and analyzed. The concatenated data matrix of seven genomic loci consisted of 3262 characters, including gaps (ACT: 237 bp, TEF-1a: 433 bp, ITS: 613 bp, BTUB: 362 bp, LSU: 822 bp, RPB2: 344 bp, and CAL: 493 bp). Both BI analysis and ML analysis showed congruent tree topology. All branches and internal nodes of the ML tree were strongly supported with bootstrap values of more than 60%. Although most of the Japanese isolates used in this study had been recorded as *Septoria* species, phylogenetic analysis reveals that they should be treated as *Sphaerulina* species, as shown in Figure 3.1. Moreover, seven novel species which includes, *Sphaerulina farfugii, Sphaerulina. hydrangeicola, Sphaerulina idesiae, Sphaerulina lapsanastri, Sphaerulina miurae, Sphaerulina styracis,* and *Sphaerulina viburnicola* and two new combinations, *Sphaerulina duchesneae* and *Sphaerulina nambuana*, are proposed as Japanese *Sphaerulina* species.

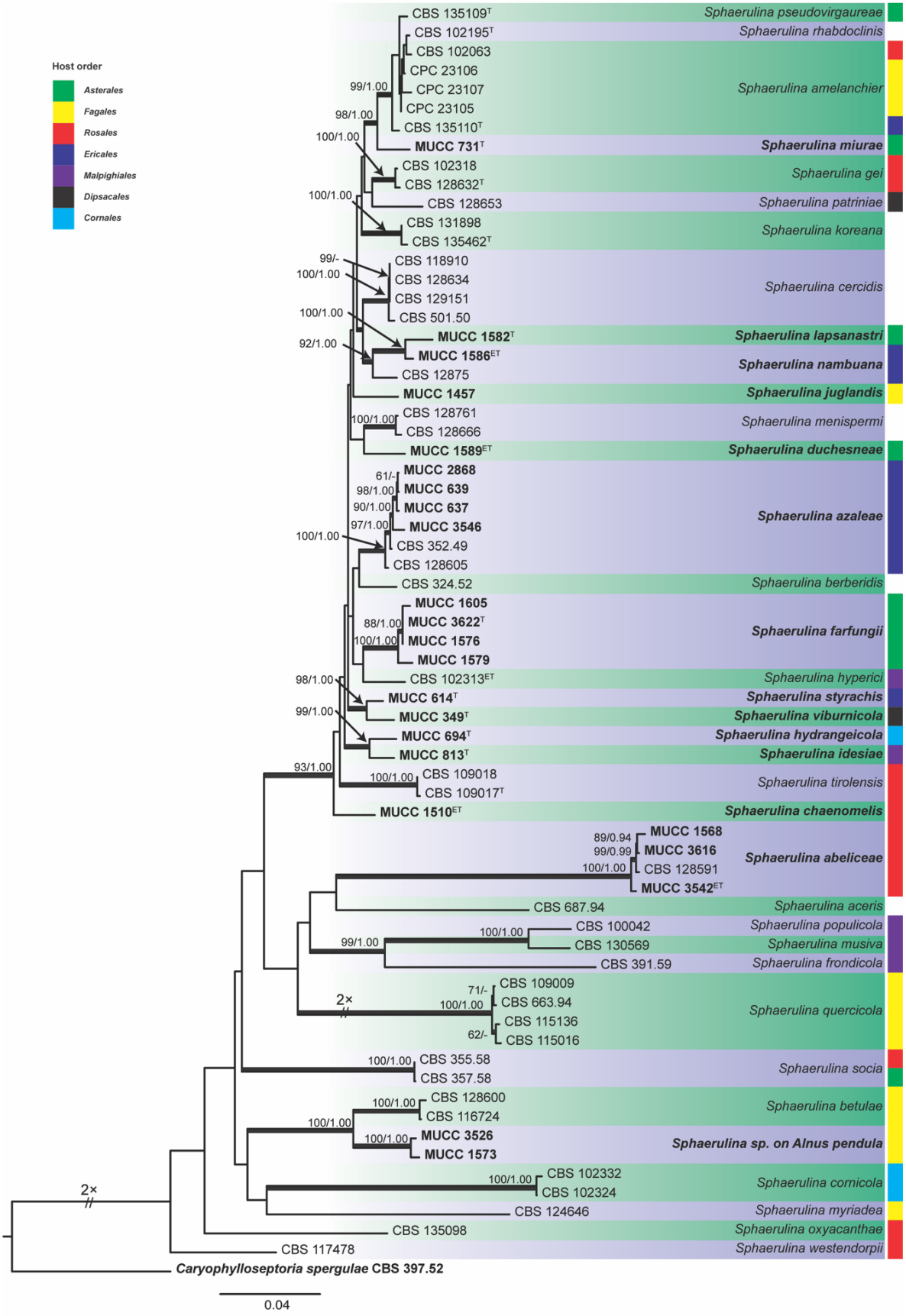

Figure 3.1. Maximum-likelihood phylogentic tree of *Sphaerulina* spp. constructed by using concatenated matrix of 7 loci. The bootstrap value of ML and posterior probability of BI are indicated near branch as BS/PP.

Taxonomy

Sphaerulina abeliceae (Hiray.) Quaedvlig, Verkley & Crous, Stud. Mycol. 75: 343, 2013. Figure 3.2

≡ *Septoria abeliceae* Hiray., Mem Col. Agr. Kyoto Imp. Univ. 13(3): 33, 1931.

Description: See Hirayama (1931)

Typus (Syntypes): On *Abelice hirta* Schn. (= *Zelkova serrata* (Thunb.) Makino), **Japan**, Nagano, Nunobiki, 11 July 1925, by K. Togashi (Kyoto University Mueseum: KYO); Ishikawa, Kanazawa, 26 August 1928, by J. Tokuda (KYO); **Lectotype** designated here, MBT 10015626, Ishikawa, Kanazawa, 26 August 1928, by J. Tokuda (KYO)(syntype); **Epitype** designated here MBT 10015627, Mie, on leaves on *Zelkova serrata*, 11 October 2022, by A.H. Ujat (TSU-MUMH 11970, culture ex-epitype MUCC 3542).

Additional specimen examined: **Japan**, Mie, Tsu, on *Zelkova serrata*, 31 May 2023, by A.H. Ujat (TSU-MUMH 11992, culture MUCC3616).

Additional culture examined: **Japan**, Kanagawa, Kawasaki, on *Zelkova serrata*, May 1999, by K. Kishi (MAFF 237886 = MUCC 1568).

Notes: The *Septoria* leaf spot on *Zelkova serrata* caused by *Sph. abeliceae* is widely distributed in Japan. Its etiological study was conducted by Ito and Hosaka (1952). They suggested the teleomorphic state rarely observed on the fallen leaves infected by *Sph. abeliceae* differ from *Mycosphaerella zelkowae* Sydow & Hori, a *Mycosphaerella* species on *Zelkova* tree based on its morphology. *Septoria abeliceae*, basionym of *Sph. abeliceae*, was described by Hirayama (1931) based on the Japanese specimens from Japan on a leaf spot pathogen of the symptomatic leaf of *Zelkova serrata* (*Abelicea hirta*). Quaedvlieg et al. (2013) transferred the genus from *Septoria* to *Sphaerulina* based on the Korean material without the designation of lectotype and epitype. The type specimens (syntypes) have been stored in the Herbarium in the Kyoto University Museum, Kyoto University, Kyoto, Japan (KYO) (Figure 7C). For further study with

specimens and its isolate, lectotype was selected from syntype, and the epitype was designated. *Zelkova* (*Ulmaceae* s.s.) is a small genus that comprises six monoecious tree species in the northern hemisphere. At present, it shows a disjunct distribution in East Asia, Western Asia, and the Mediterranean (Denk & Grimm, 2005). On the phylogenetic tree generated in this study, the isolates of the present species, including the Korean isolate (CBS 128691), formed a well-supported clade even though there were several mutations in the TEF region among isolates. There is no conflict between the habitat of the host and that of the parasitic fungus.

Spaherulina azaleae (Voglino) Quaedvlieg, Verkley & Crous, Stud. Mycol. 75: 345, 2013. Figure 3.2

≡ *Septoria azaleae* Voglino., Syll. Fung. (Abellini) 14(2): 976, 1899.

≡ *Phloeospora azalea* (Voglino) Priest, Fungi of Australia: 224, 2006.

Description: See Saccardo & Sydow, 1899

Specimens examined: **Japan**, Mie, on leaves of *Rhododendron* sp. (*Ericaceae*), 11 October 2022, by A.H. Ujat & C. Nakashima (TSU-MUMH 11974, culture MUCC 3546); **Japan**, Kagoshima, Tanegashima Is., Nishinoomote, on *Rhododendron* sp. (*Ericaceae*), 30 May 2007, C. Nakashima & K. Motohashi (TSU-MUMH 10583, culture MUCC 637 = MAFF 240680); ibid, on *Rhododendron scabrum* (*Ericaceae*), 30 May 2007, C. Nakashima & K. Motohashi (TSU-MUMH 10586, culture MUCC 639 = MAFF 240610).

Additional cultures examined: **Japan**, Mie, Tsu, on leaves of *Rhododendron* sp. (*Ericaceae*), June 2019, by K. Kuroda (MUCC 2868)

Notes: The present species widely distribute in Japan and known as *Septoria azaleae* and it male a well-supported clade with the isolates on leaves of *Rhododendron* sp. from Belgium (CBS 352.49) and South Korea (CBS 128605).

Sphaerulina chaenomelis (Y. Suto) Videira, U. Braun, H.D. Shin & Crous, Stud. Mycol. 83: 90, 2016.

≡ *Cercosporella chaenomelis* Y. Suto, Mycoscience 40: 513, 1999.

≡ *Pseudocercosporella chaenomelis* (Y. Suto) C. Nakash. et al., Stud. Mycol. 75: 70, 2013.

Description: See Crous et al. (2013), Suto (1999), Videira et al. (2016).

Specimen examined: **Japan**, Mie, Tsu, on leaves of *Chaenomeles sinensis*, 29 October 2011, C. Nakashima (epitype TFM: FPH-8101, culture ex-epitype CBS 132131 = MUCC 1510).

Notes: Present species was described by Suto (1999). Crous et al. (2013) designated the epitype from Japanese specimens for further molecular studies and transferred to the genus *Pseudocercosporella* based on the morphological characteristics. Videira et al. (2017) then analysed the phylogeny of Korean isolate of this species and transferred to the genus *Sphaerulina.* In this study, the phylogenetic position of ex-epitype was identical to that of Korean isolate.

Sphaerulina duchesneae (Hemmi & Naito) Ujat & C. Nakash., **comb. nov., MB 850278,** Figure 3.2

≡ *Septoria duchesneae* Hemmi & Naito, Mem Col. Agr. Kyoto Imp. Univ., 47: 36, 1940.

Leaf spots amphigenous, irregular, 1–5 mm in size, appear as chlorinous, with purple to brown margin, scattered, not confluent (Figure 7D). *Conidiomata* pycnidial, amphigenous, submerged, globose, herbage green to olivaceous brown, 50–80 µm in diameter; *Conidiomatal wall* 5–10 µm thick, pseudoparenchymatous, without distinctly differentiated layers. *Conidiogenous cells* hyaline, ampulliform to cylindrical, proliferating percurrently, 6–10 × 1.5–3 µm, with a few annellations. *Conidia* holoblastic, hyaline, straight or slightly curved, smooth, filiform, sometimes flexuous, 30–55 × 1–1.5 µm, 1–5-septate.

On OMA, colony brownish grey, restricted, elevated, aggregated, cerebriform, covered with white mycelial mat from the centre to the edge. *On MEA*, colony brownish black, restricted, cerebriform, forming aggregated conidiomata. *On PDA*, colony brownish black, restricted, elevated, forming aggregated conidiomata, covered with white mycelial mat. Colony on reverse side dense, cracking on media.

Typus: Syntypes **Japan**, Kyoto, Komorikami-mura, on leaves of *Duchesnea indica*, 15 May 1931, by I. Nagatomo; ibid, Kyoto, on leaves of *Duchesnea indica*, 1 June 1931, by T. Hemmi; ibid, 7 August 1932, by T. Hemmi; ibid, 23 June 1935, by N. Naito; ibid, Ohara, on leaves of *Duchesnea indica*, 1 June 1931, by T. Hemmi; ibid, Shizuichino, on leaves of *Duchesnea indica*, 7 May 1936, by T. Naito. **Lectotype** designated here MBT 10015628, **Japan**, Kyoto, Ohara, on leaves of *Duchesnea indica*, 13 November 1942, by T. Hemmi; **Epitype** designated here MBT 10015629, **Japan**, Tokyo, on leaves of *Duchesnea chrysantha* (Rosaceae), 5 June 1999, by E. Imaizumi (TSU-MUMH 11999, culture ex-epitype MUCC 1589).

Specimen examined: **Japan**, Tokyo, on leaves of *Duchesnea chrysantha* (Rosaceae), 5 June 1999, collected by E. Imaizumi, TSU-MUMH 11999, culture ex-epitype MUCC 1589.

Notes: Syntypes of *Sep*. *duchesneae* were described in the protologue. According to Naito (1940), these specimens had been deposited in the Herbarium of Plant Pathology Laboratory, Kyoto University, Kyoto, Japan. Other types, such as syntypes of *Sep. abeliceae* described in the same literature, have been maintained in the Kyoto University Museum (KYO), Kyoto, Japan. However, the type specimen of *Sep. duchesneae* have not been found in KYO throughout this study. For further molecular study, the lectotype was selected from a topotypic material of a syntype, identified by Hemmi, a co-author of *Sep. duchesneae*. An epitype and ex-epitype culture were designated.

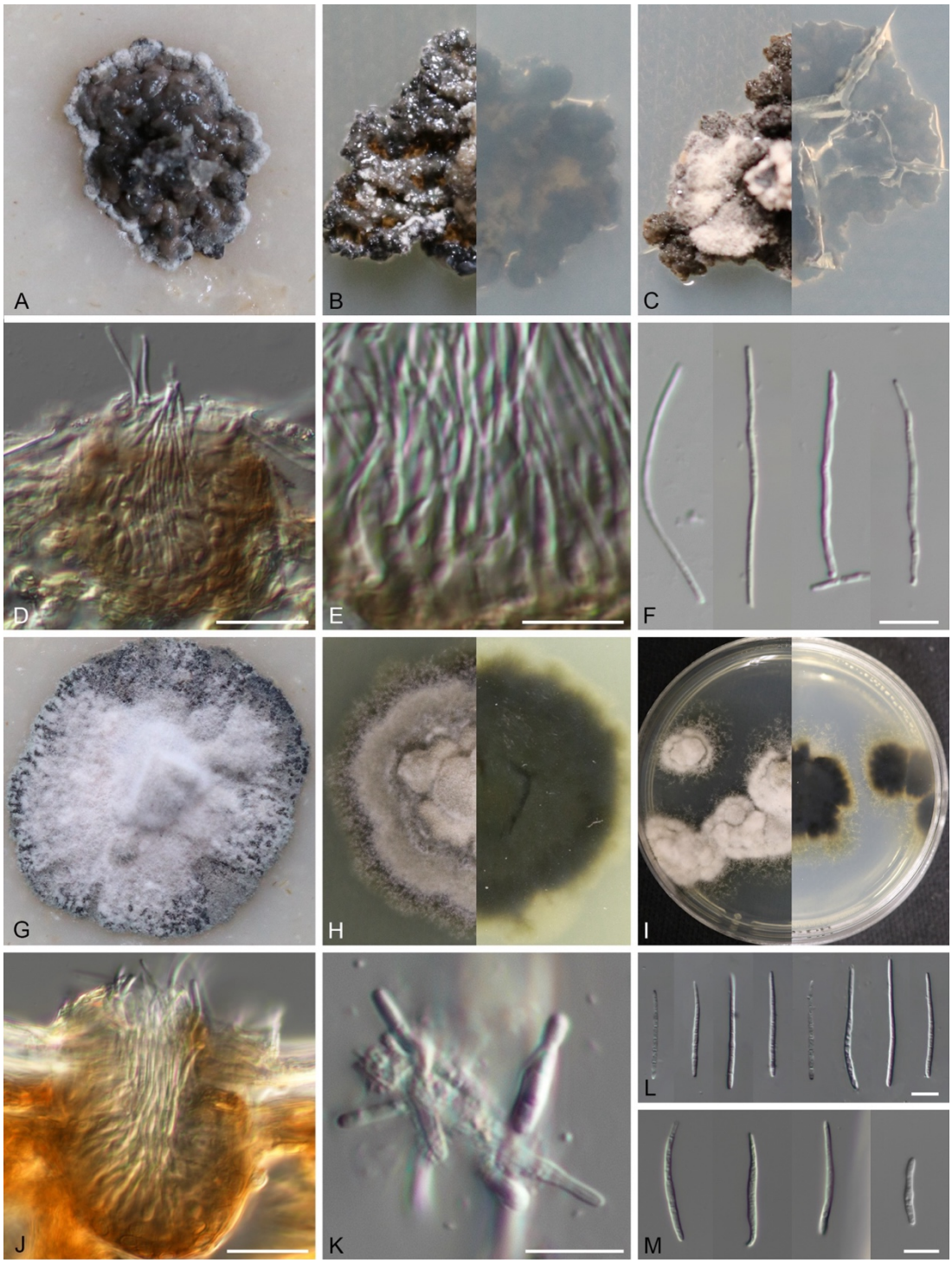

Figure 3.2. Culture characteristic of *Sphaerulina duchesnea* comb. nov., ex-epitype culture MUCC 1587 on OMA **(A)**, MEA **(B)**, and PDA **(C). D.** Hand section though a *Duchesnea chrysantha* leaves (TSU-MUMH 11999) showing the morphology of a conidioma. **E.** Conidiogenous cells. **F.** Conidia. Culture characteristic of *Sphaerulina farfugii* sp. nov., ex-type culture MUCC 1576 on OMA **(G)**, MEA **(H)**, and PDA **(I). J.** Hand section though a *Farfugium japonicum* leaf (TSU-MUMH 11998) showing the morphology of a conidioma. **K.** Conidiogenous cell. **L, M.** Conidia. Scale bar = 20 µm (D, J, K), 10 µm (E, F, L, M).

Sphaerulina farfugii Ujat & C. Nakash., **sp. nov., MB 850279,** Figure 3.2

Etymology: derived from the host plant genus, *Farfugium*.

Leaf spots circular to irregular, amphigenous, numerous, brown with dark purple margin, enlarged and coalescent, up to 2 cm in diameter (Figure 3.7E). *Conidiomata* pycnidial, submerged, olivaceous buff, 40–55 µm in diameter; *conidiomatal cell wall* thickened, composed of *textura angularis*, brown, with an ostiole, or erumpent. *Conidiogenous cells* cylindrical or elongated ampulliform, with long neck, rounded at the bottom, proliferating percurrently, 5–10 × 2–3 µm, with some annellations. *Conidia* cylindrical to acicular, hyaline, guttulate, straight, slightly curved, obconically truncated at the base, narrower towards tip, acute or rounded at the tip, 20–35 × 2–2.5 µm, 2–4-septate.

On OMA, colony greyish white, forming aggregated conidiomata. *On MEA*, colony olivaceous brown, covered with brownish white aerial mycelia, dense and short; on reverse, dense, dark green with citrine margin, appeared the folding at the centre. *On PDA*, colony greyish white, dense, elevated at the centre, covered with short aerial mycelia, on reverse, dark green.

Holotype: **Japan**, Mie, on leaves of *Farfungium japonicum* (*Asteraceae*), 7 July 2023, by C. Nakashima & A.H. Ujat (TSU-MUMH 11998, ex-type culture MUCC 3622)

Specimen examined: See typus

Cultures examined: **Japan**, Gunma, Matsuida, on *Farfugium japonicum*, April 1999, by K. Kishi (MAFF 237889 = MUCC 1576); Ibaraki, Tsukuba, on *Farfugium japonicum*, July 1999, by T. Kobayashi & T. Ono (MAFF 238285 = MUCC 1579); Kagoshima, Kanoya, on *Farfugium japonicum*, November 1994, by K. Kishi (MAFF 23233 = MUCC 1605).

Note: *Septoria tussilaginis* Westend. (Westendorp, 1852a) as a species of *Septoria* s.l., observed on the plant genus *Farfugium,* was reported by Nambu (1914) without morphological description as a collected sample in Tokyo, Japan. The examined isolates MUCC 1576 (= MAFF 237889), MUCC 1579 (= MAFF 238285) and MUCC 1605 (= MAFF 237233) had been deposited as *Septoria tussilaginis.* However, morphological characteristics of *Sep. tussilaginis* on Tussilago fragrans (= *Petasites pyrenaicus*,

Asteraceae) in the prologue seem to be the sexual state of the genus *Phyllosticta* or *Phoma* s.l.*,* which are often observed. These isolates and newly established isolate formed well supported clade (BS:PP = 100:1) and were recognised as a new species the genus *Sphaerulina* on *Farfugium* (Figure Note: *Septoria tussilaginis* Westend. (Westendorp, 1852a) as a species of *Septoria* s.l., observed on the plant genus *Farfugium,* was reported by Hennings (1904) without morphological description as a collected sample in Tokyo, Japan. The examined isolates MUCC 1576 (= MAFF 237889), MUCC 1579 (= MAFF 238285) and MUCC 1605 (= MAFF 237233) had been deposited as *Septoria tussilaginis.* However, morphological characteristics of *Sep. tussilaginis* on Tussilago fragrans (= *Petasites pyrenaicus*, *Asteraceae*) in the prologue seem to be the sexual state of the genus *Phyllosticta* or *Phoma* s.l.*,* which are often observed. These isolates and newly established isolate formed well supported clade (BS:PP = 100:1) and were recognised as a new species the genus *Sphaerulina* on *Farfugium* (Figure 1)..1).

Sphaerulina hydrangeicola Ujat & C. Nakash., **sp. nov., MB 850280,** Figure 3.3

Etymology: derived from the host plant genus, *Hydrangea*.

Leaf spots angular to irregular, limited by leaf veins, coalescent on the leaf edge, amphigenous 2–5 mm, brown, confluent (Figure 3.7F). *Conidiomata* various in shape, pycnidial, acervulous to globose, amphigenous, submerged, olivaceous to dark brown, sometimes epidermal, 40–90 µm in diameter; *conidiomatal cell wall* pseudoparenchymatous, brown, with an ostiole or erumpent. *Conidiogenous cells* ovate to cylindrical, proliferating percurrently, with indistinct annellations, 8–12 × 1.5–2 µm. *Conidia* cylindrical to obclavate, hyaline, straight, flexuous, or curved, obconical to obconically truncated at the base, acute at the tip, 25–40 × 1.5–2 µm, 2–4-septate.

On OMA, colony flat, flesh in colour, lacking to loose aerial mycelium, white to brownish grey, with dense and short aerial mycelia; on reverse, dense, dark green, appeared to be folded at the centre. *On MEA,* colony flat, dark green, citrine at the margin, covered with brownish grey short mycelial mat, bearing dense aerial mycelia at the centre; on reverse, colony dark green, with folding towards the centre. *On PDA*, colony elevated,

dark green, with brownish white mycelial mat, appearing dense folding from the centre; on reverse, colony dark green with citrine margin.

Holotype: **Japan**, Nagano, Sugadaira, on leaves of *Hydrangea paniculata* (*Hydrangeae*), 1 October 2007, by C. Nakashima, T. Akashi, I. Araki and K. Motohashi, (TSU-MUMH 10651, culture ex-type MUCC 694).

Examined specimen: See typus

Notes: On the plant genus *Hydrangea*, *Septoria hydrangeae* Bizz. is the only species of *Septoria* s.l. The morphological characteristics of the examined specimen were compared with the protologue of *Sep. hydrangeae* (Bizzozero, 1885). There were distinctive differences in the spore in width, number of septations, and shape. The conidia of *Septoria hydrangeae* are 16–22 × 1.5 µm, non-septated, and cylindrical or flexuous. The symptoms on leaves were also differentiated from those caused by *Sep. hydrangeae,* turning into a brownish-rusty colour with red border and irregular.

Sphaerulina idesiae Ujat & C. Nakash., **sp. nov., MB 850281,** Figure 3.3

Etymology: derived from the host plant genus, *Ideasia*.

Leaf spots amphigenous, angular, black, 2–4 mm, turned into brown, enlarge, coalescing with minute white spots, grey on lower leaf surface (Figure 3.7G). *Conidiomata* various in shape, pycnidial, acervulous to globose, epiphyllous, submerged, brown, sometimes epidermal, scattered, brown, splitting open at maturity, 25–40 µm in case of unmatured, up to 65 µm in diameter in mature; c*onidiomatal cell wall* pseudoparenchymatous, brown. *Conidiogenus cells* hyaline, ampulliform with long neck, proliferating percurrently, with indistinct annellations, 2–12 × 1.5–2 µm. *Conidia* hyaline, holoblastic, ovoid to cylindrical, with conically truncated or rounded at both ends, 4–18 × 1.5–1.8 µm, 0–1-septate. *Spermatogonium* globose, brown with hyaline inner cell wall 50–60 µm. *Spermatia* hyaline, dumb bell-shaped, approximately 1 µm long, and less than 0.5 µm width on both ends.

On OMA, colony elevated, flesh in colour, smooth, sparse, covered white-loose mycelial mat, folding at the edge, submerged into media. *On MEA and PDA*, colony buff to salmon on the edge, flesh at the centre, raised and slightly wrinkled, sparse, covered with short white mycelial mats, buff at the centre, white on the edge. Holotype: **Japan**, Okinawa, Kunigami, on leaves of *Idesia polycarpa* (*Salicaceae*), 17 November 2007, collected by C. Nakashima & I. Araki (TSU-MUMH 10917, culture ex-type MAFF 240634 = MUCC 813).

Specimen examined: See typus

Note: To date there is no record of *Septoria* or *Sphaerulina* species infecting *Idesia* sp. Moreover, on the phylogenetic tree, this species was sister to *Sph. hydrangeicola,* described as a new species in this study.

Sphaerulina juglandis Kaz. Ito & Tak. Kobay., Bull. Gov. For. Exp. Stn. Tokyo 96: 66, 1957.

≡ *Cercosporella juglandis* Kaz.Ito & Tak.Kobay., Bull. Gov. For. Exp. Stn. Tokyo 96: 66, 1957.

≡ *Pseudocercosporella juglandis* (Kaz.Ito & Tak.Kobay) U.Braun, A monograph of *Cercosporella*, *Ramularia* and allied genera (phytopathogenic Hyphomycetes) 1: 168, 1995.

Typus: Holotype, **Japan**, Yamagata, Kamabuchi, on leaves of *Juglans sieboldiana* (*Juglandaceae*), 15 June 1951, collected by K. Ito & T. Kobayashi (TFM-FPH 0227).

Specimen examined: **Japan**, Toyama, Nagasawa, on leaves of *Juglans sieboldiana*, 31 October 1996, collected by T. Kobayashi & C. Nakashima.

Culture examined: **Japan**, Niigata, Itoigawa, on leaves of *Juglans sieboldiana,* October 1996, by Hirata (MAFF 237622 = MUCC 1475)

Notes: Present species was described by Ito and Kobayashi (1957) with the morphological characteristics of its teleomorphic state, anamorphic state, the results of inoculation tests, and other cultural characteristics on various media. Braun (1995)

transferred the asexual state, *Cercosporella juglandis*, to *Pseudocercosporella* based on the morphological characteristics having densely fasciculate, short, hyaline conidiophores and inconspicuous conidial scars. Holotype of this species is teleomorphic state, the phylogenetic has not clear yet. In this study, we analysed the phylogenetic position using an isolate of anamorphic state.

Sphaerulina lapsanastri Ujat & C. Nakash., **sp. nov., MB 850282,** Figure 3.4

Etymology: derived from the host plant genus, *Lapsanastrum*.

Leaf spots amphigenous, circular, first observed as tiny spots in 1 mm, enlarged and confluent, turned into dark green with brown edges, up to 1 cm (Figure 3.7H). *Conidiomata* pycnidial to acervular, flattened, hypophyllous, globose to subglobose, submerged to superficial, scattered, brown, with thin wall, 35–80 µm in diameter, with an ostiole, or erumpent; c*onidiomatal cell wall* pseudoparenchymatous, brown. *Conidiogenus cells* brown, ovoid to ampulliform, proliferating percurrently or sympodially, with annellations, 4–12 × 1.5–3 µm. *Conidia* cylindrical to acicular, holoblastic, hyaline, straight or flexuous, sometimes slightly curved, acute to rounded at the tip, long-obconically truncated, narrow in width, 20–35 × 1–1.5 µm, 2–3-septate.

On OMA, colony black, covered with dense short mycelial mat, smooth, embedded into media at the edges. *On MEA*, colony dull green, forming short mycelial mat, covered with white aerial mycelia at the edge, discharging black droplets at the edge; on reverse, colony olivaceous to dull green. *On PDA*, colony herbage green, with short white aerial mycelia at the central part, forming black conidiomata; on reverse, colony greenish black at the centre, dark in other area.

Holotype: **Japan**, Tokyo, on leaves of *Lapsanastrum humile* (*Asteraceae*) 22 August 1998, collected by E. Imaizumi (TSU-MUMH 12001, culture ex-type MUCC 1582).

Specimen examined: See typus

Note: This specimen was recorded as *Septoria* sp. on *Lapsanastrum humile*. On *Lapsanastrum communis*, *Septoria lapsanae* is known from the Mediterranean area (Bernaux, 1952). Although the leaf symptoms of Japanese specimen are similar to that of *Sep. lapsanae* in forming irregular to rotundate spot, the flattened, submerged to superficial pycnidia and somewhat shorter and narrower conidia differ from those of *Septoria lapsanae,* which has ovoid pycnidia and conidia (30–50 × 1.4 µm). Moreover, the examined isolate of *Sph. lapsanastri* located in an independent clade with *Sph. nambuana*. On the other hand, molecular sequence of *Sep. lapsanae* has not been known. The further studies using fresh materials of *Sep. lapsanae* is required. In this study, this species is proposed to be a new species.

Sphaerulina miurae Ujat & C.Nakash., **sp. nov., MB 850285,** Figure 3.4

Etymology: derived from the name of Japanese mycologist, Miura Mitsushige whose work on plant pathogenic fungi in East Asia.

Leaf spots amphigenous, irregular, sometimes angular, vein-limited, dark grey, with violet margin, 5–8 mm, enlarged and coalescent (Figure 3.7I). *Ascomata* scattered, immersed, globose, papillate at the top, dark brown, 85-95 µm in diam.; *Asci* cylindrical to obclavate, rounded at the head, without paraphyses, 8-spored, 5-7 × 30–42 µm; *Ascospres* arranged 2-rows, cylindrical to fusiform, 1-3 septate, uniform in width, hyaline, 2.5–3.1 × 10–12 µm. *Conidiomata* pycnidial, hypophyllous, submerged, globose, 50–60 µm in diameter, up to 130 µm in height, with a pyriform ostiole; c*onidiomatal cell wall* composed of textura angularis, thin, 6–15 µm in thickness, brown. *Conidiogenous cells* minute, papillate, hyaline, integrated into inner wall of conidiomata, determinate, with indistinct annellations by shortly elongating conidiogenous cell. *Conidia* acicular to filiform, cylindrical, holoblastic, hyaline, straight, narrow in width, sometime slightly curved with slightly wider at the base, 35–55 × 1 µm, 1-4-septate.

Colonies on various media lacking aerial mycelium, olivaceous black to dark brown with grey olivaceous margin on surface. *On OMA*, colony smooth and flat on surface. *On MEA and PDA*, colony wrinkled, On reverse, colony white at the centre, dark olivaceous brown at the edge, without apparent margin.

Holotype: **Japan,** Wakayama, Hidakagawa, on leaves of *Aster tartaricus* (*Asteraceae*), 30 October 2007, C. Nakashima & I. Araki (TSU-MUMH 10857, culture ex-type MUCC 731) MBT 10015636.

Specimen examined: See typus

Notes: Quaedvlieg et al. (2013) and Verkley et al. (2013) showed the phylogenetic data for three species of *Septoria* s.lat infectcting *Aster* spp., *Septoria astericola* Ellis & Everh., *Sep. atropurpurea* Peck, and *Sep. stachydis* Roberge ex Desem. Shin and Sameva (2004) studied *Septoria* species on *Aster* spp. from Korea based on the morphological characteristics and they concluded that the Korean collection of *Septoria* species on *Aster* spp. as *Septoria astericola*. On the plant genus *Aster*, several Japanese species were described in previous studies. Sydow and Sydow (1914) reported the occurrence of *Sep. tatarica* on *Aster tataricus* (pycnidia 60–90 µm in diam.; conidia filiform, 22–40 × 1–1.5 µm). Miura (1928) described *Sep. pyriformis* (pycnidia 50–70 µm in diam.; conidia filiform, 35–60 × 1.5–2 µm) on *Aster scaber,* having well-developed ostiole, significantly differs from *Sep. atropurpurea* Peck (conidia filiform, 50–76 µm long, in Peck 1880). The specimens observed in this study showed similarity to *Sep. pyriformis* in the size of pycnidia and conidia.

Sphaerulina myriadea (DC.:Fr.) Sacc., Michelia l: 399, 1878.

Notes: Specimens and cultures were not examined in this study. Japanese isolates are keeping as CBS 124646 = JCM 15565 (Japan, Aomori, Kidukuri, on leaves of *Quercus dentata*). The phylogenetic position was examined in Quaedvlieg et al. (2013).

Figure 3.3. Culture characteristic of *Sphaerulina hygdrangeicola* sp nov., ex-type culture MUCC 649 on OMA **(A)**, MEA **(B)**, and PDA **(C). D.** Hand section though a *Hydrangea paniculata* leaves (TSU-MUMH 10651) showing the morphology of a conidioma. **E.** Conidia. **F.** Ascospores. Culture characteristic of *Sphaerulina idesiae* sp. nov., ex-type culture MUCC 813 on OMA **(G)**, MEA **(H)**, and PDA **(I). J.** Hand section though a *Idesia polycarpa* leaves (TSU-MUMH 10917) showing the morphology of a conidioma. **K.** Conidioma opening resembling acervuli. **L.** Conidia. Scalebar = 20 µm (D, J, K), 10 µm (E, F, L).

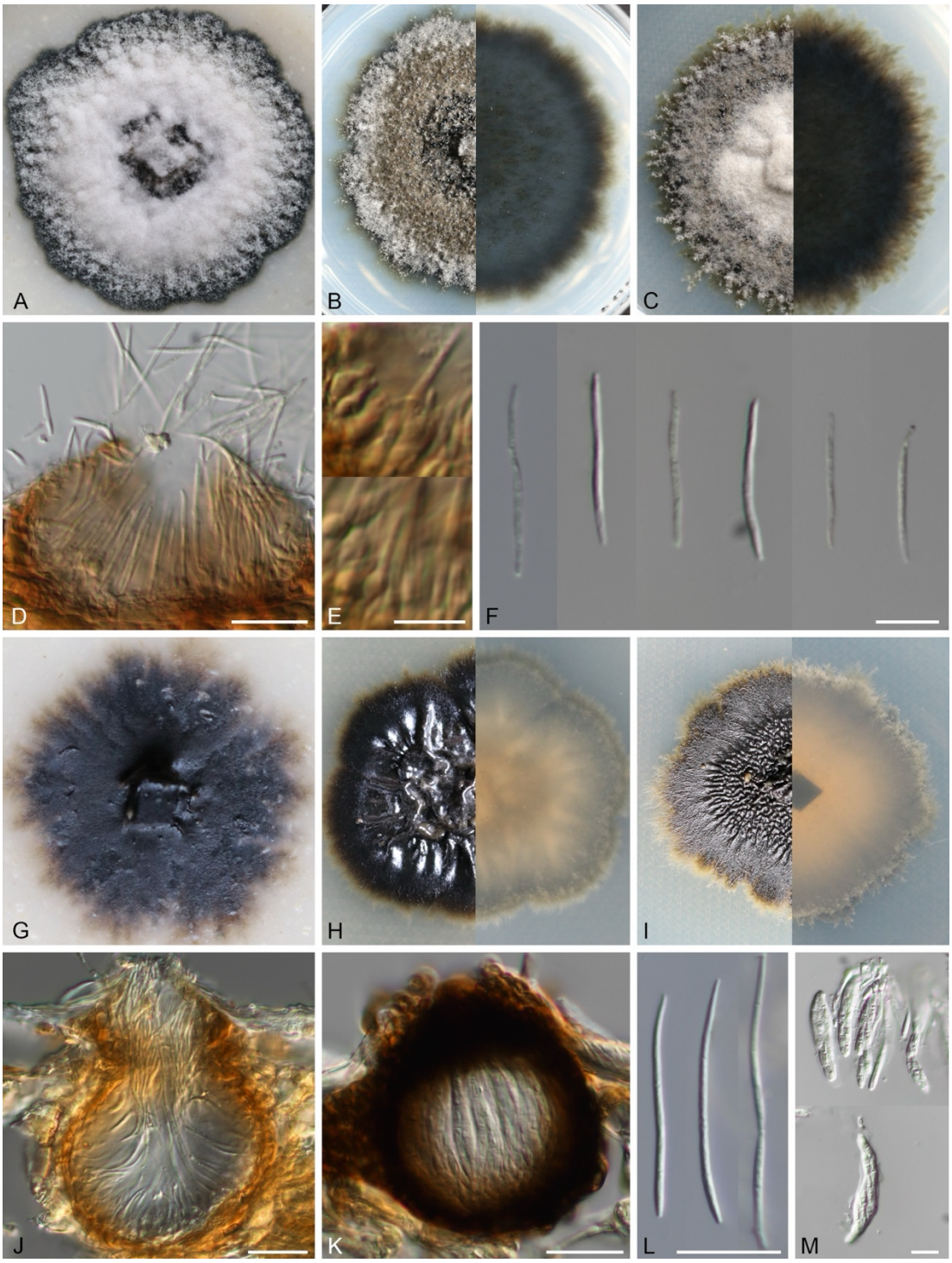

Figure 3.4. Culture characteristic of *Sphaerulina lapsanastri* sp nov., ex-type culture MUCC 1582 on OMA **(A)**, MEA **(B)**, and PDA **(C). D.** Hand section though a *Lapsanastrum humile* leaves (TSU-MUMH 12001) showing the morphology of a conidioma. **E**. Conidiogenous cell. **F.** Conidia. Culture characteristic of *Sphaerulina idesiae* sp. nov., ex-type culture MUCC 813 on OMA **(G)**, MEA **(H)**, and PDA **(I).** Hand section though an *Aster tataricus* leaves (TSU-MUMH 10857) showing the morphology of a conidiomata **(J)** and ascoma **(K)**. **L.** Conidia. **M**. Asci and ascospores. Scalebar = 20 µm (D, J, K), 10 µm (E–G, L), 1 µm (M).

Sphaerulina nambuana (P. Henn.) Ujat & C. Nakash., **comb. nov., MB 850283,** Figure 3.5

≡ *Septoria nambuana* P. Henn., Hedwigia 43: 145, 1904.

Leaf spots brown, amphigenous, circular, purple at the raised margin, with reddish purple halo, not confluent, 2–7 mm (Figure 3.7J). *Conidiomata* pseudoparenchymatous, amphigenous, submerged, globose, 40–70 µm in diameter; *Conidiomatal cell wall* pseudoparenchymatous, apricot, with a black ostiole. *Conidiogenous cells* elongated ampulliform, holoblastic, proliferating percurrently, with an indistinct annellation, 5–10 × 1–1.5 µm. *Conidia* holoblastic, hyaline, long-cylindrical, straight to curved, smooth, 3–4 septate, 35–40 × 1.5–2 µm.

On OMA, colony white at the centre, dense, covered with aerial myceliam rust to dark purple at the marginal area. *On MEA*, colony flesh in colour, lacking to loose aerial mycelium, white at both surface. *On PDA*, colony citrine, with buff to rose aerial mycelia, short and dense; on reverse, citrine, turned to white at the centre, with white margin, folded at the centre as it aged.

Typus: **Japan**, Suruga, Gotemba, on leaves of *Lysimachia barystachidis*, by N. Nambu, November 1900, S F62688; **Epitype** designated here, MBT 10015634, **Japan**, Tochigi, Nikko, On leaves of *Lysimachia fortunei* (*Primulaceae*), 3 September 1998, by E. Imaizumi (TSU-MUMH 12000, culture ex-epitype MUCC 1586).

Specimen examined: See epitype.

Notes: Type material in Herbarium S is not examined. In the previous study by Verkley et al. (2013), three species of *Septoria* s.l. on *Lysimachia* spp., which are *Septoria lysimachiae* (Lib.) Westernd. on *Lysimachia vulgaris* from Netherlands (CBS 108998), *Sep. saccardoi* Ferraris on *L. vulgaris* isolated from South Korea (CBS 128756), and *Sphaerulina* sp. on *Lysimachia clethroides* from Korea (CBS 128758). The isolate of *Septoria nambuana* used in this study is being grouped together with *Sphaerulina* sp. on *L. clethroides* from Korea (CBS 128758). For the further studies of *Sep. nambuana,*

epitype specimen and its isolate were designated based on the description by Hennings (1904). Hence, *Sep. nambuana* was transferred to the genus *Sphaerulina.*

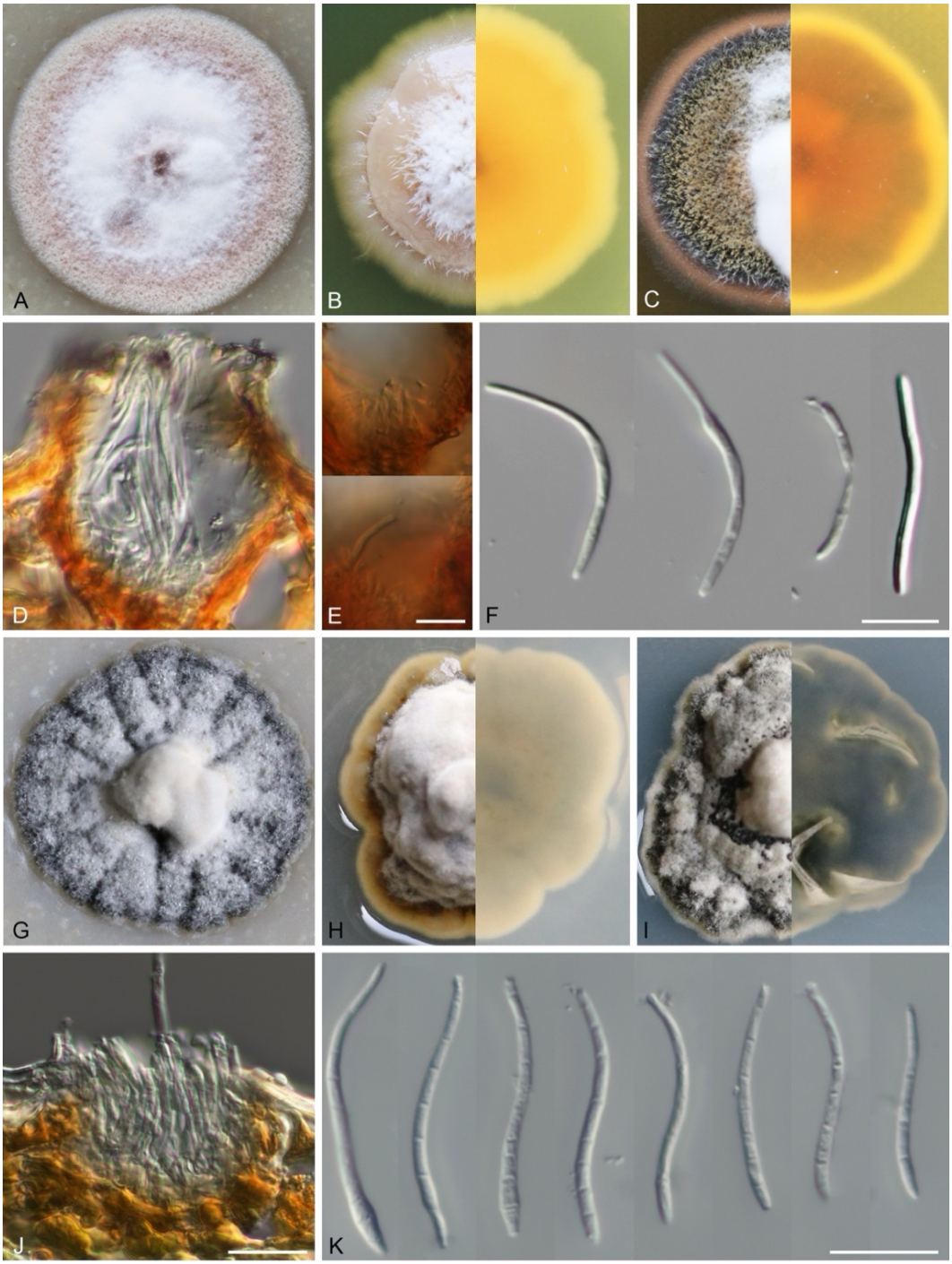

Figure 3.5. Culture characteristic of *Sphaerulina nambuana* comb nov., ex-epitype culture MUCC 1586 on OMA **(A)**, MEA **(B)**, and PDA **(C). D.** Hand section though a *Lysimachia fortunei* leaves (TSU-MUMH 12000) showing the morphology of a conidioma. **E.** Conidiogenous cell. **F.** Conidia. Culture characteristic of *Sphaerulina styrachis* sp. nov., ex-type culture MUCC 1603 on OMA **(G)**, MEA **(H)**, and PDA **(I).** Hand section though a *Styrax obassisa* leaves (TSU-MUMH 10538) showing the morphology of a conidioma. **J.** Conidiomata. **K.** Spores. Scalebar = 20 µm (D, J), 10 µm (E, F, K).

Sphaerulina pseudovirgaureae Quaedvlieg, Verkley & Crous, Stud. Mycol. 75: 346, 2013.

Notes: Two isolates of *Sph. pseudovirgaureae* are kept as MAFF 243498 and MAFF 243498, established from the fungus on *Kerria japonica* (*Rosaceae*) in MAFF. The original host of *Sph. pseudovirgaureae* is *Solidago* sp. (*Asteraceae*). Although attached reference ITS sequencing data for each isolate suggested that these isolates belong to the genus *Sphaerulina*, more detailed examination with multi loci phylogenetic analyses are required for the species identification and discussing the host range.

Sphaerulina styracis Ujat & C. Nakash., **sp. nov., MB 850284,** Figure 3.5

Etymology: derived from the host plant genus, *Styrax*.

Leaf spots amphigenous, circular, brown with dark purple margin, 2–7 mm, enlarged and confluent, up to 1.7 cm (Figure 3.7K). *Conidiomata* pycnidial, globose, epidermal, scattered on symptoms, erumpent, opening at maturity, brown, 60–100 µm in diameter; c*onidiomatal cell wall* pseudoparenchymatous, brown, paler towards inside. *Conidiogenus cells* hyaline, or pale brown, papillate to ampulliform with long neck, dull to conically truncated tip, with annellations, proliferating percurrently, 2–22.5 × 1–1.5 µm. *Conidia* cylindrical to obclavate, holoblastic, hyaline, sigmoid or slightly curved, obconically truncated at basal end, 1–1.5 µm at the base, rounded or acute at the tip, 30–60 × 3.5 µm, 4–8-septate.

On OMA, colony black, dense, covered with white aerial mycelia, embedded into the media and folded outwards. *On MEA*, colony black, covered with dense white mycelial mat, amber at the edge; on reverse, white, embedded into media. *On PDA*, colony black, rised, covered with white short mycelial mat at the centre, amber, embedded into media at the edge, bearing black aggregated conidiomata; on reverse, colony dark olivaceous green, dense, cracking.

Holotype: **Japan**, Kagoshima, Aira, on leaves of *Styrax obassia* (*Staracaceae*), 28 May 2007, collected by C. Nakashima & K. Motohashi (TSU-MUMH 10538, culture ex-type MAFF 240599 = MUCC 1603).

Specimen examined: See typus

Note: This specimen and its isolates were recorded as *Septoria* sp. on *Styrax obassia*. On *Styrax*, *Septoria styracis* Göbelez from Turkey is known as a species of *Septoria* s.lat According to protologue of *Sep. styracis*, the symptoms are similar, rounded, or angular. On the other hand, conidia of *Sep. styracis* are shorter than that of *Sph. styracis*, which is 20–27 × 2–3.5 µm (Göbelez, 1960). Moreover, the phylogenetic position of *Sph. styracis* is recognised as an independent species. Hence, this species is proposed as a new species of *Sphaerulina* infecting *Styrax*.

Sphaerulina viburnicola Ujat & C. Nakash., **sp. nov., MB 850286,** Figure 3.6

Etymology: derived from the host plant genus, *Viburnum* sp.

Leaf spots amphigenous, angular, brown, 3–5 mm, vein-limited, enlarge and coalescent (Figure 3.7L). *Ascomata* scattered, immersed, globose, papillate at the top, reddish brown, 80–90 µm in diameter; *asci* cylindrical to obclavate, rounded at the head, without paraphyses, 8-spored, bitunicate, 3–5 in perithecia, 20–25 × 5–7 µm; *Ascospores* unarrayed or arranged 2-rows, cylindrical to fusiform, 2-septate, hyaline, 0.2–0.5 × 1–1.5 µm. *Conidiomata* pycnidial to acervular, amphigenous, subglobose, epidermal, scattered, brown, 45–100 µm in diameter, erumpent; *conidiomatal cell wall* pseudoparanchymatous, brown. *Conidiogenous cells* ampulliform to cylindrical, rounded at the tip, hyaline, or pale brown, proliferating percurrently, 3–10 × 1–3.5 µm. *Conidia* holoblastic, hyaline, cylindrical to clavate, truncated at the basal end, rounded at the tip, straight, sometimes curved, 10–35 × 1–2.5 µm, 2–5-septate.

On OMA, colony herbage green, dense, covered with pale vinaceous mycelial mat, pinkish at the centre. *On MEA*, colony greenish black, olivaceous green at the edge, covered with pinkish white aerial mycelia, slightly raised, and folded towards the centre; on reverse, colony dull green, slightly cracked on folded area in concave manner. *On PDA*, colony citrine, covered with loose, short, and white aerial mycelia, raised at the centre, folding inwards; on reverse, colony black at the centre, cracking formed from the centre, raised outwards, yellowish green on the outer part, with citrine margin.

Holotype: **Japan**, Aichi, Nagoya, on leaves of *Viburnum wrightii* (*Viburnaceae*), 24 October 2005, collected by I. Araki & K. Motohashi (TSU-MUMH 10249, culture ex-type MUCC 349).

Specimen examined: See typus

Note: On the plant genus *Viburnum, Septoria viburni* Westend. and *Sep. butleri* Died has been recorded. However, measurement of the conidia of *Sep. viburni* was not described in the protologue (Westendorp, 1852b). Moreover, *Sep. butleri* is differentiated from *Sph. viburnicola* in having hypophyllous pycnidia and larger conidia, 30–50 × 2.5–3.5 µm (Sydow et al., 1914). However, these phylogenetic position of the species of *Septoria* s.l. are still unclear. On the other hand, Naito (1940) recorded *Sep. viburni* on *V. dilatatum* from Japan and described similar morphological characteristics to *Sph. viburnicola* except the longer conidia (34–52 × 1.7–2.0 µm).

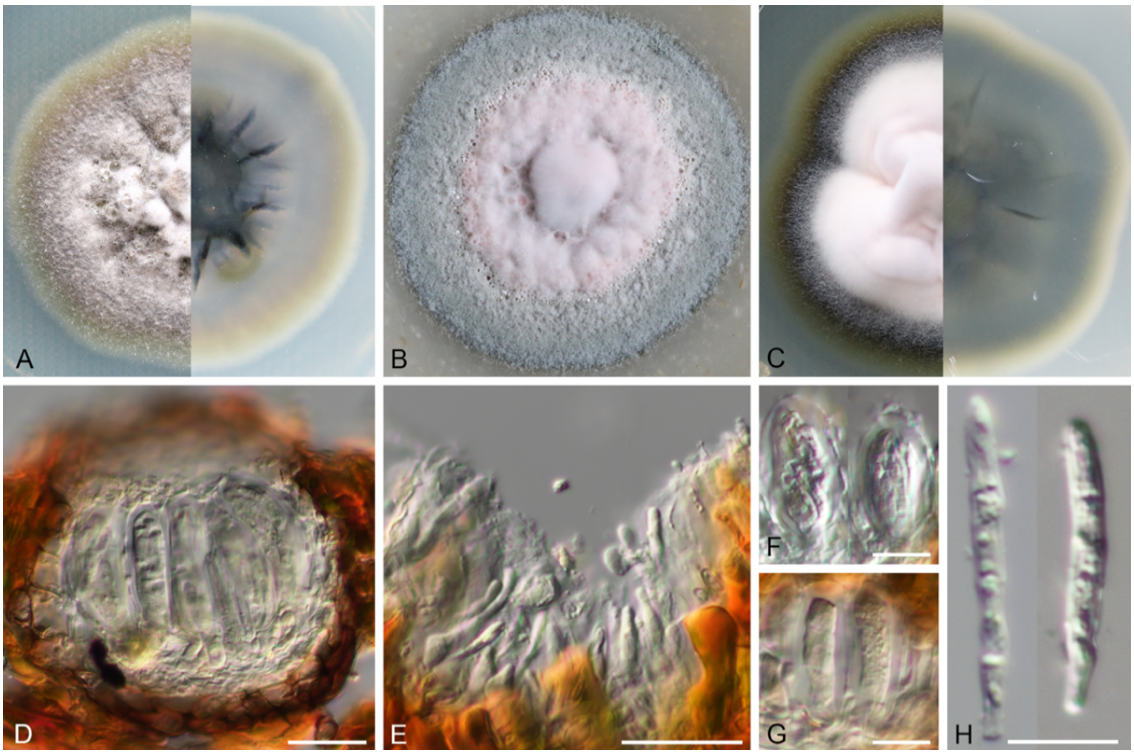

Figure 3.6. Culture characteristic of *Sphaerulina viburnicola* sp. nov., ex-type culture MUCC 349 on OMA **(A)**, MEA **(B)**, and PDA **(C).** Hand section though a *Viburnum wrightii* leaves (TSU-MUMH 10249) showing the morphology of a conidioma. **D.** Ascomata. **E.** Conidiogenous cell. **F, G.** Asci**. H.** Spores. Scalebar = 20 µm (D, E), 10 µm (F-H).

Insufficiently known species.

The following species were examined with old herbarium specimens without the isolate or examined with the isolate lacking a linked herbarium specimen. Therefore, the taxonomic position is still unclear in this study.

Sphaerulina spp. on *Alnus*

Culture examined: **Japan**, Tokyo, Meguro, on *Alnus pendula,* September 1950, T. Kobayashi (MAFF 410471 = MUCC 1573); **Japan**, Iwate, Koma, on *Alnus hirsuta*, 22 August 1957, S. Kaneko (MAFF 410476 = MUCC 3526).

On OMA, colony growth rapid, fully colonised plate with white lobate colony of mycelial mat and turning agar into maroon at the centre of growth. No sporulation observed. *On MEA*, colony striated radially, and forming white aerial mycelia. On reverse side shows maroon-red colony otherwise turning the agar into maroon red. No sporulation observed. *On PDA*, colony striated radially with white mycelial mat and forming brown exudate. On reverse, similar observation as MEA. No sporulation observed.

Notes: There are multiple records of occurrence of *Septoria* species on *Alnus* spp. including *Septoria alni* Sacc, *Septoria alnicola* Ellis & Everh, *Septoria alnifolia* Cooke and *Septoria alnigena* Sacc. The multi-locus phylogenetic analysis of isolates in MUCC, which were recorded as *Septoria alni* collected from *Alnus hirsuta* and *Alnus pendula*, shows that it belongs in the *Sphaerulina* clade. Based on this information, these isolates were suggested to be a novel species of *Sphaerulina* on *Alnus* spp. As further study, the fresh material should be examined for the morphological features.

Figure 3.7. Symptoms on different leaves. **A.** *Rhododendron* sp. **B.** *Zelkova serrata*. Herbarium specimens of **C.** *Zelkova serrata*. **D.** *Duchesnea chrysantha*. **E.** *Farfugium japonicum*. **F.** *Hydrangea paniculata*. **G.** *Idesia polycarpa*. **H.** *Lapsanastrum humile*. **I.** *Aster tataricus*. **J.** *Lysimachia fortunei*. **K.** *Styrax obassia*. **L.** *Viburnum wrightii*

DISCUSSION

Sphaerulina was first described by Saccardo (1878) as having 3 to pluriseptate ascospores. Crous et al. (2003) stated that the number of septations of ascospore is an unreliable feature for defining a genus and redefine the genus *Sphaerulina* of *Mycospharellaceae* through the study of the species of *Sphaerulina* on Eucalyptus using the ITS phylogeny. Verkley et al. (2013) and Quaedvlieg et al. (2013) indicate that the genus *Sphaerulina* is genetically distant from *Septoria* s.l. and should be classified as a separate genus. In the comprehensive study of *Mycosphaerellaceae* (Videira et al. 2017), these findings support the recognition of *Sphaerulina* and the closely related genus *Miuraea* as independent genera. In this study, Japanese isolates of hitherto known *Sphaerulina* species were already located in the *Sphaerulina* clade. Moreover, during the taxonomical re-examination of *Septoria* s.lat, several species were transferred to *Sphaerulina* and described as new species of *Sphaerulina*.

The species delimitation of the genus *Sphaerulina* is still under discussion. Many of the species in *Mycospharellaceae* are suggested to be host-specific from the various inoculation tests and phylogenetic tree consisting of species well-supported by each host plant genus (Crous et al., 2013b; Groenewald et al., 2013, Nakashima et al., 2016; Videira et al., 2017; Chen et al., 2022). The same results of the relationship among the host plant and *Sphaerulina* species were observed in this study. All isolates examined in this study formed well-supported clades by each host plant and were recognised as each species. For example, *Sph. abeliceae* was found on *Zelkova serrata*, *Sph. azaleae* on *Azalea* spp., *Sph. farfugii* on *Farfugium japonicum*, *Sph. namubana* on *Lysimachia* spp., *Sph. styracis* on *Styrax obassia*, and *Sph. viburnicola* on *Vibrunum wrightii*. Most of the *Sphaerulina* species are monophyletic, with one species infecting one host genus or host family. However, there are several different species infecting the same host family. For example, *Sph. musiva*, *Sph. populicola* and *Sph. frondicola* inhabit *Populus* species.

On the other hand, Verkley et al. (2013) and Quaedvlieg et al. (2013) indicate that some species of the genus *Sphaerulina* have a wide host range beyond the host families. *Sph. amelanchieris*, which shows a wide host range, was place in the descendent clade with one Japanese species, *Sph. miurae*, at the basal position. *Sph.*

amelanchieris isolated from the Netherlands from a well-supported clade and has host plants including *Betula* sp., *Quercus* sp., *Actinidia* sp., and *Amelanchier* sp., belonging to *Fagales*, *Ericales* and *Rosales*. Interestingly, these host orders repeatedly appear on the phylogenetic tree. These arboreous host plants might be related to the host range expansion of *Sphaerulina* species as the first step for preparing host jumping and are eventually used as a stable host.

In natural condition, *Sphaerulina* anamorph that appears predominantly often is associated with different host plant. Morphological characteristic in *Septoria* s.l., including *Sphaerulina* anamorph, are generally conserved, and the conidial morphological characters; the shape, size and septation, are more reliable for species identification (Quaedvlieg et al., 2013). These integrated criteria consisting of the host and fungal morphology on the host plant, are still effective for preliminary identification, except for species having a wide host range. However, accurate identification based on morphological analysis alone is impossible for many *Sphaerulina* species without the results of the phylogenetic analyses using a combined matrix of multi-locus sequences.

CONCLUSION

This chapter emphasize on the importance of using polyphasic approach in the identification of plant pathogenic fungi. Saccardo's initial description highlighted the ascospore septation as a defining feature of *Sphaerulina*, whereby most of modern mycologist did not agree upon but rather using the width of the spore as one of the defining feature of *Sphaerulina*. Verkley et al. (2013) and Quaedvlieg et al. (2013) are able to identify *Sphaerulina* as an independent genus from *Septoria* s.l. based on molecular identification and the result presented in this chapter aligns with the findings of previous studies, confirming the placement of Japanese isolates of *Sphaerulina* species in the clade.

Although the discussion about species delimitation in the genus *Sphaerulina* is still ongoing, the observation from this current studies shows that *Sphaerulina* are monophyletic with one species infecting one host genus or family. There are some exception as there are multiple species infecting one host family as in the *Populus* sp. This could be an indication of evolution that happens within the host plant or based on the geographical distribution of the host plants. There are opposing observation can be seen in the case of *Sph. amelanchier* as one fungi species is infecting multiple host of different genus, whereby this could be an indication of host range expansion prior to finding a suitable host to infect.

In the natural environment, the identification of *Sphaerulina* with different host plants adds remains complex as morphological characteristics remain have litter variation to that of *Septoria* s. l., making morphological characters a challenge for species identification. However, as accurate identification solely based on morphology is challenging, this study also emphasize the necessity of phylogenetic analyses using a combined matrix of multi-locus sequences.

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Chapter 4 – *Septoria*

Summary

As discussed in the previous chapter, leaf spot of *Septoria* and *Sphaerulina* are often difficult to distinguished by morphological observation alone. Although the symptoms of the are similar to those caused by *Sphaerulina* spp., the phylogenetic analysis reveals of *Septoria* at taxonomical level had revealed that many of the *Septoria* spp. catalogue in Japan are established well in the phylogeny of *Septoria*. Traditionally, *Septoria* identification relied on host plants and morphology, but molecular techniques have challenged this, revealing significant variation within the genus. The analysis shows that while *Septoria* species typically have a narrow host range, some can infect multiple hosts across different genera, suggesting host jumping plays a role in their evolution. Additionally, multiple *Septoria* species can infect a single host plant genus, highlighting complex host-pathogen associations. However, there result of this analysis also shows that there are novel species of Septoria isolated from hemp, where the name "*Septoria cannabicola*" was proposed.

Keywords: Leaf-spot disease, multi-locus phylogenies, Systematics, Taxonomy

INTRODUCTION

Fungal species of *Septoria* are among the most common and widely spread leaf-spot causing fungi worldwide. Its type species, *Septoria cytisi*, was first described by Desmazières (1847) as a pathogen of *Cytisis laburnum* (syn *Laburnum anagyroides*). Currently, *Septoria* s.lat. represents a polyphyletic assembly clustered in the Mycosphaerellaceae (Quaedvlieg et al., 2013). The name *Septoria* Sacc. was conserved over *Septaria* Fries as the spelling *Septoria* had been widely adopted and in use for an extended period. Sutton (1980) circumscribed *Septoria* with the following description: Mycelium immersed, branched septate, pale brown. Conidiomata pycnidial, immersed, separated, or aggregated (not confluent), globose, papillate (or not), brown thin-walled, or pale brown *textura angularis*, often with a small-celled inner layer, somewhat darker and more thick-walled around the ostiole. Conidiophore reduced to conidiogenous cells. Conidiogenous cell holoblastic, either determinate or indeterminate, with a limited number of sympodial proliferations. Each locus has a broad, flat, unthickened scar, discrete, hyaline, smooth, ampuliform, doliiform, or lageniform to short cylindrical. Conidia hyaline, multi-septate filiform, smooth and either continuous or constricted at septa. The morphological characteristics of *Septoria* spp. are generally conserved, making the identification by morphological characteristics alone difficult between *Septoria* and *Septoria*-like species.

The previous identification of *Septoria* relies heavily on the host plant; however, it is prone to errors, as many *Septoria* species are not restricted to a single host (Quaedvlieg et al., 2013; Verkley et al., 2013). Some species, such as *Sep. lycopersici*, are known to infect multiple host plant species of the same genus, as well as closely allied family genera. There are also *Septoria* species with distinguishable morphology that could be found infecting the same host plant species. For example, *Septoria chrysanthemella*, *Septoria leucanthemi*, and *Septoria obesa* on *Chrysanthemum* species, or *Septoria astericola* and *Septoria atropurpurea* infecting *Aster* species. Molecular phylogenetic studies on *Septoria* spp. infecting *Asteraceae* showed that species capable of infecting hosts of the same family do not always cluster in monophyletic groups (Verkley & Starink-Willemse, 2004). Verkley et al. (2013) and Quaedvlieg et al. (2013)

used 7 loci consisting of rDNA internal transcribed spacer region (ITS), nuclear large subunit rDNA (LSU), beta-tubulin gene (BTUB), the second-largest subunit of RNA polymerase II (RPB2), translation elongation factor 1-alpha (TEF-1a), calmodulin (CAL), and actin (ACT) to evaluate the relationships of *Septoria* spp. They confirmed that samples collected from the same location and host plant but under different environmental conditions could differ in average conidial size, particularly length (Verkley et al., 2013), and that conidial width is the most stable measurement to differentiate *Septoria* spp. morphologically (Priest, 2006).

This chapter aims to unveil the species diversity of the genus *Septoria* in Japan through a multi-locus phylogenetic analysis, morphology, and cultural characteristics in accordance with current taxonomical criteria.

MATERIAL AND METHOD

Sample collection and morphological study

Culture Collection of the Laboratory of Phytopathology, Mie University (MUCC) and NARO Genebank Project of the National Agriculture, Food Research Organization, Tsukuba, Japan (MAFF) along with specimens from Herbarium of the Laboratory of Phytopathology, Mie university (TSU-MUMH) and Iwate University Museum, Morioka, Iwate, Japan (IUM) were used in this study. Newly collected symptomatic specimens and those isolates are shown in Table 4.1. Cultural characteristics of isolates were observed on Oatmeal agar (OMA; Becton Dickinson, MD, USA), Potato Dextrose Agar (PDA; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), and 2% Malt Extract Agar (MEA; Becton Dickinson) for 25 days and maintained on Malt Agar (MA; Becton Dickinson). The hand section of the herbarium specimens was prepared to observe the morphological characteristics of fungal bodies on the host plant and observed under a compound microscope Axio Imager A1 with the Shear's solution (Chupp, 1940a) as mounting medium.

DNA extraction, amplification, and sequencing

The genomic DNA was extracted using DNeasy Ultra Clean Microbial Kit (Qiagen, Hilden, Germany) from mycelia of growing culture according to the manufacturer's instructions. Seven genomic loci of the DNA regions, including ITS, LSU, BTUB, RPB2, TEF-1a, CAL, and ACT, were amplified via polymerase chain reaction using the T100 thermal cycler. 12.5 µL of PCR final mixture was prepared as follows: 1–10 ng of genomic DNA, 0.1 µL of 0.25 U Taq DNA polymerase (Bioline, London, UK), 2.5 mM of $MgCl_2$, 1.25 µL of 10× NH_4 reaction buffer (Bioline), 40 µM dNTPs (Bioline) and 0.2 µM of each primer. For RPB2, 12.5 µL PCR final mixture was prepared as follows: 20-25 ng of genomic DNA, 0.07 µL of TaKaRa Taq hot start version (TaKaRa Bio, Shiga, Japan), 1.25 µL of Buffer $MgCl_2$ mixture (TaKaRa Bio), 1 µL of dNTPs (TaKaRa Bio) and 0.25 µL of each primer. All PCR conditions and primers used in this study were as follows with different annealing temperatures as listed in Table 4.2: Initial denaturation at 94°C for 5 minutes, followed by denaturation at 94°C for 5 minutes, annealing at designated temperature for 30 seconds, elongation

Table 4.1. Host plant, Isolate number, voucher number and locality.

Isolate name	Recorded name	Isolate number	Voucher number	Locality	Host Plant
Septoria bupleuricola	*Septoria bupleuricola*	MUCC 640; MAFF 240611	TSU-MUMH 10588	Kumage, Kagoshima	*Bupleurum scorzonerifolium*
Sep. bupleuricola	*Sep. bupleuricola*	MUCC 725	TSU-MUMH 10852	Hidakagawa, Wakayama	*Bupleurum scorzonerifolium*
Sep. cannabicola	*Sep. cannabicola*	MUCC 3619	TSU-MUMH 11996	Kirihara, Mie	*Cannabis sariva*
Sep. convolvuli	*Sep. convolvuli*	MUCC 1569; MAFF 238308		Tateyama, Chiba	*Calystegia soldanella*
Sep. crepidis	*Sep. crepidis*	MUCC 1566; MAFF 237806		Kumejima, Okinawa	*Youngia japonica*
Sep. dolichospora	*Septoria* sp.	MUCC 3544	TSU-MUMH 11972	Tsu, Mie	*Splidago altissima*
Sep. erigeronitis	*Sep. erigeronitis*	MUCC 1588; MAFF 237726		Tsukuba, Ibaraki	*Erigeron annuus*
Sep. erigeronitis	*Sep. erigeronitis*	MUCC 1590; MAFF 237728		Kominato, Kagoshima	*Erigeron annuus*
Sep. erigeronitis	*Sep. erigeronitis*	MUCC 1602; MAFF 237807		Fuchu, Toyama	*Erigeron annuus*
Sep. erigeronitis	*Septoria* sp.	MUCC 1712	TSU-MUMH 11472	Morioka, Iwate	*Bellis prennis*
Sep. glycine	*Sep. glycine*	MUCC 1574; MAFF 306150		Tsu, Mie	*Glycine max*
Sep. glycine	*Sep. glycine*	MUCC 1598; MAFF 241246		Tsukuba, Ibaraki	*Glycine max*
Sep. obesa	*Sep. obesa*	MUCC 1599; MAFF 237299		Showa, Gunma	*Chrysanthemum morifolium*
Sep. oenanthicola	*Septoria apiicola*	MUCC 1581; MAFF 305588		Miyagi	*Oenanthe javanica*
Sep. polygonarum	*Sep. polygonarum*	MUCC 1600; MAFF 237803		Kitakata, Fukushima	*Persicaria longiseta*
Septoria sp.	*Septoria* sp.	MUCC 628; MAFF 240605	TSU-MUMH 10554	Kirishima, Kagoshima	*Gnaphalium japonicum*
Septoria sp.	*Septoria* sp.	MUCC 698	TSU-MUMH 10683	Sugadaira, Nagano	*Hydrocotyle sibthorpioides*
Septoria sp.	*Sep. centellae*	MUCC 1564; MAFF 237729		Tateyama, Chiba	*Centella asiatica*
Septoria sp.	*Sep. centellae*	MUCC 1565; MAFF 237744		Kumejima, Okinawa	*Centella asiatica*
Septoria sp.	*Septoria* sp.	MUCC 1601; MAFF 237804		Kunigami, Okinawa	*Clematis javana*
Septoria sp.	*Septoria taraxacicola*	MUCC 1604; MAFF 237805		Koriyama, Fukushima	*Taraxacum officinale*
Septoria sp.	*Septoria* sp.	MUCC 1711	TSU-MUMH 11470	Morioka, Iwate	*Persicaria posumbu*
Septoria sp.	*Septoria* sp.	MUCC 2889	TSU-MUMH 11887	Hokkaido	*Cacalia auriculata*

at 72°C for 45 seconds and final extension at 72°C for 7 minutes. Amplicons were sequenced in both directions using the respective PCR primers and BigDye terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an Applied Biosystems 3730xl DNA analyser installed at the Mie University Advanced Science Research Promotion Centre (Tsu, Mie, Japan).

Table 4.2. Primer used in this study for amplification and sequencing.

Region	Primer	Sequences	Annealing temp.
Actin	ACT-512F	ATGTGCAAGGCCGGTTTCGC	52
	ACT2Rd	ARRTCRCGDCCRGCCATGTC	52
TEF	EF1-728F	CATCGAGAAGTTCGAGAAGG	52
	EF2	GGARGTACCAGTSATCATGTT	52
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	52
	ITS4	TCCTCCGCTTATTGATATGC	52
β-tubulin	T1	AACATGCGTGAGATTGTAAGT	52
	β-Sandy-R	GCRCGNGGVACRTACTTGTT	52
LSU	LSU1Fd	GRATCAGGTAGGRATACCCG	52
	LR5	TCCTGAGGGAAACTTCG	52
RPB2	fRPB2-5F	GAYGAYMGWGATCAYTTYGG	49
	fRPB2-414	ACMANNCCCCARTGNGWRTTRTG	49
Calmodulin	CAL-235F	TTCAAGGAGGCCTTCTCCCTCTT	50
	CAL2Rd	TGRTCNGCCTCDCGGATCATCTC	50

Phylogenetic analysis

The newly analysed sequences were assembled with sequences retrieved from previous studies listed in Table 4.3, conducted by Quaedvlieg et al. (2013), Verkley et al. (2013) and Rahmana et al. (2021) on MEGA X software package (Kumar et al., 2016) . The matrix was aligned using MAFFT online software package (citation) and edited manually using AliView (Larsson, 2014). Phylogenetic trees generated by Maximum-likelihood (ML) and Bayesian Inference analyses were used in this study to estimate the phylogenetic relationship. The best substitution model for each region in the analysis was evaluated using ModelTest-NG (Darriba et al., 2020) and applied. ML analyses were performed using RAxML-NG (Kozlov et al., 2019) with 1000 replications of bootstrap analysis to test for tree strength. BI analysis was performed by using MrBayes (Ronquist et al., 2012). BI analysis was performed using MrBayes (Ronquist et al., 2012). To estimate the posterior probability of tree topologies, Metropolis-Coupled Markov Chain Monte Carlo

(MCMCMC) searches were run for 10 million generations, with trees sampled and saved every 1000 generations. The first 25% of the tree was discarded as a burn-in phase based on the Average Standard Deviation of Split Frequencies (Below 0.01), and the PPs were determined using the remaining trees. *Caryophyllospetoria spergulae* (CBS 397.52) was used as an outgroup in this study, and the generated trees were viewed using FigTree v 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Table 4.3: List of isolates and reference sequence used in this study.

Fungal Species	Isolate number	Country	Host	GenBank Accession number						
				EF	Tub	RPB2	LSU	ITS	Act	Cal
Septoria abei	CBS 128598	South Korea	*Hibiscus syriacus*	KF253280	KF252805	KF252336	KF251837	KF251333	KF253638	KF253985
Sep. aegopodina	CBS 123740	Czech Republic	*Aegopodium podagraria*	KF253281	KF252806	–	KF251838	KF251334	KF253639	KF253986
Sep. agrimoniicola	CBS 128601	South Korea	*Agrimonia pilosa*	KF253284	KF252809	KF252338	KF251841	KF251337	–	KF253988
Sep. anthrisci	CBS 109020	Austria	*Anthriscus* sp.	KF253286	KF252811	KF252340	KF251843	KF251339	KF253643	KF253991
Sep. anthurii	CBS 346.58	Germany	*Anthurium* sp.	KF253288	KF252813	KF252342	KF251845	KF251341	KF253645	KF253993
Sep. apiicola	CBS 400.54	Netherlands	*Apium graveolens*	KF253292	KF252817	KF252346	KF251849	KF251345	KF253649	KF253997
Sep. astericola	CBS 128593	South Korea	*Aster yomena*	KF253294	KF252819	KF252348	KF251851	KF251347	KF253651	KF253999
Sep. astragali	CBS 123878	Czech Republic	*Astragalus glycyphyllos*	KF253297	KF252822	KF252351	KF251854	KF251350	KF253654	KF254002
Sep. atropurpurea	CBS 348.58	Germany	*Aster canus*	KF253299	KF252824	KF252353	KF251856	KF251352	KF253656	KF254004
Sep. bothriospermi	CBS 128599	South Korea	*Bothriospermum tenellum*	KF253301	KF252826	KF252355	KF251858	KF251354	KF253658	KF254006
Sep. bupleuricola	CBS 128603	South Korea	*Bupleurum falcatum*	KF253303	KF252828	KF252357	KF251860	KF251356	KF253660	KF254008
Sep. bupleuricola	**MUCC 640**	**Japan**	***Bupleurum scorzonerifolium***	–	–	–	–	–	–	–
Sep. bupleuricola	**MUCC 725**	**Japan**	***Bupleurum scorzonerifolium***	–	–	–	–	–	–	–
Sep. calendulae	CBS 349.58	Italy	*Calendula arvensis*	KF253304	KF252829	KF252358	KF251861	KF251357	KF253661	KF254009
Sep. callistephi	CBS 128590	South Korea	*Callistephus chinensis*	KF253305	KF252830	KF252359	KF251862	KF251358	KF253662	KF254010
Sep. campanulae	CBS 128604	South Korea	*Campanula takesimana*	KF253308	KF252833	KF252362	KF251865	KF251361	KF253665	KF254013
Sep. cannabicola	**MUCC 3619**	**Japan**	***Cannabis sativa***	**OR759783**	**OR759784**	**OR759785**	**OR755918**	**OR755916**	**OR759782**	**OR759786**
Sep. cannabis	17JS002	USA	*Cannabis sativa*	MW556605	MW556608	MW556602	MW556614	MW556611	MW526952	MW526955
Sep. cannabis	18CL004	USA	*Cannabis sativa*	MW556603	MW556606	MW556600	MW556612	MW556609	MW526950	MW526953

Sep. cannabis	18MF001	USA	*Cannabis sativa*	MW556604	MW556607	MW556601	MW556613	MW556610	MW526951	MW526954
Sep. cerastii	CBS 128612	South Korea	*Cerastium holosteoides*	KF253311	KF252836	KF252365	KF251868	KF251364	KF253668	KF254016
Sep. cf. *rubi*	CBS 128646	South Korea	*Rubus crataegifolius*	KF253314	KF252839	KF252368	KF251871	KF251367	KF253671	KF254019
Sep. cf. *sonchi*	CBS 128757	South Korea	*Sonchus asper*	KF253500	KF253020	KF252546	KF252057	KF251552	KF253855	KF254204
Sep. chamaecisti	CBS 350.58	Germany	*Helianthemum hybridum*	KF253318	KF252843	KF252372	KF251875	KF251371	KF253675	KF254023
Sep. chelidonii	CBS 128607	South Korea	*Chelidonium majus*	KF253319	KF252844	KF252373	KF251876	KF251372	KF253676	KF254024
Sep. chromolaenae	CBS 113373	Cuba	*Chromolaena odorata*	KF253321	KF252846	KF252375	KF251878	KF251374	KF253678	KF254026
Sep. chrysanthemella	CBS 128617	South Korea	*Chrysanthemum morifolium*	KF253322	KF252847	KF252376	KF251879	KF251375	KF253679	KF254027
Sep. chrysanthemella	CBS 128622	South Korea	*Chrysanthemum boreale*	KF253323	KF252848	KF252377	KF251880	KF251376	KF253680	KF254028
Sep. cirsii	CBS 128621	South Korea	*Cirsium setidens*	KF253328	KF252853	KF252382	KF251885	KF251381	KF253685	KF254033
Sep. citri	CBS 177.77	New Zealand	*Fragaria* sp.	KF253463	KF252984	KF252509	KF252019	KF251514	KF253818	KF254167
Sep. citri	CBS 566.88	France	*Hedera helix*	KF253470	KF252990	KF252515	KF252026	KF251521	KF253825	KF254174
Sep. citricola	CBS 356.36	Italy	*Citrus sinensis*	KF253329	KF252854	KF252383	KF251886	KF251382	KF253686	KF254034
Sep. clematidis	CBS 108983	Germany	*Clematis vitalba*	KF253330	KF252855	KF252384	KF251887	KF251383	KF253687	KF254035
Sep. codonopsidis	CBS 128620	South Korea	*Codonopsis lanceolata*	KF253333	KF252858	KF252387	KF251890	KF251386	KF253690	KF254038
Sep. convolvuli	CBS 128627	South Korea	*Calystegia soldanella*	KF253336	KF252861	KF252390	KF251893	KF251389	KF253693	KF254041
Sep. convolvuli	**MUCC 1569**	**Japan**	***Calystegia soldanella***	–	–	–	–	–	–	–
Sep. coprosmae	CBS 113391	New Zealand	*Coprosma robusta*	KF253255	KF252787	KF252313	KF251812	KF251308	KF253617	KF253971
Sep. crepidis	CBS 128608	South Korea	*Youngia japonica*	KF253337	KF252862	KF252391	KF251894	KF251390	KF253694	KF254042
Sep. crepidis	**MUCC 1566**	**Japan**	***Youngia japonica***	–	–	–	–	–	–	–

Sep. cruciatae	CBS 123747	Czech Republic	*Galium odoratum*	KF253340	KF252865	KF252394	KF251897	KF251393	KF253697	KF254045
Sep. cucubali	CBS 102386	Netherlands	*Saponaria officinalis*	KF253344	KF252869	KF252398	KF251901	KF251397	KF253701	KF254049
Sep. cucurbitacearum	CBS 178.77	New Zealand	*Cucurbita maxima*	KF253346	–	KF252400	KF251903	KF251399	KF253703	KF254051
Sep. dearnessii	CBS 128624	South Korea	*Angelica dahurica*	KF253347	KF252871	KF252401	KF251904	KF251400	KF253704	KF254052
Sep. digitalis	CBS 391.63	Czech Republic	*Digitalis lanata*	KF253349	KF252873	KF252403	KF251906	KF251402	KF253706	KF254054
Sep. dolichospora	CBS 129152	South Korea	*Solidago virgaurea*	KF253350	KF252874	–	KF251907	KF251403	KF253707	KF254055
Sep. dysentericae	CBS 131892 ; CPC 12328	South Korea	*Inula britannica*	KF253353	KF252877	KF252406	KF251910	KF251406	KF253710	KF254058
Sep. ekmaniana	CBS 113612	Mexico	*Chromolaena odorata*	KF253355	KF252879	–	KF251912	KF251408	KF253712	KF254060
Sep. epambrosiae	CBS 128629	South Korea	*Ambrosia trifida*	KF253356	KF252880	KF252407	KF251913	KF251409	KF253713	KF254061
Sep. epilobii	CBS 109085	Austria	*Epilobium fleischeri*	KF253359	KF252883	KF252410	KF251916	KF251412	KF253716	KF254064
Sep. erigerontis	CBS 186.93	Italy	*Erigeron annuus*	KF253364	KF252887	KF252537	KF252048	KF251543	KF253893	KF254244
Sep. erigerontis	**MUCC 1588**	**Japan**	***Erigeron annuus***	–	–	–	–	–	–	–
Sep. erigerontis	**MUCC 1590**	**Japan**	***Erigeron annuus***	–	–	–	–	–	–	–
Sep. erigerontis	**MUCC 1602**	**Japan**	***Erigeron annuus***	–	–	–	–	–	–	–
Sep. erigerontis	**MUCC 1712**	**Japan**	***Bellis prennis***	–	–	–	–	–	–	–
Sep. eucalyptorum	CBS 118505	India	*Eucalyptus* sp.	KF253365	KF252889	KF252415	KF251921	KF251417	KF253721	KF254069
Sep. exotica	CBS 163.78	New Zealand	*Hebe speciosa*	KF253366	KF252890	KF252416	KF251922	KF251418	KF253722	KF254070
Sep. galeopsidis	CBS 102314	Netherlands	*Galeopsis tetrahit*	KF253371	KF252895	KF252421	KF251927	KF251423	KF253727	KF254075
Sep. gentianae	CBS 128633	South Korea	*Gentiana scabra*	KF253374	KF252898	KF252424	KF251930	KF251426	KF253730	KF254078
Sep. glycines	CBS 336.53	Japan	–	KF253377	KF252901	–	KF251933	KF251429	KF253733	KF254081
Sep. glycines	**MUCC 1574**	**Japan**	***Glycine max***	–	–	–	–	–	–	–
Sep. glycines	**MUCC 1598**	**Japan**	***Glycine max***	–	–	–	–	–	–	–

Sep. glycinicola	CBS 128618	South Korea	*Glycine max*	KF253378	KF252902	KF252427	KF251934	KF251430	KF253734	KF254082
Sep. helianthi	CBS 123.81	–	*Helianthus annuus*	KF253379	KF252903	KF252428	KF251935	KF251431	KF253735	KF254083
Sep. helianthicola	CBS 122.81	–	*Helianthus annuus*	KF253380	KF252904	KF252429	KF251936	KF251432	KF253736	KF254084
Sep. hibiscicola	CBS 128615	South Korea	*Hibiscus syriacus*	KF253382	KF252906	KF252431	KF251938	KF251434	KF253738	KF254086
Sep. hippocastani	CBS 411.61	Germany	*Aesculus hippocastanum*	KF253383	KF252907	KF252432	KF251939	KF251435	KF253739	KF254087
Sep. justiciae	CBS 128625	South Korea	*Justicia procumbens*	KF253385	KF252909	KF252434	KF251941	KF251437	KF253741	KF254089
Sep. lactucae	CBS 352.58	Germany	*Lactuca sativa*	KF253388	KF252912	KF252437	KF251944	KF251440	KF253744	KF254092
Sep. lamiicola	CBS 123884	Czech Republic	*Lamium* sp.	KF253397	KF252921	KF252446	KF251953	KF251449	KF253753	KF254101
Sep. lepidiicola	CBS 128635	South Korea	*Lepidium virginicum*	KF253398	KF252922	KF252447	KF251954	KF251450	KF253754	KF254102
Sep. leptostachyae	CBS 128628	South Korea	*Phryma leptostachya*	KF253400	KF252924	KF252449	KF251956	KF251452	KF253756	KF254104
Sep. leucanthemi	CBS 109090	Austria	*Chrysanthemum leucanthemum*	KF253403	KF252927	KF252452	KF251959	KF251455	KF253759	KF254107
Sep. limonum	CBS 419.51	Italy	*Citrus limonium*	KF253407	KF252931	KF252456	KF251963	KF251459	KF253763	KF254111
Sep. linicola	CBS 316.37	–	*Linum usitatissimum*	KF253408	KF252932	KF252457	KF251964	KF251460	KF253764	KF254112
Sep. lycoctoni	CBS 109089	Austria	*Aconitum vulparia*	KF253409	KF252933	KF252458	KF251965	KF251461	KF253765	KF254113
Sep. lycopersici	CBS 128654	South Korea	*Lycopersicon esculentum*	KF253410	KF252934	KF252459	KF251966	KF251462	KF253766	KF254114
Sep. lycopicola	CBS 128651	South Korea	*Lycopus ramosissimus*	KF253412	KF252936	KF252461	KF251968	KF251464	KF253768	KF254116
Sep. lysimachiae	CBS 102315	Netherlands	*Lysimachia vulgaris*	KF253413	KF252937	KF252462	KF251969	KF251465	KF253769	KF254117
Sep. malagutii	CBS 106.80	Peru	*Solanum* sp.	KF253418	–	KF252467	KF251974	KF251470	KF253774	KF254122
Sep. matricariae	CBS 109001	Netherlands	*Matricaria discoidea*	KF253420	KF252943	KF252469	KF251976	KF251472	KF253776	KF254124
Sep. mazi	CBS 128755	South Korea	*Mazus japonicus*	KF253422	KF252945	KF252471	KF251978	KF251474	KF253778	KF254126

Sep. melissae	CBS 109097	Netherlands	*Melissa officinalis*	KF253423	KF252946	KF252472	KF251979	KF251475	KF253779	KF254127
Sep. menthae	CBS 404.34	Japan	–	KF253424	KF252947	–	KF251980	KF251476	KF253780	KF254128
Sep. napelli	CBS 109105	Austria	*Aconitum napellus*	KF253426	KF252949	KF252474	KF251982	KF251478	KF253782	KF254130
Sep. obesa	CBS 128588	South Korea	*Artemisia lavandulaefolia*	KF253428	KF252951	KF252476	KF251984	KF251480	KF253784	KF254132
Sep. obesa	CBS 128623	South Korea	*Chrysanthemum indicum*	KF253429	KF252952	KF252477	KF251985	KF251481	KF253785	KF254133
Sep. obesa	**MUCC 1599**	**Japan**	***Chrysanthemum morifolium***	–	–	–	–	–	–	–
Sep. oenanthis	CBS 128667	South Korea	*Cicuta virosa*	KF253432	KF252953	KF252481	KF251989	KF251485	KF253788	KF254136
Sep. oenanthicola	CBS 128649	South Korea	*Oenanthe javanica*	KF253433	KF252954	KF252480	KF251988	KF251484	KF253789	KF254137
Sep. orchidearum	CBS 128631	South Korea	*Cyclamen fatrense*	KF253434	KF252955	KF252482	KF251990	KF251486	KF253790	KF254138
Sep. orchidearum	CBS 457.78	France	*Listera ovata*	KF253435	KF252956	KF252483	KF251991	KF251487	KF253791	KF254139
Sep. pachyspora	CBS 128652	South Korea	*Zyathoxylum schinifolium*	KF253437	KF252958	KF252485	KF251993	KF251488	KF253792	KF254141
Sep. paridis	CBS 109111	Austria	*Paris quadrifolia*	KF253438	KF252959	KF252486	KF251994	KF251489	KF253793	KF254142
Sep. paridis	CBS 109108	Austria	*Viola* sp.	KF253440	KF252961	KF252488	KF251996	KF251491	KF253795	KF254144
Sep. passifloricola	CBS 102701	New Zealand	*Passiflora edulis*	KF253442	KF252963	KF252490	KF251998	KF251493	KF253797	KF254146
Sep. perillae	CBS 128655	South Korea	*Perilla frutescens*	KF253444	KF252965	KF252491	KF252000	KF251495	KF253799	KF254148
Sep. petroselini	CBS 182.44	Netherlands	*Petroselinum sativum*	KF253446	KF252967	KF252493	KF252002	KF251497	KF253801	KF254150
Sep. phlogis	CBS 128663	South Korea	*Phlox paniculata*	KF253448	KF252969	KF252495	KF252004	KF251499	KF253803	KF254152
Sep. polygonorum	CBS 347.67	Netherlands	*Polygonum persicaria*	KF253455	KF252976	KF252502	KF252011	KF251506	KF253810	KF254159
Sep. polygonorum	**MUCC 1600**	**Japan**	***Persicaria longiseta***	–	–	–	–	–	–	–
Sep. posoniensis	CBS 128645	South Korea	*Chrysosplenium japonicum*	KF253456	KF252977	KF252503	KF252012	KF251507	KF253811	KF254160

Sep. protearum	CBS 390.59	Italy	*Ligustrum vulgare*	KF253467	KF252987	KF252513	KF252023	KF251518	KF253822	KF254171
Sep. protearum	CBS 778.97	South Africa	*Protea cynaroides*	KF253472	KF252992	KF252517	KF252028	KF251523	KF253827	KF254176
Sep. pseudonapelli	CBS 128664	South Korea	*Aconitum pseudolaeve*	KF253475	KF252995	KF252520	KF252031	KF251526	KF253830	KF254179
Sep. putrida	CBS 109088	Austria	*Senecio nemorensis*	KF253477	KF252997	KF252522	KF252033	KF251528	KF253832	KF254181
Sep. rumicum	CBS 503.76	France	*Rumex acetosa*	KF253478	KF252998	KF252523	KF252034	KF251529	KF253833	KF254182
Sep. saccardoi	CBS 128756	South Korea	*Lysimachia vulgaris*	KF253479	KF252999	KF252524	KF252035	KF251530	KF253834	KF254183
Sep. scabiosicola	CBS 102336	Netherlands	*Knautia arvensis*	KF253483	KF253003	KF252528	KF252039	KF251534	KF253838	KF254187
Sep. scabiosicola	CBS 109093	Austria	*Knautia dipsacifolia*	KF253487	KF253007	KF252532	KF252043	KF251538	KF253842	KF254191
Sep. senecionis	CBS 102366	Netherlands	*Senecio fluviatilis*	KF253492	KF253012	KF252538	KF252049	KF251544	KF253847	KF254196
Sep. siegesbeckiae	CBS 128659	South Korea	*Siegesbeckia glabrescens*	KF253494	KF253014	KF252540	KF252051	KF251546	KF253849	KF254198
Sep. siegesbeckiae	CBS 128661	South Korea	*Siegesbeckia pubescens*	KF253495	KF253015	KF252541	KF252052	KF251547	KF253850	KF254199
Sep. sii	CBS 102370	Netherlands	*Berula erecta*	KF253497	KF253017	KF252543	KF252054	KF251549	KF253852	KF254201
Sep. sisyrinchii	CBS 112096	New Zealand	*Sysirinchium* sp.	KF253499	KF253019	KF252545	KF252056	KF251551	KF253854	KF254203
Septoria sp.	CPC 19976	Italy	*Feijoa sellowiana*	KF253509	KF253030	–	KF252067	KF251562	KF253863	KF254214
Septoria sp.	CBS 109114	Austria	*Campanula glomerata*	KF253501	KF253021	KF252547	KF252058	KF251553	KF253856	KF254205
Septoria sp.	CBS 120739	Italy	*Eucalyptus* sp.	KF253503	KF253023	KF252549	KF252060	KF251555	KF253858	KF254207
Septoria sp.	CBS 128650	South Korea	*Taraxacum officinale*	KF253504	KF253024	KF252550	KF252061	KF251556	KF253859	KF254208
Septoria sp.	CBS 128658	South Korea	*Chrysoplenium japonicum*	KF253505	KF253025	KF252551	KF252062	KF251557	KF253860	KF254209
Septoria sp.	CBS 135472 ; CPC 19304	Austria	*Vigna unguiculata ssp. sesquipedalis*	KF253506	KF253026	KF252552	KF252063	KF251558	KF253861	KF254210

Septoria sp.	CBS 135474 ; CPC 19485	Brazil	*Conyza canadensis*	KF253507	KF253027	KF252553	KF252064	KF251559	KF253862	KF254211
Septoria sp.	CBS 135478 ; CPC 19716	South Africa	*Searsia laevigatum*	KF253508	KF253028	KF252554	KF252065	KF251560	–	KF254212
Septoria sp.	CBS 135479 ; CPC 19793	South Africa	*Syzygium cordatum*	–	KF253029	KF252555	KF252066	KF251561	–	KF254213
Septoria sp.	CPC 23103; MP11	Netherlands	*Aesculus* sp.	KF253510	KF253031	KF252556	KF252068	KF251563	KF253864	KF254215
***Septoria* sp.**	**MUCC 628**	Japan	***Gnaphalium japonicum***	–	–	–	–	–	–	–
***Septoria* sp.**	**MUCC 698**	Japan	***Hydrocotyle sibthorpioides***	–	–	–	–	–	–	–
***Septoria* sp.**	**MUCC 1564**	Japan	***Centella asiatica***	–	–	–	–	–	–	–
***Septoria* sp.**	**MUCC 1565**	Japan	***Centella asiatica***	–	–	–	–	–	–	–
***Septoria* sp.**	**MUCC 1581**	**Japan**	***Oenanthe javanica***	–	–	–	–	–	–	–
***Septoria* sp.**	**MUCC 1601**	Japan	***Clematis javana***	–	–	–	–	–	–	–
***Septoria* sp.**	**MUCC 1604**	Japan	***Taraxacum officinale***	–	–	–	–	–	–	–
***Septoria* sp.**	**MUCC 1711**	Japan	***Persicaria posumbu***	–	–	–	–	–	–	–
***Septoria* sp.**	**MUCC 2889**	Japan	***Cacalia auriculata***	–	–	–	–	–	–	–
***Septoria* sp.**	**MUCC 3544**	**Japan**	***Solidago altissima***	–	–	–	–	–	–	–
Sep. stachydicola	CBS 128668	South Korea	*Stachys riederi*	KF253512	KF253033	KF252558	KF252070	KF251565	KF253866	KF254217
Sep. stachydis	CBS 109115	Austria	*Campanula glomerata*	KF253502	KF253022	KF252548	KF252059	KF251554	KF253857	KF254206
Sep. stachydis	CBS 102326	Netherlands	*Stachys sylvatica*	KF253514	KF253035	KF252560	KF252072	KF251567	KF253868	KF254219
Sep. stachydis	CBS 109127	Austria	*Stachys sylvatica*	KF253517	KF253038	KF252563	KF252075	KF251570	KF253871	KF254222
Sep. stachydis	CBS 347.58	Germany	*Aster canus*	KF253295	KF252820	KF252349	KF251852	KF251348	KF253652	KF254000
Sep. stellariae	CBS 102376	Netherlands	*Stellaria media*	KF253521	KF253042	KF252567	KF252079	KF251574	KF253875	KF254226
Sep. taraxaci	CBS 567.75	Armenia	*Taraxacum* sp.	KF253524	KF253045	KF252570	KF252082	KF251577	KF253878	KF254229

Sep. tinctoriae	CBS 129154	South Korea	*Serratula coronata*	KF253525	KF253046	KF252571	KF252083	KF251578	KF253879	KF254230
Sep. tormentillae	CBS 128647	South Korea	*Potentilla fragarioides*	KF253527	KF253048	KF252573	KF252085	KF251580	KF253881	KF254232
Sep. urticae	CBS 102316	Netherlands	*Glechoma hederacea*	KF253528	KF253049	KF252574	KF252086	KF251581	KF253882	KF254233
Sep. urticae	CBS 102375	Netherlands	*Urtica dioica*	KF253530	KF253051	KF252576	KF252088	KF251583	KF253884	KF254235
Sep. verbascicola	CBS 102401	Netherlands	*Verbascum nigrum*	KF253531	KF253052	KF252577	KF252089	KF251584	KF253885	KF254236
Sep. verbenae	CBS 113438	New Zealand	*Verbena officinalis*	KF253532	KF253053	KF252578	KF252090	KF251585	KF253886	KF254237
Sep. villarsiae	CBS 514.78	Netherlands	*Nymphoides peltata*	KF253534	KF253055	KF252580	KF252092	KF251587	KF253888	KF254239
Sep. violae-palustris	CBS 128644	South Korea	*Viola selkirkii*	KF253537	KF253058	KF252583	KF252095	KF251590	KF253891	KF254242
Sep. violae-palustris	CBS 128660	South Korea	*Viola yedoensis*	KF253538	KF253059	KF252584	KF252096	KF251591	KF253892	KF254243
Caryophylloseptoria spergulae	CBS 397.52	Netherlands	*Dianthus caryophyllus*	KF253243	KF252777	KF252301	KF251799	KF251295	KF253604	KF253958

RESULT

Phylogeny

A total of 151 OTUs belonging to the genus *Septoria*, including 23 OTUs obtained in this study, were aligned, and analysed. The concatenated data matrix of seven genomic loci consisted of 3377 characters, including gaps (ACT: 254 bp, TEF-1a: 529 bp, ITS: 509 bp, BTUB: 345 bp, LSU: 826 bp, RPB2: 344 bp, and CAL: 569 bp). BI analysis and ML analysis showed congruent tree topology. All branches and internal nodes of the ML tree were strongly supported with bootstrap values of more than 60%. The result from this analysis reveals that the *Septoria* spp. from Japan infecting herbaceous host plant, mainly of Asterales and Apiales, as shown in Figure 4.1. One new species was proposed as addition to the Japan mycoflora.

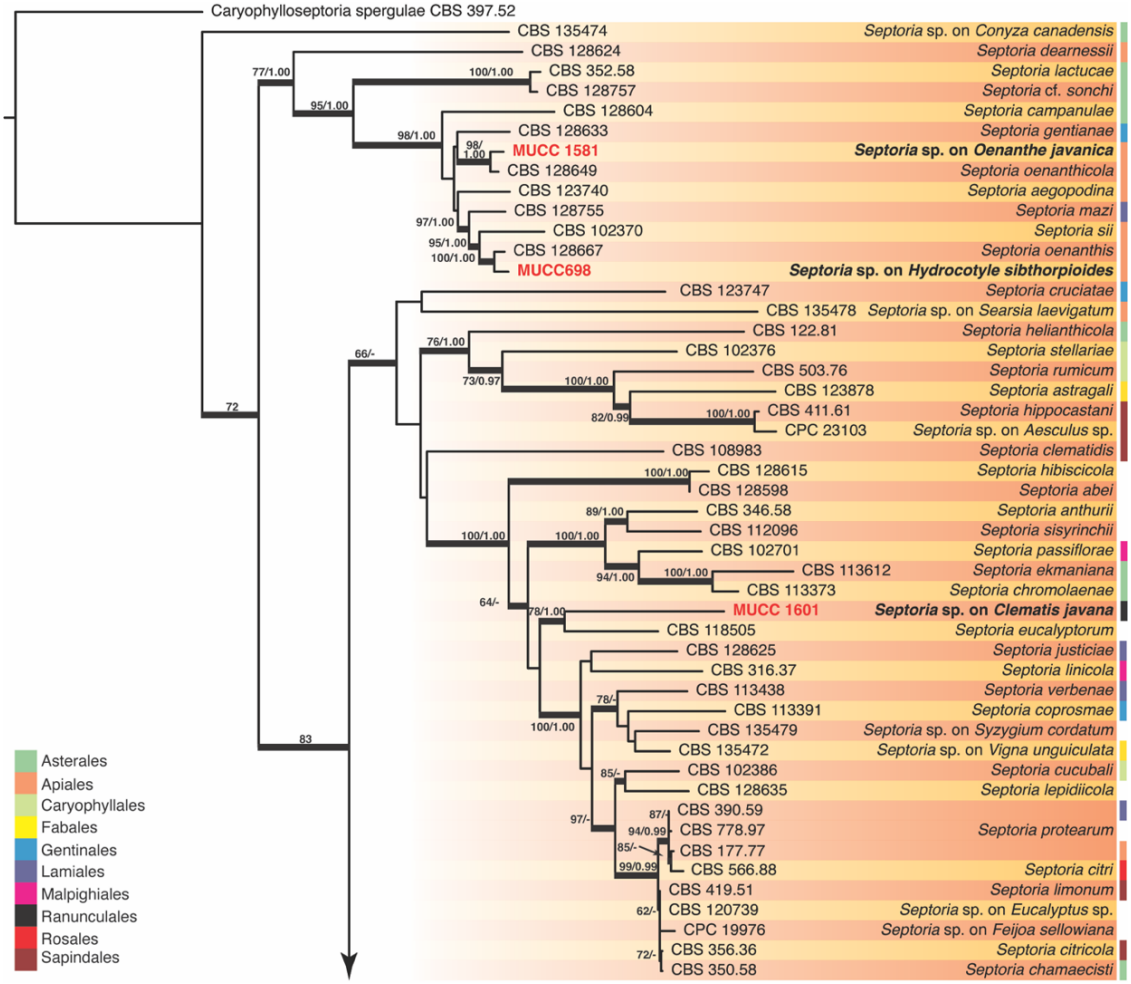

Figure 4.1: Maximum likelihood phylogenetic tree of *Septoria* spp. constructed by using concatenated matrix of 7 loci. The bootstrap value of ML and posterior probability of BI are indicated near branch as BS/PP.

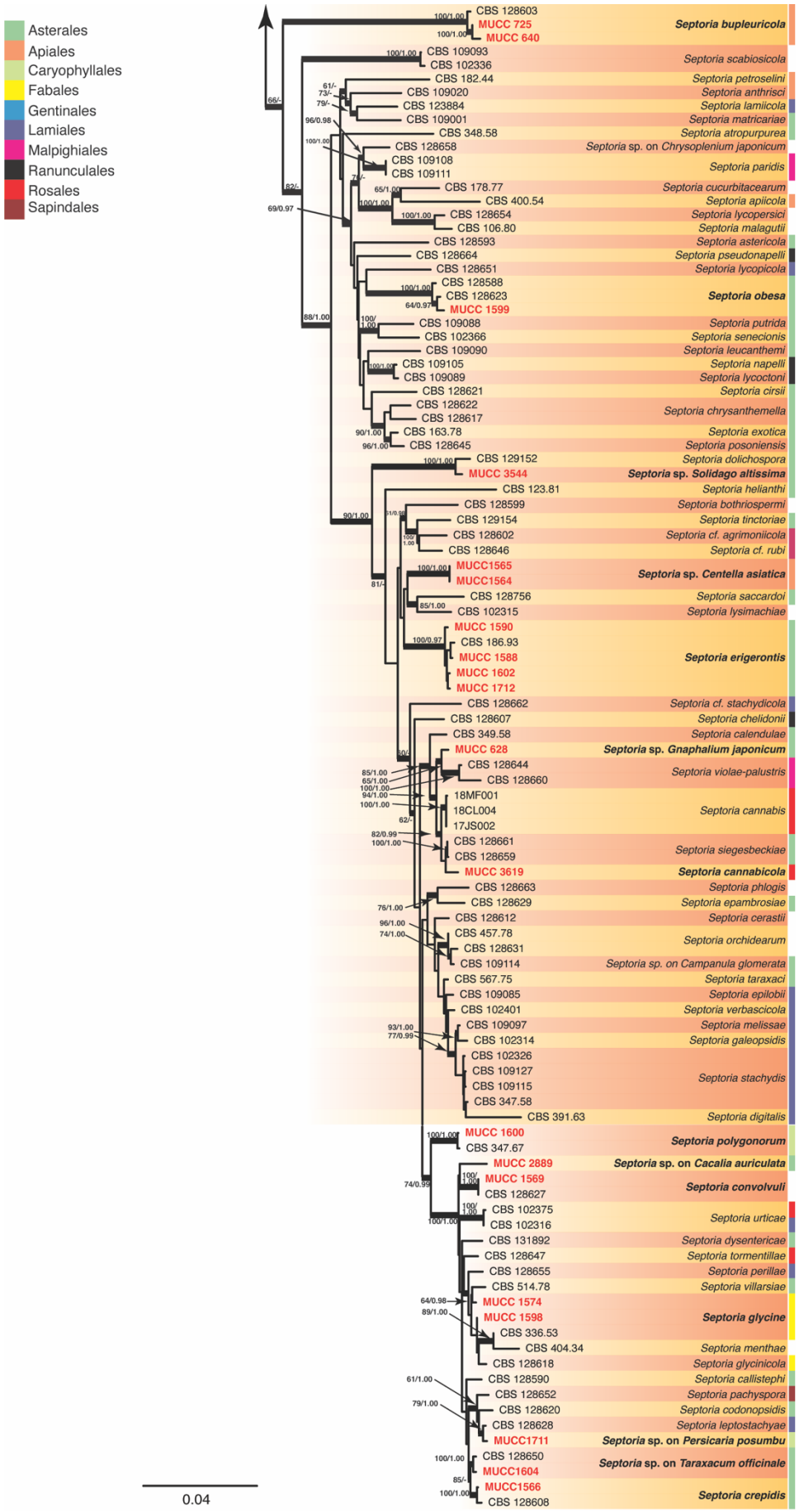

Figure 4.1: Continue.

Taxonomy

Septoria bupleuricola Sacc., Syll Fung. 3: 529 (1884)

Description: See Saccardo (1884)

Specimens examined: **Japan**, Wakayama, Hidakagawa, on leaves of *Bupleurum scorzonerifolium* (Apiceae), 30 October 2007, collected by E. Imaizumi (TSU-MUMH 10852, culture MUCC 725); **Japan**, Kagoshima, Kumage, on leaves of *Bupleurum scorzonerifolium*, 31 May 2005, C. Nakashima (TSU-MUMH 10588, culture MUCC 640 = MAFF 240611)

Note: Isolate MUCC 640 and MUCC 725 forms well supported clade with *Septoria bupleuricola* (CBS 128603), and the morphological description by Saccardo is similar with the specimens examined in this study. Hirayama (1931) recorded *Septoria* species infecting *Bupleurum falcatum* as *Sep. bupleuri* Desm. and treated it as a synonym of Sep. *buplericola* Sacc. However, Shin and Sameva (2004) mentioned *Sep. bupleuricola* differes from *Sep. bupleuri* by having larger ostiole, longer and wider conidia and concluded that the material recorded by Hirayama should be *Sep. bupleuricola*.

Septoria cannabicola Ujat & C. Nakash. **sp.nov. , MB 850821,** Figure 4.2.

Etymology: derived from the host plant genus, *Cannabis*.

Leaf spots on the lower position leaves, causing early defoliation, amphigenous, angular, yellow with indistinct border at early stage, later becoming brown, circular to irregular, surrounded by yellow halo, 2–8 mm. *Mycelium* hyaline or pale brown, 2–2.5 µm in width. *Conidiomata* pycnidial, amphigenous, mainly hypogenous, pale brown to brown, epidermal or subepidermal, submerged or erumpent through epidermis, globose to subglobose, 88–125 µm in diam., with an ostiole erumpent through epidermis, with opening 20–25 µm in diam.; conidiomatal wall 1–2 cell layers wide, composed of *textura angularis*, 2–2.5 µm. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* ampulliform, pale brown, paler towards apex, lining inner cavity of basal half of conidiomata, percurrently proliferating, 2.5–5 × 2–3 µm. *Conidia* holoblastic, solitary,

hyaline, cylindrical to obclavate, straight to slightly curved, pointed at the tip, obconical truncated at the base, not thickened, 30–40 × 1–2.5 µm, 0–4-septate.

Colony on MA greyish white to cream buff, floccose, with loose aerial mycelia at the edge; *pycnidial* conidiomata rarely formed on the surface under diffuse natural light at room temperature; reverse black to olivaceous black, with concentric patterns.

Holotype: **Japan**, Mie, Minami Ise, Kirihara, on Cannabis sativa, L., 07 Jul 2023, collected by C. Nakashima (TSU-MUMH 11996, Ex-type culture: MUCC3619)

Additional specimens examined: on C. sativa, JAPAN, Shida Path, 07 Jul 1905, K. Sawada (4836) (IUM*-FY914); Iwate, Morioka, 12 Sep 1911, G. Yamada (4824) (IUM-FY915); Iwate, Morioka, 05 Oct 1905, G. Yamada (4823) (IUM-FY916); Iwate, Morioka, 17 Oct 1904, G. Yamada (4822) (IUM-FY917); Hokkaido, Otaru, 17 Jul 1896, G. Yamada (4817) (IUM-FY918); Hokkaido, Otaru, 01 Aug 1898, G. Yamada (4818) (IUM-FY919); Hokkaido, Maruyama, 13 Oct 1896, G. Yamada (4819) (IUM-FY920); Hokkaido, Maruyama, 10 Jul 1896, G. Yamada (4820) (IUM-FY921); Hokkaido, Sapporo, 03 Oct 1896, G. Yamada (4821) (IUM-FY922); Iwate, Morioka, 06 Jun 1947, K. Sawada (IUM-FY946).

Note: Morphological characteristics of specimens examined in this study, including specimens collected from 1896 to 1947 in the northern part of Japan, show that the conidiomata size of Japanese fungus (88–125 µm in diam) is larger than that of *Sep. cannabis* (90 µm in diam.; McPartland, 1995) and *Sep. neocannabina* McPartl. (66 µm in diam.; McPartland, 1995) on *C. sativa*. Apart from conidiomata shape, observable differences include conidial shape and size as described in report by McPartland (1995), where conidia of *Sep. cannabina* are longer and wider compared to Japanese fungus at 30–55 × 2.0–2.5 µm and those of *Sep. neocannabina* are shorter with about the same width 20–30 × 1.0–2.0 µm. From these results, Japanese *Septoria* species on *C. cannabis* should be treated as a novel species.

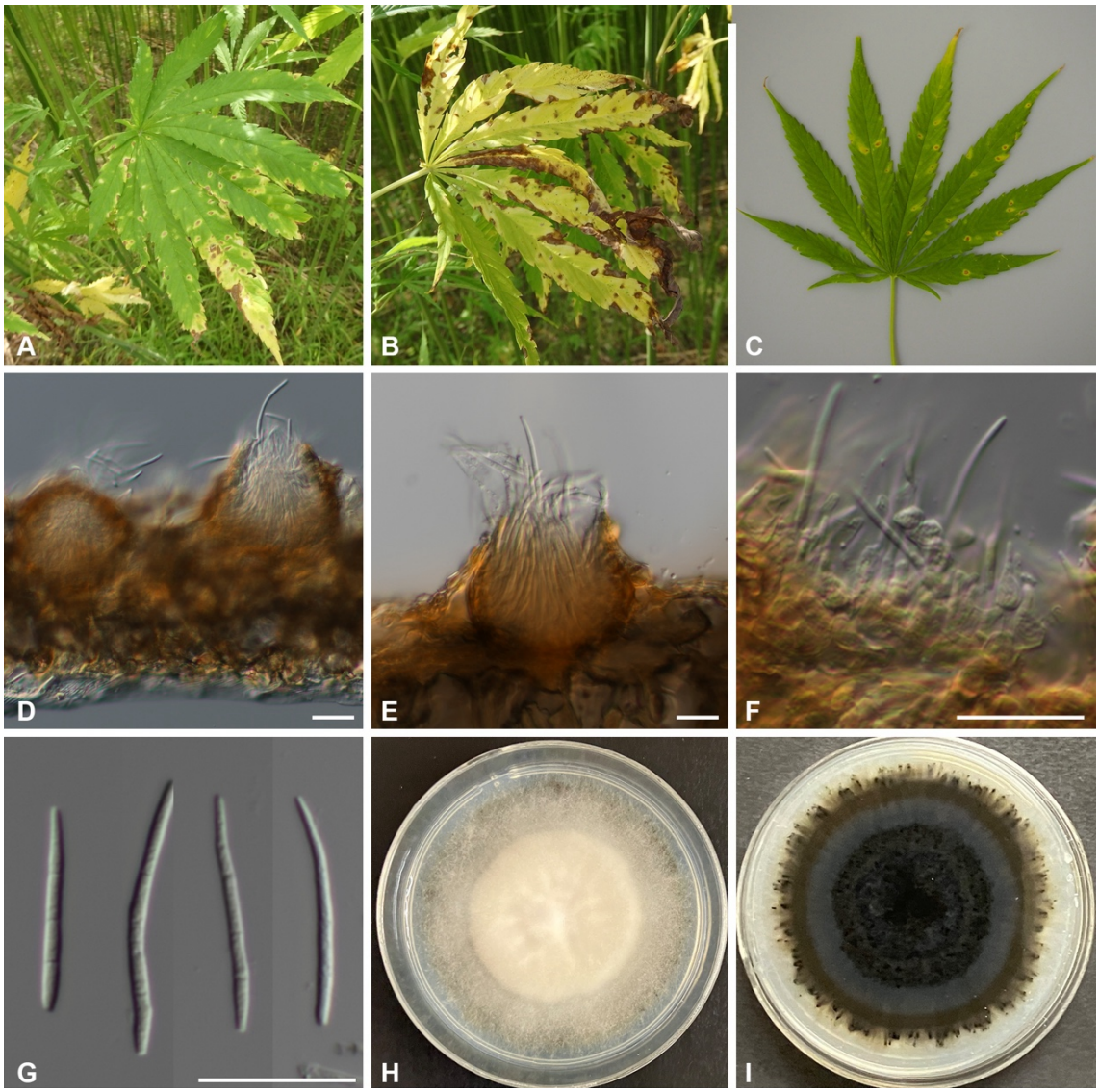

Figure 4.2: *Septoria cannabicola* (TSU-MUMH 11996). A–B: Natural symptoms of leaf spot disease on *Cannabis sativa* L. caused by *Septoria cannabicola*. C: Symptoms on artificially inoculated leaves of C. sativa. D–E: Conidiomata F: Conidiogenous cells. G: Conidia. H–I: Colony characteristics of *Septoria cannabicola* in culture on MA. Bars: D–G 20 µm.

Septoria convolvuli Desm., Ann. Sci. Nat., Bot. 17:108 (1842)

= *Ascochyta convolvuli* Lib. Plantae Cryptogamicae quas in Arduenna collegot m.A. Libert Fasc. 1: no 56 (1830)

= *Sep. convolvuli* Desm var *dolichospora* Sacc.

Description: See Desmazières (1842)

Culture examined: **Japan**, Chiba, Tateyama, on leaves of *Calystergia soldanella* (Convolvulaceae), July 1998, collected by E. Imaizumi (culture MUCC 1569 = MAFF 238308)

Note: Isolate MUCC 1569 forms well supported clade with *Septoria convolvuli* (CBS 128627) isolated from *Calystegia soldanella* from South Korea. The original specimens for the culture MUCC 1569 was not examined in this study. Apart from *Sep. convolvuli*, there are other *Septoria* recorded to infect *Calystegia* spp. and *Convolvulus* spp. includes *Septoria flagellaris* Ellis and Everh. Whereby it differs morphologically by having longer and wider spores of 35–114µm x 1.5 µm, 4–8 septate and boarder at one end and attenuated to the other end. It also differes from *Septoria sepium* Desm. by having cylindrical, obtuse, curved or nearly straight spore with truncated base. Other record include *Septoria septulata* Beach where it was described as having pycnidia mostly epiphyllous, innate, globose, 60–90µm, ostiole prominent and protruding, 20–30µm, spores curved or flexuous, one end narrow with more acute tip, 3–5 septate, 30–50µm long by 1–2 µm width.

Shin and Sameva (2004) mention that there are more than 10 species of *Septoria* infecting *Calytegia* spp. and *Convolvulus* spp. Priest (2006) summarised other *Septoria* spp. infecting Convolvulaceae including *Septoria. convolvulina* Speg (conidia 40–45 µm × 2.5–3.0 µm, 1–3 septate), *Septoria fabletina* Speg (conidia 25–35 × 1µm) and *Septoria longispora* Bondartsev (conidia 70–130 × 2.5–3.0, 5 septate).

Septoria crepidis Vestergr., Bih. Kongl. Svenska Vetensk.-Akad. Handl. 22 (6): 24 (1896)

Description: Vestergren (1896).

Culture examined: **Japan**, Okinawa, Kumejima, on leaves of *Youngia japonica* (Asteraceae), March 1998, collected by E. Imaizumi (culture MUCC 1599 = MAFF 237806)

Note: Sawada (1943) mention that *Septoria* infecting *Crepis japonica* in Tamsui, Taipei, Taiwan was different morphologically from *Septoria crepidis* infecting *Crepis tectorum* described by Vestergren, and design their collection as *Septoria crepidis-japonica.* Vestergren described the spore of *Septoria crepidis* as 25–35 × 1 µm, multi-guttulated with septation, while Sawada describe *Septoria crepidis-japonicum* as 21–41 × 2µm. Hirayama (1931) described *S. crepidis* as a pathogen of *Youngia japonica* with description of spore of 28.3–45 × 1.7–2.0 µm. While Hirayama description is similar to Sawada, the material provided by Shin and Sameva (2004) was kept as *Septoria crepidis*, which was used as a reference material in this study.

Septoria erigeronitis Peck., Ann. Rep. N. Y. State. Mus. Nat. Hist. 24:67 (1872)

Description: See Shin and Sameva (2004)

Specimens examined: Japan, Iwate, Morioka, on leaves of *Bellis prennis* (Asteraceae), 09 July 2012, collected by C. Nakashima (TSU-MUMH 11472, culture MUCC 1712).

Note: Records of *Septoria* species causing leaf spot on *Bellis prennis* include *Septoria bellidis* Roberge ex Desm., *Septoria bellidicola* Roberge ex Desm., and *Septoria bellidiastri* Allesch. However, the specimens examined in this study shows difference with protologue description in spore size, 35-40 µm × 1.2-1.4, wider than other *Septoria* describe on *Bellis* spp. Molecular phylogeny show isolate used in this study are cladded together with *Septoria erigeronitis*, isolated from *Erigeron annuus*. Shin and Sameva (2004) gives an update to the description based on the Korean specimens.

Insufficiently Known Species

Septoria sp. *on Oenanthe javanica*

Note: Honkura et al., (1988) initially recorded this species as *Septoria apiicola* Speg. On *Oenanthe javanica* as *Septoria apiicola* are known to infect Apiceae or Umbellifererae. However, their description is slightly different than of *Septoria apiicola* by Spegazzini Whereby Honkura described their isolate as having conidiomata of approximate 75–175 µm in diam., condia length ranging from 10–90 × 1.5–3.0 µm with 0–6 septa, while Spegazzini (1888) described *Septoria apiicola* with conidiomata of 60–80 µm in diam., conidia filiform, slightly pointed at the end, non-constricted, flexous, filiform, 3–7 septate, 30–45 × 1.5 µm. Shin and Sameva (2004) describe their collection as *Septoria oenanthes* Ellis & Everhart, however, the conidia of Korean collection (30–60 × 1.5–2.5) are much larger than the American type material (20–35 × 1.5–2.0 µm; Saccardo 1895), hence Quaedvlieg et al., (2013) design the Korean material as a new species of *Septoria*. Although the phylogenetic analysis shows strong bootstrap support of (100/1.00), the herbarium material was not examined in this study, hence, it is recorded as *Septoria* sp. on *Oenanthe javanica*.

Septoria sp. on *Gnaphalium japonicum*

Conidiomata pycnidial, epiphyllous, pale brown to brown, submerged, globose to subglobose, 65–90 µm in diam., with an ostiole erumpent through epidermis, with opening up to 40 µm in diam.; *conidiomatal wall* 1–2 cell layers wide, composed of textura angularis, 3–5 µm. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* ampulliform, hyaline, lining inner cavity of basal half of conidiomata, percurrently proliferating, 2.5–4 × 1–1.2 µm. *Conidia* holoblastic, solitary, hyaline, cylindrical to obclavate, straight, rounded and narrower at the tip, truncated at the base, 25–45 × 1.2–1.5 µm, 3–4-septate.

Specimens examined: **Japan**, Kagoshima, Kirishima, on leaves on Gnaphalium japonicum, 29 May 2007, C. Nakashima (TSU-MUMH 10554, culture MUCC 628 = MAFF 240605)

Note: Sawada (1942) describe *Septoria gnaphalii-indici* on *Gnaphalium indicum*, isolated in Taiwan. However, the present isolate examined exhibit variation in conidiomata and spore size compare to protologue description of *Sep. gnaphalii-indici*, and there is no holotype material sequence available for comparison. Hence this species was not described formally.

Septoria sp. on *Hydrocotyle sibthopioides*

Conidiomata pycnidia., epiphyllous, amphigenous, dark brown, submerged, globose to subglobose, 45–76 µm in diam., ostiole erumpent through epidermis, with opening up to 25–30 µm in diam. *Conidiomatal* wall 2–3 cell layers wide, composed of *textura angularis*, 1.5–2.0 µm. *Conidiophores* reduced to conidiogenous cells. Conid*iogenous cells* ampulliform, hyaline, percurrently proliferating, 2.5–3.5 × 1–1.3 µm. *Conidia* holoblastic, solitary, hyaline, cylindrical, obclavate, straight, slightly curved, rounded tip, truncated base, 13–25 × 1.3–2.5 µm, 0–1-septate.

Specimens examined: TSU-MUMH 10683

Note: Shin and Sameva (2004) recorded *Septoria hydrocotyle* Desem. as the pathogen of *Hydrocotyle ramiflora*. *Septoria hydrocotyle* conidia are recorded as containing oil drops, subhyaline, 16–30 × 1.5–2µm. Other *Septoria* spp on *Hydrocotyle* spp includes *Septoria spegazzainii* Sacc. (conida: 45–50 × 1.5µm, Saccardo, 1884), *Septoria hydrocotylicola* Sepg. (conidia: 40–50 × 1 µm, Spegazzini, 1908), *Septoria fusiformis* Muthumary (conidia: 35–60 × 3–4 µm, Muthumary, 1999).

Septoria sp. on *Taraxacum officiniale*

Note: The isolate used in this study clustered together with *Septoria* isolates from *Taraxacum officinale* in South Korea (CBS 128650). While both of these isolates were recorded as *Septoria taraxacicola*, the holotype material collected by Miura could not

be accessed, and there are no records of isolates being kept. Specimens of the current isolate are also not accessible.

Septoria sp. on *Cacalia auriculata*

Conidiomata pycnidia, amphigenous, dark brown, submerged, globose to subglobose, 70–85 µm in diam., *Conidiomatal wall* 2–3 cell layers wide, composed of *textura angularis*, 1.0–2.5 µm. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the basal of conidiomatal wall, ampulliform, ovate, hyaline, percurrently proliferating, 5–6.5 × 1.5–2.0 µm. *Conidia* holoblastic, solitary, hyaline, cylindrical, slightly curved, narrower at the apex, obconical truncated base, 40–85 × 1.8–2.2 µm, 7–11-septate.

Specimens examined: Japan, Hokkaido, on leaves of *Cacalia auriculata*, 04 July 2019, collected by Y. Hattori and T. Shirouzu (TSU-MUMH 11887, culture MUCC 2889)

Note: the current isolate examined differs from *Septoria cacaliae* Ellis & Kellerm. by having longer and wider spore size.

Septoria sp. on *Clematis javana*

Note: The isolate MUCC 1601 forms an independent clade, next to sister clade *Septoria eucalyptorum* (CBS 118505). However, the morphological characteristic are not examined in this study. This isolate is designated as *Septoria* sp. at the moment.

Septoria sp. on *Centella asiatica*

Note: The isolated used in this study forms independent lineage in the phylogenetic tree. It is closely related to sister clade of *Septoria saccardoi* CBS 128756 and *Septoria lysimachiae* CBS 102315. On *Centella asiatica*, *Septoria centellae* G. Winter was recorded as pathogen, however, there are no sequence of holotype material for comparison.

Septoria sp. on *Persicaria possumbu*

Note: The isolate MUCC 1601 forms an independent lineage, next to *Septoria leptostachyae* (CBS 128628). However, the morphological characteristic are not examined in this study. This isolate is designated as *Septoria* sp. at the moment.

Septoria sp. on *Solidago altissima*

Conidiomata pycnidial, amphigenous, epiphyllous, pale brown to brown, submerged or erumpent through epidermis, globose to subglobose, 72–88 µm in diam., with an *ostiole* erumpent through epidermis, with opening 23–27 µm in diam.; *conidiomatal wall* 1–2 cell layers wide, composed of *textura angularis*, 4–5.5 µm. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* ampulliform, hyaline, lining inner cavity of basal half of conidiomata, percurrently proliferating, 2.5–5 × 1–1.5 µm. *Conidia* holoblastic, solitary, hyaline, cylindrical to obclavate, straight to slightly curved, rounded at the tip, truncated at the base, 40–56 × 1–1.5 µm, 3–7-septate.

Holotype: **Japan**, Mie, Tsu, on *Solidago altissima*, 11 October 2022, collected by A. H. Ujat (TSU-MUMH 11996, culture=MUCC 3544)

Note: There are more than 13 records of *Septoria* spp. infecting *Solidago* spp. from the fungus-host records (https://agdatacommons.nal.usda.gov). Shin and Sameva (2004) recorded *Septoria dolichospora* as the *Septoria* infecting *Solidago serotina* and *Solidago virgaurea* (Verkley et al., 2013) in South Korea.

DISCUSSION

Traditionally, Septoria was linked to the teleomorph *Mycosphaerella* based on observations. However, molecular techniques have shown that *Mycosphaerella* is only restricted to species of the *Ramularia* anamorph (Videira et al., 2016). The previous identification of *Septoria* relied mainly on host and morphological characteristics, with host specificity as the decisive criterion in the delimitation of *Septoria*. Sutton (1980) had previously commented that *Septoria* contains significant morphological variation, mentioning that the genus should be revised as it contains conidiomata ranging from acervuli to pycnidia. Quaedvlieg et al. (2011) managed to extract the DNA of the type material *Septoria cytisi* and confirmed that it was in the main *Septoria* clade. The study to resolve the *Septoria*-like complex was continued and subsequently led to the demarcation of *Sphaerulina* from *Septoria*.

In this study, most *Septoria* species are pathogens of the orders Asterales, Apiales, and Lamiales. At the base of the phylogenetic tree, with *Septoria dearnessii* as the basal clade, the host order is predominantly composed of Apiales. Moving towards the descendant clade, the host orders are Asterales and Lamiales. This repetitive host order pattern is especially evident on the descendant clade with *Septoria polygonorum* as the basal clade. It could be hypothesized that host jumping is the primary factor contributing to evolution in *Septoria* spp. (Verkely et al., 2013) as it does not seem to consistently co-evolve with the host family. Although most *Septoria* species have a limited host range, mostly infecting a single genus of host plant or a few genera within the same host plant family, it is expected that the addition of more molecular data will reveal more taxa to be plurivorous. An example in this study is the accommodation of *Septoria erigeronitis* on *Bellis perennis* and *Erigeron annuus*, which belong to different genera within Asteraceae. Another example in this study can be observed with *Septoria urticae* and *Septoria citri* infecting hosts from Rosales and Lamiales.

In this study, it is evident that one host plant genus can accommodate multiple species of *Septoria*. While some host plants have been extensively studied for their economic importance, others lack sufficient details. For instance, numerous studies have explored the species complex and pathogenicity of Septoria causing diseases on

Chrysanthemum spp. (Punithalingam & Wheeler, 1965; Waddell & Weber, 1963). These studies led to the discovery of a broad host range for certain *Septoria* species, such as *Septoria obesa*, *Septoria helianthi*, and *Septoria leucospermi*. In *Solidago* spp., at least 13 different species have been recorded; however, the scarcity of molecular data and fresh specimens has impeded the taxonomic revision of *Septoria* infecting *Solidago* spp.

Current study reveals that *Septoria* spp. infecting the same host plant exhibit different disease symptoms and morphology yet are placed near each other in the phylogenetic tree. For instance, *Septoria cannabicola* and *Septoria cannabis*, both infecting *Cannabis sativa*, exhibit distinct disease symptoms and morphology. McPartland (1995) described another *Septoria* species infecting *Cannabis sativa*; however, due to the lack of isolate and access to holotype material, the position of *Septoria neocannabina* could not be determined.

Although molecular identification has revealed the position of *Septoria* species in the phylogenetic tree, instances exist where species infecting the same host plant exhibit distinct morphological characteristics. The *Septoria* spp. infecting *Solidago altissima*, as examined in this study, displays morphological traits notably different from those of *Sep. dolichospora* Trail infecting *Solidago virgaurea*, as described by Shin and Sameva (2004). A similar phenomenon is observed in the case of *Septoria* sp. infecting *Oenanthe javanica*, illustrated by instances involving MUCC 1581 and CBS 128649 (*Septoria* sp. and *Septoria oenanthicola* Quaedvl., H.D. Shin, Verkley & Crous., respectively). However, owing to the absence of specimens for Septoria sp. (MUCC 1581), the classification of this isolate as a novel species or as *Septoria oenanthecola* cannot be definitive. This underscores the significance and urgency of specimens and literature as crucial evidentiary support for the taxonomic elucidation of *Septoria* species.

CONCLUSION

This study had revealed that the traditional association of host to pathogen in *Septoria* had been challenged by molecular method, revealing the complexity in the host-pathogen association. Host-specificity as a decisive criterion in delimitation of *Septoria* was highlighted as there are significant variation in the morphological characteristics.

Phylogenetic analysis in this study highlights that although the host range of *Septoria* is generally narrow, there could be one species having broad shot range. Contrasting relationship could also be seen when one host plant could accommodate different *Septoria* species. The juxtaposition of the molecular, morphological data and host specificity provided us with more question than answer in the elucidation of the systematics of *Septoria* and allied genera.

While molecular method is important in confirming the position of *Septoria* in the phylogeny of Mycosphaerellaceae, morphological assessment remains crucial in the typification of species. This would require not only recollection of fresh materials and comparison with protologue material, but it would also require the oldest name recorded on host plant to be studied as part of such work.

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Chapter 5 – *Diaporthe*

Summary

The genus *Diaporthe* encompasses numerous species recorded as pathogens, saprobes, and endophytes. With more than 1,200 species of *Diaporthe* recorded to date, in Japan, *Diaporthe* was recorded as pathogens on economically important plants only. This chapter focuses on elucidating the diversity of endophytic *Diaporthe* spp. from asymptomatic *Prunus* sensu lato (s.l.) commonly known as the Sakura tree. Polyphasic approach was employed in this study by using multi-locus analysis of 5 loci, including internal transcribed spacer (ITS) region, partial sequence of translation elongation factor 1–alpha (TEF), beta–tubulin (TUB), histone H3 (HIS) and calmodulin (CAL) gene along with morphological identification which also conducted by sporulating the isolate in artificial media. Additionally, in this chapter, coalescent-based Poisson Tree Phylogeny (PTP) method was employed to aid in the species delimitation. This resulted in the introduction of two novel species, *Diaporthe endoprunicola* and *Diaporthe pseudoamygdalii*, and the identification of several other *Diaporthe* species which have been described previously.

Keywords: Coalescent-Based Phylogeny, Multi-locus phylogenies, Systematics, Taxonomy

INTRODUCTION

Species of the genus *Diaporthe* (Diaporthaceae, Diaporthales) have attracted the interest of many mycologists and plant pathologists in recent years. It is known to occur as plant pathogens, saprobes, and endophytes (Gomes et al., 2013; Udayanga et al., 2012). The distribution of *Diaporthe* spp. is worldwide. Published an annotated list of *Diaporthe* species, which include 832 species and information of their morphology, ecology, geographic distribution, molecular data, and pathogenesis. The initial species concept of *Diaporthe* assumes host specificity, where a new species had been established when observed from one new host plant (Gao et al., 2017). The phenotypic plasticity, and morphological characteristics, and host association are shown to be insufficient to delimit species in the genus *Diaporthe* (Gomes et al., 2013). Currently, the circumscription of the delimitation of *Diaporthe* species relies on morphological characteristics of the isolates and multi-locus phylogeny based on the internal transcribed spacer region (ITS) and partial sequences of several protein-coded genes, including translation elongation factor 1-α (TEF), β-tubulin (TUB), histone H3 (HIS) and calmodulin (CAL) (Guarnaccia & Crous, 2017).

While a single species of *Diaporthe* could colonise multiple hosts, one single host species could also accommodate multiple species of *Diaporthe* (Sessa et al., 2017). Guarnaccia & Crous (2017) revealed that, through inoculation test, most *Citrus* species are susceptible to *Diaporthe* species, such as *D. baccae* and *D. novum*. The abundance of *Diaporthe* species identified leads to the proposal of some species comprise into species complexes, such as *D. eres* (Hilário et al., 2021a; Udayanga et al., 2014), *D. amygdali* (Hilário et al., 2021b), *D. sojae* (Udayanga et al., 2015), and *D. areacae* (Pereira et al., 2023). This taxonomical treatment prompts the recommendation of careful introduction of *Diaporthe* species (Gao et al., 2017; Santos et al., 2017) and recollection and typification of old records to ensure the stability of *Diaporthe* taxonomy (Dissanayake et al., 2017a; Hongsanan et al., 2023). Moreover, many of the newly described species and hitherto known species known species based on the phylogeny were synonymized under *D. eres* as the result of the re-examination of the taxonomical position of *Diaporthe* species.

According to Guarnaccia et al., (2022), in European countries, *D. eres* is known as a ubiquitous and dominant species of the causal pathogen to fruit trees, including cherry (*P. avium*) and peach (*P. persica*). Nekrasov et al., (2022) also reported the occurrence of *D. eres* in *P. mandschuria*. In addition, numerous *Diaporthe* species have been recorded on *Prunus* s.l.; such as *D. amygdali* on *P. dulcis* (Guarnaccia et al., 2022) and on *P. persica* (Sessa et al., 2017), *D. foeniculina* on *P. amygdalus* (Gomes et al., 2013) and *P. dulcis* (Guarnaccia et al., 2022), *D. hongkongensis* on *P. persica* (Zhang et al., 2021) , *D. jinxiu* on *P. persica* (Wang et al., 2021), *D. mahothocarpus* on *P. avium* (Bien & Damm, 2020) , *D. momicola* on *P. persica* (Dissanayake et al., 2017b) *D. noveum* on *P. dulcis* (Hongsanan et al., 2023), *D. oligocarpa* on *P.spinosa* (fide Hongsanan et al., 2023), *D. oxe* on *P*. *persica* (Sessa et al., 2017) and *P. dulcis* (Gomes et al., 2013), *D. padicola* on *P. padus* (Petrak 1916), *P. paranesis* on *P. persica* (Gomes et al., 2013), *P. pardalota* on *P. divaricata* (fide Hongsanan et al., 2023), *D. pennsylvanica* on *P. pensylvanica, P. serotina* and *Prunus* sp.(fide Hongsanan et al., 2023), *D. perniciosa* on *P. cerasus* (fide Hongsanan et al., 2023), *D. rudis* on *P. avium* and *P. salicina* (Guarnaccia et al., 2023), *D. taoicola* on *P. persica* (Dissanayake et al., 2017b). On the other hand, in Japan, only two species of *Diaporthe*, *D. amygdali* on *P. persica* and *D. eres* on *Prunus* spp. have been reported (The Phytopathological Society of Japan, 2023). This study describes the morphological and molecular characteristics and evaluates the diversity of *Diaporthe* species isolated from intact branches of *Prunus* sensu lato (s.l.) in Japan.

MATERIAL AND METHOD

Fungal Isolate Collection and Morphological Observation

In 2023, field surveys were conducted to collect *Diaporthe* species associated with the plant genus *Prunus* spp. in Japan were conducted. Intact branches of Prunus spp. and Cerasus spp. were sampled from six areas (Sapporo, Hokkaido; Morioka, Iwate; Kitaibaraki and Tsukuba, Ibaraki; Shimonita, Gunma; Hachioji, Tokyo; Seto, Aichi) in Japan. *Diaporthe* spp. were isolated from intact branches using the surface sterilization method. Branches were cut into 3 mm pieces, immersed in 70% ethanol for 30 s, and washed in sterilized water for 60 s. These sterilized pieces were placed on 3% water agar. Following culture at room temperature (22°C) for 3 days, only a tip of hyphae growing from the piece was transferred by a flame-sterilized needle under the microscope onto a potato dextrose agar medium plate (PDA; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). Growing colonies of *Diaporthe* were selected based on their cultural characteristics and transferred onto new Malt Agar (MA: Becton Dickinson, MD, USA) plates. Morphological characters of the conidiomata formed on potato carrot agar (PCA: Simmons, 2007) were examined under a compound microscope Axio Imager A1 (Zeiss, Göttingen, Germany) with Shear's solution (Chupp, 1940b) as the mounting medium. The colonies' colour was assessed using a colour chart by Rayner (1970) , and the isolates were deposited into Mie University Culture Collection (MUCC).

DNA extraction, amplification, and sequencing

All 31 fungal isolates were subjected to genomic DNA extraction using DNeasy Ultra Clean Microbial Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR mixture of 12.5 µL was prepared as follows for all of the reactions; 1–10 ng of genomic DNA, 5.6% DMSO, 2 mM $MgCl_2$ (Bioline, London, UK), 1.25 µL of 10× NH_4 reaction buffer (Bioline), 40 µM dNTPs (Bioline), 0.25 U Bioline *Taq* DNA polymerase (Bioline), and 0.25 µM of each forward and reverse primer. All 31 isolates were subjected to PCR amplification on a T100 thermal cycler (Bio-Rad, Tokyo, Japan), with Initial denaturation of 94 °C at 5 minutes, followed by 35 cycles of denaturation at 94 °C at 30 seconds, annealing, extension at 72 °C at 30 seconds, and final extension of 72 °C at 10 minutes. The annealing temperature of PCR and primer were as listed in Table 5.1.

Amplicons were analysed in both directions using BigDye Terminator version 3.1 cycle Sequencing Kit (Applied Biosystem, Foster City, CA) on an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystem) installed at Mie University Advance Science Research Promotion Centre, Tsu, Mie, Japan.

Table 5.1: Annealing temperature and primer set used in PCR amplification.

Locus	PCR primers	Annealing temperature	Reference
ITS	ITS1/ITS4	52 °C	White et al., (1990)
CAL	CAL228F/CAL737R	56 °C	Carbone & Kohn (1999)
HIS	CYLH3F/H3-1b	56 °C	Glass & Donaldson (1995)
TEF	EF1-728F/EF1-986R	56 °C	Carbone & Kohn (1999) O'Donnell & Cigelnik (1997)
BTUB	T1 or Bt2a/Bt2b	55 °C	Glass & Donaldson (1995) O'Donnell & Cigelnik (1997)

Phylogenetic analysis

The resulting sequences were assembled and aligned with sequence retrieved from NCBI DNA GenBank, as indicated in Table 5.2. Taxonomic novelties are indicated in bold italic and the GenBank accession numbers of the newly generated sequences are indicated in bold. The individual matrix of each locus was aligned by MAFFT online service (Katoh et al., 2018b). The best substitution model for each locus was determined by ModelTest-NG (Darriba et al., 2020). The matrix was concatenated by Concatenator (Vences et al., 2022) before being subjected to further analysis. Maximum-likelihood analysis (ML) was conducted on RAxML-NG (Kozlov et al., 2019) with 100 bootstraps. Bayesian Inference (BI) analysis was performed on MrBayes v.3.2.5 (Ronquist et al., 2012). Posterior probability was estimated by Metropolis Coupled Monte Carlo Markov Chain (MCMCMC) option. The convergence was judged when the Average Standard Deviation of Split Frequencies was below 0.01, and the posterior probability (PP) were determined using the remaining tree. *Diaporthella corylina* was used as an outgroup. After evaluation of the inferred tree, the alignment was split into three sections (Figure 5.1, shown in pink, green, and blue) and realigned using MAFFT. The aligned sequence was then manually edited using AliView (Larsson, 2014). The separated sections were subjected to ML of 100 bootstrap replication and BI analysis with 10 million generations. The tree was sampled and saved at every 100 generations. The first 25% of the tree was

discarded as a burn-in phase of analysis. The split sections of the are viewed with FigTree (http://tree.bio.ed.ac.uk/software/figtree/). The alignment and respective phylogenetic tree were deposited in TreeBASE (S31006).

Coalescent-Based Species Delimitation Analysis

Species boundaries of restricted analysis were analysed by coalescent-based models Poisson Tree processed (PTP) (Zhang et al., 2013), which accommodate different degrees of intraspecific genetic diversity within the phylogeny. Non-annotated Newick format restricted analysis of ML trees was used for PTP analysis performed with 500,000 MCMC generations, 100 set of thinning, and 10% burn-in on the webserver for PTP (https://species.h-its.org/ptp/).

Table 5.2: List of reference sequence used in this study.

Species	Isolates	ITS	BTUB	H3	TEF1-a	CAL
Diaporthe absenteum	LC 3924^{T}	KP267897	KP293477	KP293547	KP267971	NA
D. acaciarum	CBS 138862^{T}	KP004460	KP004509	KP004504	–	–
D. acaciigena	CBS 129521^{T}	KC343005	KC343973	KC343489	KC343731	KC343247
D. acericola	MFLUCC 17-0956^{T}	KY964224	KY964074	–	KY964180	KY964137
D. acerigena	CFCC 52554^{T}	MH121489	–	MH121449	MH121531	MH121413
D. acerina	CBS 137.27	KC343006	KC343974	KC343490	KC343732	KC343248
D. actinidiae	ICMP 13683^{T}	KC145886	–	–	KC145941	–
D. acuta	PSCG 047^{T}	MK626957	MK691225	MK726161	MK654802	MK691125
D. acutispora	CGMCC 3.18285^{T}	KX986764	KX999195	KX999235	KX999155	KX999274
D. aestuarium	BRIP 59930a^{T}	OM918686	OM960613	–	OM960595	–
D. africana	CBS 150080^{T}	OR198681	OR225229	OR225231	OR225227	OR225233
D. afzeliae	SDBR-CMU 467^{T}	OQ600199	OQ678279	OQ646886	OQ603502	OQ646882
D. aitkeniae	BRIP 58827a^{T}	OR019750	OR039647	–	OR039640	–
D. alangii	CFCC 52556^{T}	MH121491	MH121573	MH121451	MH121533	MH121415
D. albosinensis	CFCC 53066^{T}	MK432659	MK578059	MK443004	MK578133	MK442979
D. alleghaniensis	CBS 495.72^{T}	FJ889444	KC843228	KC343491	GQ250298	KC343249
D. alnea	CBS 146.46^{T}	KC343008	KC343976	KC343492	KC343734	KC343250
D. amaranthophila	MAFF 246900	LC459575	LC459579	LC459581	LC459577	LC459583
D. ambigua	CBS 114015^{T}	KC343010	KC343978	KC343494	KC343736	KC343252
D. ampelina	CBS 114016^{T}	AF230751	JX275452	–	GQ250351	JX197443
D. amygdali	CBS 126679^{T}	KC343022	KC343990	KC343506	KC343748	KC343264
D. amygdali	**MUCC 3587**	**OR897079**	**OR913139**	**OR913170**	**OR913201**	**OR913231**
D. amygdali	**MUCC 3589**	**OR897081**	**OR913141**	**OR913172**	**OR913203**	**OR913233**
D. amygdali	**MUCC 3590**	**OR897082**	**OR913142**	**OR913173**	**OR913204**	**OR913234**

D. amygdali	**MUCC 3592**	**OR897084**	**OR913144**	**OR913175**	**OR913206**	**OR913236**
D. amygdali	**MUCC 3598**	**OR897090**	**OR913150**	**OR913181**	**OR913212**	**OR913241**
D. amygdali	**MUCC 3600**	**OR897092**	**OR913152**	**OR913183**	**OR913214**	**OR913243**
D. amygdali	**MUCC 3603**	**OR897095**	**OR913155**	**OR913186**	**OR913217**	**OR913246**
D. anacardii	CBS 720.97^{T}	KC343024	KC343992	KC343508	KC343750	KC343266
D. angelicae	CBS 111592^{T}	KC343026	KC343994	KC343511	KC343752	KC343268
D. anhuiensis	CNUCC 201902^{T}	MN219727	MN227009	MN224550	MN224669	MN224556
D. annellsiae	BRIP 59731a^{T}	OM918687	OM960614	–	OM960596	–
D. antonovae	BRIP 58824b^{T}	OR019751	OR039648	–	OR039641	–
D. apiculata	LC 3418^{T}	KP267896	KP293476	KP293550	KP267970	–
D. apiculatum	CFCC 53068	MK432651	MK578054	MK442998	MK578127	MK442973
D. aquatica	IFRDCC 3051^{T}	JQ797437	–	–	–	–
D. araucanorum	CBS 145285^{T}	MN509711	MN509722	–	MN509733	MN974277
D. arctii	CBS 136.25	KC343031	KC343999	KC343515	KC343757	KC343273
D. arecae	CBS 161.64^{T}	KC343032	KC344000	KC343516	KC343758	KC343274
D. arengae	CBS 114979^{T}	KC343034	KC344002	KC343518	KC343760	KC343276
D. arezzoensis	MFLU 19-2880^{T}	MT185503	MT454055	–	–	–
D. aseana	MFLUCC 12-0299a^{T}	KT459414	KT459432	–	KT459448	KT459464
D. asheicola	CBS 136967^{T}	KJ160562	KJ160518	–	KJ160594	KJ160542
D. aspalathi	CBS 117169^{T}	KC343036	KC344004	KC343520	KC343762	KC343278
D. atlantica	CECT 21217^{T}	ON159893	ON364040	ON398810	ON398831	ON364019
D. australafricana	CBS 111886^{T}	KC343038	KC344006	KC343522	KC343764	KC343280
D. australiana	BRIP 66145^{T}	MN708222	MN696530	–	MN696522	–
D. australpacifica	BRIP 60163d^{T}	OM918688	OM960615	–	OM960597	–
D. averrhoae	SCHM 3605^{T}	AY618930	–	–	–	–
D. baccae	CBS 136972^{T}	KJ160565	MF418509	MF418264	KJ160597	–

D. batatas	CBS 122.21	KC343040	KC344008	KC343524	KC343766	KC343282
D. bauhiniae	CFCC 53071^{T}	MK432648	MK578051	MK442995	MK578124	MK442970
D. beasleyi	BRIP 59326a^{T}	OM918689	OM960616	–	OM960598	–
D. beckhausii	CBS 138.27	KC343041	KC344009	KC343525	KC343767	KC343283
D. beilharziae	BRIP 54792^{T}	JX862529	KF170921	–	JX862535	–
D. benedicti	ATCC MYA-4970^{T}	KM669929	–	–	KM669785	KM669862
D. berteroae	BRIP 57900a^{T}	OR019752	OR039649	–	OR039642	–
D. betulae	CFCC 50469^{T}	KT732950	KT733020	KT732999	KT733016	KT732997
D. betulicola	CFCC 51128^{T}	KX024653	KX024657	KX024661	KX024655	KX024659
D. betulina	CFCC 52562^{T}	MH121497	MH121579	MH121457	MH121539	MH121421
D. bicincta	CBS 121004^{T}	KC343134	KC344102	KC343618	KC343860	KC343376
D. biconispora	CGMCC 3.17252^{T}	KJ490597	KJ490418	KJ490539	KJ490476	–
D. biguttulata	CFCC 52584	MH121519	MH121598	MH121477	MH121561	MH121437
D. bohemiae	CBS 143347^{T}	MG281015	MG281188	MG281361	MG281536	MG281710
D. bombacis	SDBR-CMU 468^{T}	OQ600198	OQ678278	OQ646885	OQ603501	OQ646881
D. bounty	BRIP 59361a^{T}	OM918690	OM960617	–	OM960599	–
D. brasiliensis	CBS 133183^{T}	KC343042	KC344010	KC343526	KC343768	KC343284
D. breyniae	CBS 148910^{T}	ON400846	ON409186	ON409187	ON409188	ON409189
D. brideliae	CBS 148911^{T}	OR348649	OR468827	OR468807	OR468817	OR468837
D. brumptoniae	BRIP 59403a^{T}	OM918702	OM960629	–	OM960611	–
D. butterlyi	BRIP 59194a^{T}	OR019753	OR039650	–	OR039643	–
D. caatingaensis	CBS 141542^{T}	KY085927	KY115600	KY115605	KY115603	KY115597
D. camelliae-sinensis	SAUCC 194.92^{T}	MT822620	MT855817	MT855588	MT855932	MT855699
D. cameroonensis	CBS 148913^{T}	OR348650	OR468826	OR468806	OR468816	OR468836
D. camporesii	JZB 320143^{T}	MN535309	MN561316	–	–	–
D. canthii	CBS 132533^{T}	JX069864	KC843230	–	KC843120	KC843174

D. careyae	SDBR-CMU469^{T}	OQ600196	OQ678276	OQ646883	–	OQ646879
D. carpini	CBS 114437	KC343044	KC344012	KC343528	KC343770	KC343286
D. carriae	BRIP 59932a^{T}	OM918691	OM960618	–	OM960600	–
D. caryae	CFCC 52563^{T}	MH121498	MH121580	MH121458	MH121540	MH121422
D. cassines	CBS 136440^{T}	KF777155	–	–	KF777244	–
D. caulivora	CBS 127268^{T}	KC343045	KC344013	KC343529	KC343771	KC343287
D. celastrina	CBS 139.27^{T}	KC343047	KC344015	KC343531	KC343773	KC343289
D. celeris	CBS 143349^{T}	MG281017	MG281190	MG281363	MG281538	MG281712
D. celticola	CFCC 53074^{T}	MK573948	MK574643	MK574603	MK574623	MK574587
D. celtidis	NCYU 19-0357^{T}	MW114346	MW148266	–	MW192209	–
D. ceratozamiae	CBS 131306^{T}	JQ044420	–	–	–	–
D. cercidis	CFCC 52565^{T}	MH121500	MH121582	MH121460	MH121542	MH121424
D. cerradensis	CMRP 4331^{T}	MN173198	MW751671	MW751663	MT311685	MW751655
D. chamaeropis	CBS 454.81	KC343048	KC344016	KC343532	KC343774	KC343290
D. changpingensis	CFCC 58812^{T}	OQ912925	OQ910292	OQ910234	OQ910264	OQ910202
D. charlesworthii	BRIP 54884m^{T}	KJ197288	KJ197268	–	KJ197250	–
D. chensiensis	CFCC 52567^{T}	MH121502	MH121584	MH121462	MH121544	MH121426
D. chiangmaiensis	MFLUCC 18-0544^{T}	OK393703	–	–	OL439483	–
D. chimonanthi	SCHM 3614^{T}	AY622993	–	–	–	–
D. chinensis	MFLUCC 19-0101^{T}	MW187324	MW245013	–	MW205017	MW294199
D. chongqingensis	PSCG 435^{T}	MK626916	MK691321	MK726257	MK654866	MK691209
D. chromolaenae	MFLUCC 17-1422^{T}	MH094275	–	–	–	–
D. chrysalidocarpi	SAUCC 194.35^{T}	MT822563	MT855760	MT855532	MT855876	MT855646
D. cichorii	MFLUCC 17-1023^{T}	KY964220	KY964104	–	KY964176	KY964133
D. cinerascens	CBS 719.96	KC343050	KC344018	KC343534	KC343776	KC343292
D. cinnamomi	CFCC 52569^{T}	MH121504	MH121586	MH121464	MH121546	–

D. cissampeli	CBS 141331^{T}	KX228273	KX228384	KX228366	–	–
D. citri	CBS 135422^{T}	KC843311	KC843187	MF418281	KC843071	KC843157
D. citriasiana	CBS 134240^{T}	JQ954645	KC357459	MF418282	JQ954663	KC357491
D. citrichinensis	CBS 134242^{T}	JQ954648	MF418524	KJ420880	JQ954666	KC357494
D. clematidina	MFLUCC 17-2060^{T}	MT310657	MT394623	–	MT394669	MT394624
D. collariana	MFLUCC 17-2636^{T}	MG806115	MG783041	–	MG783040	MG783042
D. compacta	LC3083^{T}	KP267854	KP293434	KP293508	KP267928	–
D. conica	CFCC 52571^{T}	MH121506	MH121588	MH121466	MH121548	MH121428
D. constrictospora	CGMCC 3.20096^{T}	MT385947	MT424702	MW022487	–	MT424718
D. convolvuli	CBS 124654	KC343054	KC344022	KC343538	KC343780	KC343296
D. coryli	CFCC 53083^{T}	MK432661	MK578061	MK443006	MK578135	MK442981
D. corylicola	CFCC 53986	MW839880	MW883977	MW836717	MW815894	MW836684
D. crataegi	CBS 114435	KC343055	KC344023	KC343539	KC343781	KC343297
D. crotalariae	CBS 162.33^{T}	KC343056	KC344024	KC343540	KC343782	KC343298
D. crousii	CAA823^{T}	MK792311	MK837932	MK871450	MK828081	MK883835
D. cucurbitae	DAOM 42078^{T}	KM453210	KP118848	KM453212	KM453211	–
D. cuppatea	CBS 117499^{T}	AY339322	JX275420	KC343541	AY339354	JX197414
D. cynaroidis	CBS 122676	KC343058	KC344026	KC343542	KC343784	KC343300
D. cytosporella	CBS 137020^{T}	KC843307	KC843221	MF418283	KC843116	KC843141
D. decedens	CBS 109772	KC343059	KC344027	KC343543	KC343785	KC343301
D. delonicis	MFLU 16-1059^{T}	MT215490	MT212209	–	–	–
D. destruens	ZJUPD 06	MN708229	MN696537	–	MN696526	–
D. detrusa	CBS 109770	KC343061	KC344029	KC343545	KC343787	KC343303
D. diospyricola	CBS 136552^{T}	KF777156	–	–	–	–
D. diospyrina	CFCC 58820^{T}	OQ912929	OQ910296	OQ910236	OQ910268	OQ910206
D. discoidispora	ICMP 20662^{T}	KJ490624	KJ490445	KJ490566	KJ490503	–

D. donglingensis	CFCC 56581^{T}	OM956090	ON158021	ON157951	ON157986	–
D. drenthii	BRIP 66524^{T}	MN708229	MN696537	–	MN696526	–
D. durionigena	VTCC 930005^{T}	MN453530	MT276159	–	MT276157	–
D. elaeagni-glabrae	CGMCC 3.18287^{T}	KX986779	KX999212	KX999251	KX999171	KX999281
D. eleagni	CBS 504.72	KC343064	KC344032	KC343548	KC343790	KC343306
D. ellipsospora	CGMCC 3.20099^{T}	MT385949	MT424704	MW022488	MT424684	MT424720
D. endocitricola	ZHKUCC 20-0012^{T}	MT355682	MT409290	–	MT409336	MT409312
D. endophytica	CBS 133811^{T}	KC343065	KC344033	KC343549	KC343791	KC343307
D. endoprunicola	**MUCC 3584^{T}**	**OR897076**	**OR913136**	**OR913167**	**OR913198**	–
D. eres	CBS 138594^{T}	KJ210529	KJ420799	KJ420850	KJ210550	KJ434999
D. eres	**MUCC 3586**	**OR897078**	**OR913138**	**OR913169**	**OR913200**	**OR913230**
D. eres	**MUCC 3588**	**OR897080**	**OR913140**	**OR913171**	**OR913202**	**OR913232**
D. eres	**MUCC 3594**	**OR897086**	**OR913146**	**OR913177**	**OR913208**	**OR913238**
D. eres	**MUCC 3595**	**OR897087**	**OR913147**	**OR913178**	**OR913209**	–
D. eres	**MUCC 3596**	**OR897088**	**OR913148**	**OR913179**	**OR913210**	**OR913239**
D. eres	**MUCC 3599**	**OR897091**	**OR913151**	**OR913182**	**OR913213**	**OR913242**
D. eres	**MUCC 3601**	**OR897093**	**OR913153**	**OR913184**	**OR913215**	**OR913244**
D. eres	**MUCC 3602**	**OR897094**	**OR913154**	**OR913185**	**OR913216**	**OR913245**
D. eres	**MUCC 3604**	**OR897095**	**OR913156**	**OR913187**	**OR913218**	–
D. eres	**MUCC 3605**	**OR897097**	**OR913157**	**OR913188**	**OR913219**	**OR913247**
D. eres	**MUCC 3606**	**OR897098**	**OR913158**	**OR913189**	**OR913220**	**OR913248**
D. eres	**MUCC 3607**	**OR897099**	**OR913159**	**OR913190**	**OR913221**	**OR913249**
D. eres	**MUCC 3608**	**OR897100**	**OR913160**	**OR913191**	**OR913222**	**OR913250**
D. eres	**MUCC 3609**	**OR897101**	**OR913161**	**OR913192**	**OR913223**	**OR913251**
D. eres	**MUCC 3610**	**OR897102**	**OR913162**	**OR913193**	**OR913224**	**OR913252**
D. eres	**MUCC 3611**	**OR897103**	**OR913163**	**OR913194**	**OR913225**	**OR913253**

D. eres	**MUCC 3613**	**OR897105**	**OR913165**	**OR913196**	**OR913227**	**OR913255**
D. eres[1]	CGMCC 3.17089	KF576267	KF576291	–	KF576242	–
D. eres[2]	MFLUCC 16-0113	KU557563	KU557587	–	KU557631	KU557611
D. eres[3]	CGMCC 3.15181	KC153096	–	–	KC153087	–
D. eres[4]	CGMCC 3.17084	KF576270	KF576296	–	KF576245	–
D. eres[5]	CGMCC 3.17081	KF576282	KF576306	–	KF576257	–
D. eres[6]	CFCC 51632	KY203726	KY228893	KY228881	KY228887	KY228877
D. etinsideae	BRIP 64096a^{T}	OM918692	OM960619	–	OM960601	–
D. eucalyptorum	CBS 132525^{T}	JX069862	–	–	–	–
D. eugeniae	CBS 444.82	KC343098	KC344066	KC343582	KC343824	KC343340
D. fibrosa	CBS 109751	KC343099	KC344067	KC343583	KC343825	KC343341
D. fici-septicae	MFLU 18-2588^{T}	MW114348	MW148268	–	MW192211	–
D. foeniculina	CBS 111553^{T}	KC343101	KC344069	KC343585	KC343827	KC343343
D. foeniculina[7]	CBS 129528	JF951146	KC843205	–	KC843100	KC843124
D. foikelawen	CBS 145289^{T}	MN509713	MN509724	–	MN509735	MN974278
D. forlicesenica	MFLUCC 17-1015^{T}	KY964215	KY964099	–	KY964171	–
D. fraxini- angustifoliae	BRIP 54781^{T}	JX862528	KF170920	–	JX862534	–
D. fraxinicola	CFCC 52582^{T}	MH121517	–	–	MH121559	MH121435
D. fujianensis	JZB 320149^{T}	MW010212	MW056008	–	MW205231	MW205212
D. fukushii	MAFF 625034	JQ807469	–	–	JQ807418	–
D. fulvicolor	PSCG 051^{T}	MK626859	MK691236	MK726163	MK654806	MK691132
D. fusicola	CGMCC 3.17087^{T}	KF576281	KF576305	–	KF576256	KF576233
D. fusiformis	JZB 320156^{T}	MW010218	MW056014	–	MW205234	MW205218
D. ganjae	CBS 180.91^{T}	KC343112	KC344080	KC343596	KC343838	KC343354
D. ganzhouensis	CFCC 53087^{T}	MK432665	MK578065	MK443010	MK578139	MK442985
D. gardeniae	CBS 288.56	KC343113	KC344081	KC343597	KC343839	KC343355

D. garethjonesii	MFLUCC 12-0542a^{T}	KT459423	KT459441	–	KT459457	KT459470
D. glabrae	SCHM 3622^{T}	AY601918	–	–	–	–
D. globoostiolata	MFLUCC 23-0025^{T}	OQ600200	OQ678280	–	OQ603503	–
D. gossiae	BRIP 59730a^{T}	OM918693	OM960620	–	OM960602	–
D. goulteri	BRIP 55657a^{T}	KJ197290	KJ197270	–	KJ197252	–
D. grandiflori	SAUCC 194.84^{T}	MT822612	MT855809	MT855580	MT855924	MT855691
D. griceae	BRIP 67014a^{T}	OM918694	OM960621	–	OM960603	–
D. guangdongensis	ZHKUCC 20-0014^{T}	MT355684	MT409292	–	MT409338	MT409314
D. guangxiensis	JZB 320094^{T}	MK335772	MK500168	–	MK523566	MK736727
D. guizhouensis	GZAAS 20-0338^{T}	OM060254	OL961762	–	OL961761	OL961763
D. gulyae	BRIP 54025^{T}	JF431299	KJ197271	–	JN645803	–
D. guttulata	CGMCC 3.20100^{T}	MT385950	MT424705	MW022491	MT424685	MW022470
D. hartii	BRIP 60285e^{T}	OR019754	OR039651	–	OR039644	–
D. helianthi	CBS 592.81^{T}	KC343115	KC344083	KC343599	KC343841	JX197454
D. helicis	CBS 138596^{T}	KJ210538	KJ420828	KJ420875	KJ210559	KJ435043
D. heliconiae	SAUCC 194.77^{T}	MT822605	MT855802	MT855573	MT855917	MT855684
D. heterophyllae	CBS 143769^{T}	MG600222	MG600226	MG600220	MG600224	MG600218
D. heterostemmatis	SAUCC 194.85^{T}	MT822613	MT855810	MT855581	MT855925	MT855692
D. hickoriae	CBS 145.26^{T}	KC343118	KC344086	KC343602	KC343844	KC343360
D. hispaniae	CBS 143351^{T}	MG281123	MG281296	MG281471	MG281644	MG281820
D. hongkongensis	CBS 115448^{T}	KC343119	KC344087	KC343603	KC343845	KC343361
D. hordei	CBS 481.92	KC343120	KC344088	KC343604	KC343846	KC343362
D. howardiae	BRIP 59697a^{T}	OM918695	OM960622	–	OM960604	–
D. hsinchuensis	NTUPPMCC 18-153-1^{T}	MZ268409	MZ268430	MZ268493	MZ268472	MZ268451
D. huairouensis	CFCC 56808	ON188788	ON158051	ON157982	ON158016	ON157945
D. huangshanensis	CNUCC 201903^{T}	MN219729	MN227010	MN224558	MN224670	–

D. hubeiensis	JZB 320123^{T}	MK335809	MK500147	–	MK523570	MK500235
D. humulicola	CT2018-1^{T}	MN152927	–	MN180213	MN180207	MN180204
D. hunanensis	HNZZ 023^{T}	MZ509550	MZ504713	MZ504691	MZ504702	MZ504680
D. hungariae	CBS 143353^{T}	MG281126	MG281299	MG281474	MG281647	MG281823
D. iberica	CECT 21218^{T}	ON159902	ON364049	ON398819	ON398841	ON364028
D. ilicicola	FPH 2015502^{T}	MH171064	MH171074	MH171084	–	–
D. impulsa	CBS 114434	KC343121	KC344089	KC343605	KC343847	KC343363
D. incompleta	CGMCC 3.18288^{T}	KX986794	KX999226	KX999265	KX999186	KX999289
D. inconspicua	CBS 133813^{T}	KC343123	KC344091	KC343607	KC343849	KC343365
D. infecunda	CBS 133812^{T}	KC343126	KC344094	KC343610	KC343852	KC343368
D. infertilis	CBS 230.52^{T}	KC343052	KC344020	KC343536	KC343778	KC343294
D. irregularis	CGMCC 3.20092^{T}	MT385951	MT424706	–	MT424686	MT424721
D. isoberliniae	CBS 137981^{T}	KJ869133	KJ869245	–	–	–
D. italiana	MFLUCC 18-0090^{T}	MH846237	MH853688	–	MH853686	MH853690
D. jinxiu	CGMCC 3.20269^{T}	MW477881	MW480877	MW480865	MW480873	MW480869
D. juglandicola	CFCC 51134^{T}	KU985101	KX024634	–	KX024628	KX024616
D. kadsurae	CFCC 52586^{T}	MH121521	MH121600	MH121479	MH121563	MH121439
D. kochmanii	BRIP 54033^{T}	NR111614	–	–	JN645809	–
D. kongii	BRIP 54031^{T}	NR111616	KJ197272	–		–
D. krabiensis	MFLUCC 17-2481^{T}	MN047101	MN431495	–	MN433215	
D. lenispora	CGMCC 3.20101^{T}	MT385952	MT424707	MW022493	MT424687	MW022472
D. leptostromiformis	CBS 558.93	KC343244	KC344212	KC343728	KC343970	KC343486
D. leucospermi	CBS 111980^{T}	JN712460	KY435673	KY435653	KY435632	KY435663
D. limonicola	CBS 142549^{T}	MF418422	MF418582	MF418342	MF418501	MF418256
D. liquidambaris	SCHM 3621^{T}	AY601919	–	–	–	–
D. litchicola	BRIP 54900^{T}	JX862533	KF170925	–	JX862539	–

D. litchii	SAUCC 194.22^{T}	MT822550	MT855747	MT855519	MT855863	MT855635
D. lithocarpi	CGMCC 3.15175^{T}	KC153104	KF576311	–	KC153095	–
D. litoricola	MFLUCC 16-1195^{T}	MF190139	–	–	–	–
D. longicolla	FAU 599^{T}	KJ590728	KJ610883	KJ659188	KJ590767	KJ612124
D. longispora	CBS 194.36^{T}	KC343135	KC344103	KC343619	KC343861	KC343377
D. lonicerae	MFLUCC 17-0963^{T}	KY964190	KY964073	–	KY964146	KY964116
D. lovelaceae	BRIP 60163a^{T}	OM918696	OM960623	–	OM960605	–
D. lusitanicae	CBS 123212^{T}	KC343136	KC344104	KC343620	KC343862	KC343378
D. lutescens	SAUCC 194.36^{T}	MT822564	MT855761	MT855533	MT855877	MT855647
D. macadamiae	BRIP 66526^{T}	MN708230	MN696539	–	MN696528	–
D. machili	SAUCC 194.111^{T}	MT822639	MT855836	MT855606	MT855951	MT855718
D. macintoshii	BRIP 55064a^{T}	KJ197289	KJ197269	–	KJ197251	–
D. malorum	CBS142383^{T}	KY435638	KY435668	KY435648	KY435627	KY435658
D. manihotia	CBS 505.76	KC343138	KC344106	KC343622	KC343864	KC343380
D. marina	MFLU 17-2622^{T}	MN047102	–	–	–	–
D. maritima	DAOMC 250563^{T}	KU552025	KU574615	–	KU552023	–
D. masirevicii	BRIP 57892a^{T}	KJ197276	KJ197257	–	KJ197239	–
D. mayteni	CBS 133185^{T}	KC343139	KC344107	KC343623	KC343865	KC343381
D. maytenicola	CBS 136441^{T}	KF777157	KF777250	–	–	–
D. mclennaniae	BRIP 60072a^{T}	OM918697	OM960624	–	OM960606	–
D. mediterranea	DAL-34	MT007489	MT006686	MT007095	MT006989	MT006761
D. megalospora	CBS 143.27	KC343140	KC344108	KC343624	KC343866	KC343382
D. melastomatis	SAUCC 194.55^{T}	MT822583	MT855780	MT855551	MT855896	MT855664
D. meliae	CFCC 53089^{T}	MK432657	MK578057	ON081662	ON081654	–
D. melitensis	CBS 142551^{T}	MF418424	MF418584	MF418344	MF418503	MF418258
D. melonis	CBS 507.78^{T}	KC343142	KC344110	KC343626	KC343868	KC343384

D. micheliae	SCHM 3603	AY620820	–	–	–	–
D. middletonii	BRIP 54884e^{T}	KJ197286	KJ197266	–	KJ197248	–
D. millettiae	GUCC 9167^{T}	MK398674	MK460488	–	MK480609	MK502086
D. minima	CGMCC 3.20097^{T}	MT385953	MT424708	MW022496	MT424688	MT424722
D. minusculata	CGMCC 3.20098^{T}	MT385957	MT424712	MW022499	MT424692	MW022475
D. miriciae	BRIP 54736j^{T}	KJ197283	KJ197263	–	KJ197245	–
D. monetii	MF-Ha18-049^{T}	MW008494	MW008505	MZ671965	MW008516	MZ671939
D. moorei	BRIP 61500b^{T}	OR019755	OR039652	–	OR039645	–
D. moriniae	BRIP 60190a^{T}	OM918698	OM960625	–	OM960607	–
D. multigutullata	ICMP 20656^{T}	KJ490633	KJ490454	KJ490575	KJ490512	–
D. musigena	CBS 129519^{T}	KC343143	KC344111	KC343627	KC343869	KC343385
D. myracrodruonis	URM 7972^{T}	MK205289	MK205291	–	MK213408	MK205290
D. neatei	BRIP 60289a^{T}	OR019756	OR039653	–	OR039646	–
D. nebulae	PMM 1681^{T}	KY511337	KY511369	–	MH708552	–
D. neilliae	CBS 144.27^{T}	KC343144	KC344112	KC343628	KC343870	KC343386
D. neoarctii	CBS 109490	KC343145	KC344113	KC343629	KC343871	KC343387
D. neoraonikayaporum	MFLUCC 14-1136^{T}	KU712449	KU743988	–	KU749369	KU749356
D. nigra	JZB 320170^{T}	MN653009	MN887113	–	MN892277	–
D. nobilis	CBS 587.79	KC343153	KC344121	KC343637	KC343879	KC343395
D. nomurai	CBS 157.29	KC343154	KC344122	KC343638	KC343880	KC343396
D. norfolkensis	BRIP 59718a^{T}	OM918699	OM960626	–	OM960608	–
D. nothofagi	BRIP 54801^{T}	JX862530	KF170922	–	JX862536	–
D. novem	CBS 127269^{T}	KC343155	KC344123	KC343639	KC343881	KC343397
D. obtusifoliae	CBS 143449^{T}	MG386072	–	MG386137	–	–
D. ocoteae	CBS 141330^{T}	KX228293	KX228388	–	–	–
D. oculi	HHUF 30565^{T}	LC373515	LC373519	–	LC373517	–

D. oncostoma	CBS 589.78	KC343162	KC344130	KC343646	KC343888	KC343404
D. oraccinii	LC 3166[T]	KP267863	KP293443	KP293517	KP267937	–
D. orixae	HKAS 121465[T]	OK283041	OK432278	OK484486	OK432279	OK484485
D. osmanthi	GUCC 9165[T]	MK398675	MK502091	–	MK480610	MK502087
D. ovalispora	ICMP 20659[T]	KJ490628	KJ490449	KJ490570	KJ490507	–
D. ovoidea	CGMCC 3.17092[T]	KF576264	KF576288	–	KF576239	KF576222
D. oxe	CBS 133186[T]	KC343164	KC344132	KC343648	KC343890	KC343406
D. pachirae	COAD 2074[T]	MG559537	MG559541	–	MG559539	MG559535
D. padi var. *padi*	CBS 114200	KC343169	KC344137	KC343653	KC343895	KC343411
D. padina	CFCC 52590[T]	MH121525	MH121604	MH121483	MH121567	MH121443
D. pandanicola	MFLUCC 17-0607[T]	MG646974	MG646930	–	–	–
D. paranensis	CBS 133184	KC343171	KC344139	KC343655	KC343897	KC343413
D. parapterocarpi	CBS 137986[T]	KJ869138	KJ869248	–	–	–
D. parvae	PSCG 034[T]	MK626919	MK691248	MK726210	MK654858	–
D. pascoei	BRIP 54847[T]	JX862532	KF170924	–	JX862538	–
D. passiflorae	CBS 132527[T]	JX069860	KY435674	KY435654	KY435633	KY435664
D. passifloricola	CBS 141329[T]	KX228292	KX228387	KX228367	–	–
D. patagonica	CBS 145291[T]	MN509717	MN509728	–	MN509739	MN974279
D. penetriteum	LC 3353	KP714505	KP714529	KP714493	KP714517	–
D. perjuncta	CBS 109745[T]	KC343172	KC344140	KC343656	KC343898	KC343414
D. perniciosa	CBS 124030	KC343149	KC344117	KC343633	KC343875	KC343391
D. perseae	CBS 151.73	KC343173	KC344141	KC343657	KC343899	KC343415
D. pescicola	MFLUCC 16-0105[T]	KU557555	KU557579	–	KU557623	KU557603
D. phaseolorum	CBS 113425	KC343174	KC344142	KC343658	KC343900	KC343416
D. phillipsii	CAA 817[T]	MK792305	MN000351	MK871445	MK828076	MK883831
D. phragmitis	CBS 138897[T]	KP004445	KP004507	KP004503	–	–

D. phyllanthicola	SCHM 3680^{T}	AY620819	–	–	–	–
D. pimpinellae	JZB320131^{T}	MK874656	MT373072	MT373073	MT373074	–
D. platzii	BRIP 60353a^{T}	OM918700	OM960627	–	OM960609	–
D. podocarpi- macrophylli	CGMCC 3.18281^{T}	KX986774	KX999207	KX999246	KX999167	KX999278
D. poincianellae	URM 7932^{T}	MH989509	MH989537	MH989539	MH989538	MH989540
D. pometiae	SAUCC 194.72^{T}	MT822600	MT855797	MT855568	MT855912	MT855679
D. portugallica	CBS 144228^{T}	MH063905	MH063917	MH063899	MH063911	MH063893
D. pseudoalnea	CFCC 54190^{T}	MZ727037	MZ753487	MZ781302	MZ816343	MZ753468
D. pseudoamygdali	**MUCC 3612^{T}**	**OR897104**	**OR913164**	**OR913195**	**OR913226**	**OR913254**
D. pseudoanacardii	CBS 148909^{T}	OR348655	OR468821	OR468801	OR468811	OR468831
D. pseudobiguttulata	ICMP 20657^{T}	KJ490582	KJ490403	KJ490524	KJ490461	–
D. pseudoinconspicua	URM 7874^{T}	MH122538	MH122524	MH122517	MH122533	MH122528
D. pseudomangiferae	CBS 101339^{T}	KC343181	KC344149	KC343665	KC343907	KC343423
D. pseudooculi	HHUF 30617^{T}	LC373515	LC373519	–	LC373517	–
D. pseudophoenicicola	CBS 462.69^{T}	KC343184	KC344152	KC343668	KC343910	KC343426
D. pseudotsugae	MFLU 15-3228^{T}	KY964225	KY964108	–	KY964181	KY964138
D. psoraleae	CBS 136412^{T}	KF777158	KF777251	–	KF777245	–
D. psoraleae-pinnatae	CBS 136413^{T}	KF777159	KF777252	–	–	–
D. pterocarpi	MFLUCC 10-0571	JQ619899	JX275460	–	JX275416	JX197451
D. pterocarpicola	MFLUCC 10-0580a	JQ619887	JX275441	–	JX275403	JX197433
D. pulla	CBS 338.89^{T}	KC343152	KC344120	KC343636	KC343878	KC343394
D. pungensis	SAUCC 194.112^{T}	MT822640	MT855837	MT855607	MT855952	MT855719
D. pustulata	CBS 109742	KC343185	KC344153	KC343669	KC343911	KC343427
D. pyracanthae	CBS 142384^{T}	KY435635	KY435666	KY435645	KY435625	KY435656
D. quercicola	CSUFTCC 104^{T}	ON076567	–	ON081667	ON081659	ON081670
D. racemosae	CBS 143770^{T}	MG600223	MG600227	MG600221	MG600225	MG600219

D. raonikayaporum	CBS 133182^{T}	KC343188	KC344156	KC343672	KC343914	KC343430
D. rauvolfiae	CBS 148912^{T}	OR348658	OR468818	OR468798	OR468808	OR468828
D. ravennica	MFLUCC 15–0479^{T}	KU900335	KX432254	–	KX365197	–
D. rhodomyrti	CFCC 53101^{T}	MK432643	MK578046	MK442990	MK578119	MK442965
D. rhoina	CBS 146.27	KC343189	KC344157	KC343673	KC343915	KC343431
D. rizhaoensis	CFCC 57562^{T}	OP955930	OP959773	OP959785	OP959767	OP959782
D. rosae	MFLUCC 17-2658^{T}	MG828894	MG843878	–	–	MG829273
D. rosicola	MFLU 17-0646^{T}	MG828895	MG843877	–	MG829270	MG829274
D. rosiphthora	COAD 2913^{T}	MT311196	–	–	MT313692	MT313690
D. rossmaniae	CAA 762^{T}	MK792290	MK837914	MK871432	MK828063	MK883822
D. rostrata	CFCC 50062^{T}	KP208847	KP208855	KP208851	KP208853	KP208849
D. rudis	CBS 113201	KC343234	KC344202	KC343718	KC343960	KC343476
D. rumicicola	MFLUCC 18-0739^{T}	MK066126	MK049555	–	MK049554	–
D. saccarata	CBS 116311^{T}	KC343190	KC344158	KC343674	KC343916	KC343432
D. sackstonii	BRIP 54669b^{T}	KJ197287	KJ197267	–	KJ197249	–
D. salicicola	BRIP 54825^{T}	JX862531	KF170923	–	JX862537	–
D. salinicola	MFLU 18-0553^{T}	MN047098	–	–	MN077073	–
D. samaneae	SDBR-CMU470^{T}	OQ600197	OQ678277	OQ646880	OQ603500	OQ646884
D. sambuci	CFCC 51986	KY852495	KY852511	KY852503	KY852507	KY852499
D. sapindicola	CFCC 55344^{T}	MW881507	MW898937	MW898940	MW898934	MW898943
D. schimae	CFCC 53103^{T}	MK432640	MK578043	MK442987	MK578116	MK442962
D. schini	CBS 133181^{T}	KC343191	KC344159	KC343675	KC343917	KC343433
D. schisandrae	CFCC 51988^{T}	KY852497	KY852513	KY852505	KY852509	KY852501
D. schoeni	MFLU 15-1279^{T}	KY964226	KY964109	–	KY964182	KY964139
D. sclerotioides	CBS 296.67^{T}	KC343193	KC344161	KC343677	KC343919	KC343435
D. scobina	CBS 251.38	KC343195	KC344163	KC343679	KC343921	KC343437

D. searlei	BRIP 66528^{T}	MN708231	MN696540	–	–	–
D. sennae	CFCC 51636^{T}	KY203724	KY228891	–	KY228885	KY228875
D. sennicola	CFCC 51634^{T}	KY203722	KY228889	–	KY228883	KY228873
D. serafiniae	BRIP 55665a^{T}	KJ197274	KJ197254	–	KJ197236	–
D. shaanxiensis	CFCC 53106	MK432654	–	MK443001	MK578130	MK442976
D. shawiae	BRIP 64534a^{T}	OM918701	OM960628	–	OM960610	–
D. shennongjiaensis	CNUCC 201905^{T}	MN216229	MN227012	MN224559	MN224672	MN224551
D. siamensis	MFLUCC 10-0573a	JQ619879	JX275429	–	JX275393	–
D. silvicola	CFCC 54191^{T}	MZ727041	MZ753491	MZ753481	MZ816347	MZ753472
D. sinensis	CGMCC 3.19521^{T}	MK637451	MK660447	–	MK660449	–
D. smilacicola	CFCC 54582^{T}	OP955933	OP959776	OP959788	OP959770	OP959779
D. sojae	CBS 139282^{T}	KJ590719	KJ610875	KJ659208	KJ590762	KJ612116
D. sojae	**MUCC 3591**	**OR897083**	**OR913143**	**OR913174**	**OR913205**	**OR913235**
D. sojae	**MUCC 3593**	**OR897085**	**OR913145**	**OR913176**	**OR913207**	**OR913237**
***Diaporthe* sp. 1**	**MUCC 3585**	**OR897077**	**OR913137**	**OR913168**	**OR913199**	**OR913229**
***Diaporthe* sp. 2**	**MUCC 3597**	**OR897089**	**OR913149**	**OR913180**	**OR913211**	**OR913240**
***Diaporthe* sp. 3**	**MUCC 3583**	**OR897075**	**OR913135**	**OR913166**	**OR913197**	**OR913228**
D. spartinicola	CBS 140003^{T}	KR611879	KR857695	KR857696	–	–
D. spinosa	PSCG 383^{T}	MK626849	MK691234	MK726156	MK654811	MK691129
D. sterilis	CBS 136969^{T}	KJ160579	KJ160528	MF418350	KJ160611	KJ160548
D. stewartii	CBS 193.36	FJ889448	–	–	GQ250324	–
D. stictica	CBS 370.54	KC343212	KC344180	KC343696	KC343938	KC343454
D. subclavata	ICMP 20663^{T}	KJ490630	KJ490451	KJ490572	KJ490509	–
D. subcylindrospora	KUMCC 17-0151^{T}	MG746629	MG746631	–	MG746630	–
D. subellipicola	KUMCC 17-0153^{T}	MG746632	MG746634	–	MG746633	–
D. subordinaria	CBS 101711	KC343213	KC344181	KC343697	KC343939	KC343455

D. taoicola	MFLUCC 16-0117^{T}	KU557567	KU557591	–	KU557635	–
D. tarchonanthi	CBS 146073^{T}	MT223794	MT223733	MT223759	–	–
D. tecomae	CBS 100547	KC343215	KC344183	KC343699	KC343941	KC343457
D. tectonae	MFLUCC 12-0777^{T}	KU712430	KU743977	–	KU749359	KU749345
D. tectonendophytica	MFLUCC 13-0471^{T}	KU712439	KU743986	–	KU749367	KU749354
D. tectonigena	MFLUCC 12-0767^{T}	KU712429	KU743976	–	KU749371	KU749358
D. terebinthifolii	CBS 133180^{T}	KC343216	KC344184	KC343700	KC343942	KC343458
D. ternstroemia	CGMCC 3.15183^{T}	KC153098	–	–	KC153089	–
D. thunbergiae	MFLUCC 10-0756a	JQ619893	JX275449	–	JX275409	JX197440
D. thunbergiicola	MFLUCC 12-0033^{T}	KP715097	–	–	KP715098	–
D. tibetensis	CFCC 51999^{T}	MF279843	MF279873	MF279828	MF279858	MF279888
D. torilicola	MFLUCC 17-1051^{T}	KY964212	KY964096	–	KY964168	KY964127
D. toxica	CBS 534.93^{T}	KC343220	KC344188	KC343704	KC343946	KC343462
D. toxicodendri	FFPRI 420987	LC275192	LC275224	LC275216	LC275216	LC275200
D. trevorrowii	BRIP 70737a^{T}	OM918703	OM960630	–	OM960612	–
D. tulliensis	BRIP 62248a	KR936130	KR936132	–	KR936133	–
D. ueckeri	FAU 656	KJ590726	KJ610881	KJ659215	KJ590747	KJ612122
D. ukurunduensis	CFCC 52592^{T}	MH121527	–	MH121485	MH121569	MH121445
D. ulmina	CFCC 58828^{T}	OQ912957	OQ910324	OQ910262	OQ910290	OQ910232
D. undulata	CGMCC 3.18293^{T}	KX986798	KX999230	KX999269	KX999190	–
D. unshiuensis	CGMCC 3.17569^{T}	KJ490587	KJ490408	KJ490529	KJ490466	–
D. vaccinii	CBS 160.32^{T}	AF317578	KC344196	KC343712	GQ250326	KC343470
D. vacuae	CAA 830^{T}	MK792309	MK837931	MK871449	MK828080	MK883834
D. vangoghii	MF-Ha18-046^{T}	MW008492	MW008503	MZ671963	MW008514	MZ671937
D. vangueriae	CBS 137985^{T}	KJ869137	KJ869247	–	–	–
D. vawdreyi	BRIP 57887a	KR936126	KR936128	–	KR936129	–

D. velutina	CGMCC 3.18286^{T}	KX986790	KX999223	KX999261	KX999182	–
D. verniciicola	CFCC 53109^{T}	MK573944	MK574639	MK574599	MK574619	MK574583
D. vexans	CBS 127.14	KC343229	KC344197	KC343713	KC343955	KC343471
D. viciae	JZB 320179^{T}	OP626092	OP627281	OP627279	OP627280	–
D. viniferae	JZB 320071^{T}	MK341551	MK500112	–	MK500107	MK500119
D. virgiliae	CBS 138788^{T}	KP247573	KP247582	–	–	–
D. vitimegaspora	STE-U 2675	AF230749	–	–	–	–
D. vochysiae	LGMF 1583^{T}	MG976391	MK007527	MK033323	MK007526	MK007528
D. woolworthii	CBS 148.27	KC343245	KC344213	KC343729	KC343971	KC343487
D. xishuangbanica	CGMCC 3.18282^{T}	KX986783	KX999216	KX999255	KX999175	–
D. xunwuensis	CFCC 53085^{T}	MK432663	MK578063	MK443008	MK578137	MK442983
D. yunnanensis	CGMCC 3.18289^{T}	KX986796	KX999228	KX999267	KX999188	KX999290
D. zaobaisu	PSCG 031^{T}	MK626922	MK691245	MK726207	MK654855	–
D. zaofenghuang	CGMCC 3.20271^{T}	MW477883	MW480875	–	MW480871	MW480867
Diaporthella corylina	CBS 121124	KC343004	KC343972	KC343488	KC343730	KC343246

[1] type strain of *D. longicicola*; [2] type strain of *D. momicola*; [3] strain originally named *D. mahothocarpi* Nom. Inval.; [4] type strain of *D. ellipicola*; [5] type strain of *D. biguttusis*; [6] type strain of *D. campthothecicola;* [7] type strain of *D. rhusicola*; ^{T} ex-type material

Boldface type font indicate isolate used in this study.

RESULT

Isolation and Phylogeny

A total of 31 isolates were obtain from the sample in this study (Table 5.3) and the morphological characteristic were described in the taxonomy sections. Preliminary analysis of the combine matrix of combine loci included of 432 OTUs was composed of 3159 sites, including gaps (ITS: 456 bp, TUB: 643 bp, HIS: 711 bp, TEF: 707 bp, CAL: 642 bp). Figure 5.1 shows the phylogenetic trees generated from combine dataset, revealing isolates obtained from *Prunus* spp. were located in three major clades; the first subclade contains isolates of *Diaporthe eres* species complex (DESC), the second subclade contains *Diaporthe amygdali* species complex (DASC), and the third subclade consists of *Diaporthe sojae* species complex (DSSC). Maximum-Likelihood bootstrap (ML BS ≥ 50%) and Bayesian Inference posterior probability (BI PP ≥ 0.95) have been shown above the branches. Then, in the following analyses, the matrix was split into three sections and realigned. The reference taxa retrieved from GenBank were reextracted for more detailed analyses.

Table 5.3: List of isolates, host, and location of sampling

Isolates MUCC	Species	Host species	Location
3587	*D. amygdali*	*Cerasus speciosa*	Tsukuba, Ibaraki
3589	*D. amygdali*	*Cerasus* Sato-zakura Group '*Sekiyama*'	Tsukuba, Ibaraki
3590	*D. amygdali*	*Cerasus* Sato-zakura Group '*Hisakura*'	Tsukuba, Ibaraki
3592	*D. amygdali*	*Cerasus* Sato-zakura Group '*Sekiyama*'	Tsukuba, Ibaraki
3598	*D. amygdali*	*Cerasus* Sato-zakura Group '*Grandiflora*'	Hachioji, Tokyo
3600	*D. amygdali*	*Prunus* sp.	Morioka, Iwate
3603	*D. amygdali*	*Cerasus* Sato-zakura Group '*Albo-rosea*'	Shimonita, Gunma
3584	***D. endoprunicola***	*Cerasus* × *yedoensis*	Tsukuba, Ibaraki
3586	*D. eres*	*Cerasus* × *yedoensis*	Tsukuba, Ibaraki
3588	*D. eres*	*Cerasus itosakura* 'Plena-rosea'	Tsukuba, Ibaraki
3594	*D. eres*	*Padus grayana*	Tsukuba, Ibaraki
3595	*D. eres*	*Padus grayana*	Tsukuba, Ibaraki
3596	*D. eres*	*Prunus* sp.	Seto, Aichi
3599	*D. eres*	*Cerasus* Sato-zakura Group '*Nobilis*'	Hachioji, Tokyo
3601	*D. eres*	*Cerasus* '*Yoko*'	Morioka, Iwate

3602	*D. eres*	*Cerasus jamasakura* var. *jamasakura*	Kitaibaraki, Ibaraki
3604	*D. eres*	*Prunus* sp.	Shimonita, Gunma
3605	*D. eres*	*Padus ssiori*	Sapporo, Hokkaido
3606	*D. eres*	*Cerasus maximowiczii*	Sapporo, Hokkaido
3607	*D. eres*	*Padus ssiori*	Sapporo, Hokkaido
3608	*D. eres*	*Cerasus* × *yedoensis*	Sapporo, Hokkaido
3609	*D. eres*	*Cerasus* × *yedoensis*	Sapporo, Hokkaido
3610	*D. eres*	*Cerasus* × *yedoensis*	Sapporo, Hokkaido
3611	*D. eres*	*Padus ssiori*	Sapporo, Hokkaido
3613	*D. eres*	*Padus ssiori*	Sapporo, Hokkaido
3612	***D. pseudoamygdali***	*Cerasus* × *yedoensis*	Sapporo, Hokkaido
3591	*D. sojae*	*Cerasus* × *yedoensis*	Tsukuba, Ibaraki
3593	*D. sojae*	*Cerasus* × *yedoensis*	Tsukuba, Ibaraki
3585	*Diaporthe* sp. 1	*Cerasus itosakura* '*Pendula*'	Tsukuba, Ibaraki
3597	*Diaporthe* sp. 2	*Prunus* sp.	Seto, Aichi
3583	*Diaporthe* sp. 3	*Cerasus* × *yedoensis*	Tsukuba, Ibaraki

The reconstructed resultant tree for DESC was generated with the matrix of the total length of the alignment featured 2245 sites, including gaps (ITS: 410 bp, TUB: 439 bp, HIS: 503 bp, TEF: 407 bp, CAL: 486 bp) (Figure 5.2). It consists of a total of 73 OTUs, including 18 Japanese isolates, of which 17 isolates are recognized as *D. eres* s.l. of DESC and one new species. The second restricted tree features DASC species composed of 43 OTU and was generated from 2145 sites, including gaps (ITS: 410 bp, TUB: 425 bp, HIS: 424 bp, TEF: 311 bp, CAL: 575 bp) of (Figure 5.3) with eight Japanese isolates from *Prunus* spp. were recognized under *D. amygdali* and one new species. The third restricted tree characterized by DSSC, composed of 46 OTUs, was generated with 2247 sites, including gaps (ITS: 400 bp, TUB: 513 bp, HIS: 504 bp, TEF: 360 bp, CAL: 470 bp) (Figure 5.4). The tree topologies of split trees showed congruency between ML and BI, where ML tree was shown in the figure with BS and PP indicated at the branch. Species delimitations were suggested by the Poisson Tree Processes Method. Only the split trees were subjected to coalescent-based PTP.

Figure 5.1: Maximum-likelihood tree obtained from the combine ITS, TUB, TEF, HIS, and CAL sequence of isolates used in this study and reference strain. *Diaporthella corylina* CBS 121124 was used as outgroup for this tree. Bootstrap value ≥ 60% are indicated along the branch with thicken. Legend refers to nucleotide substitution per site.

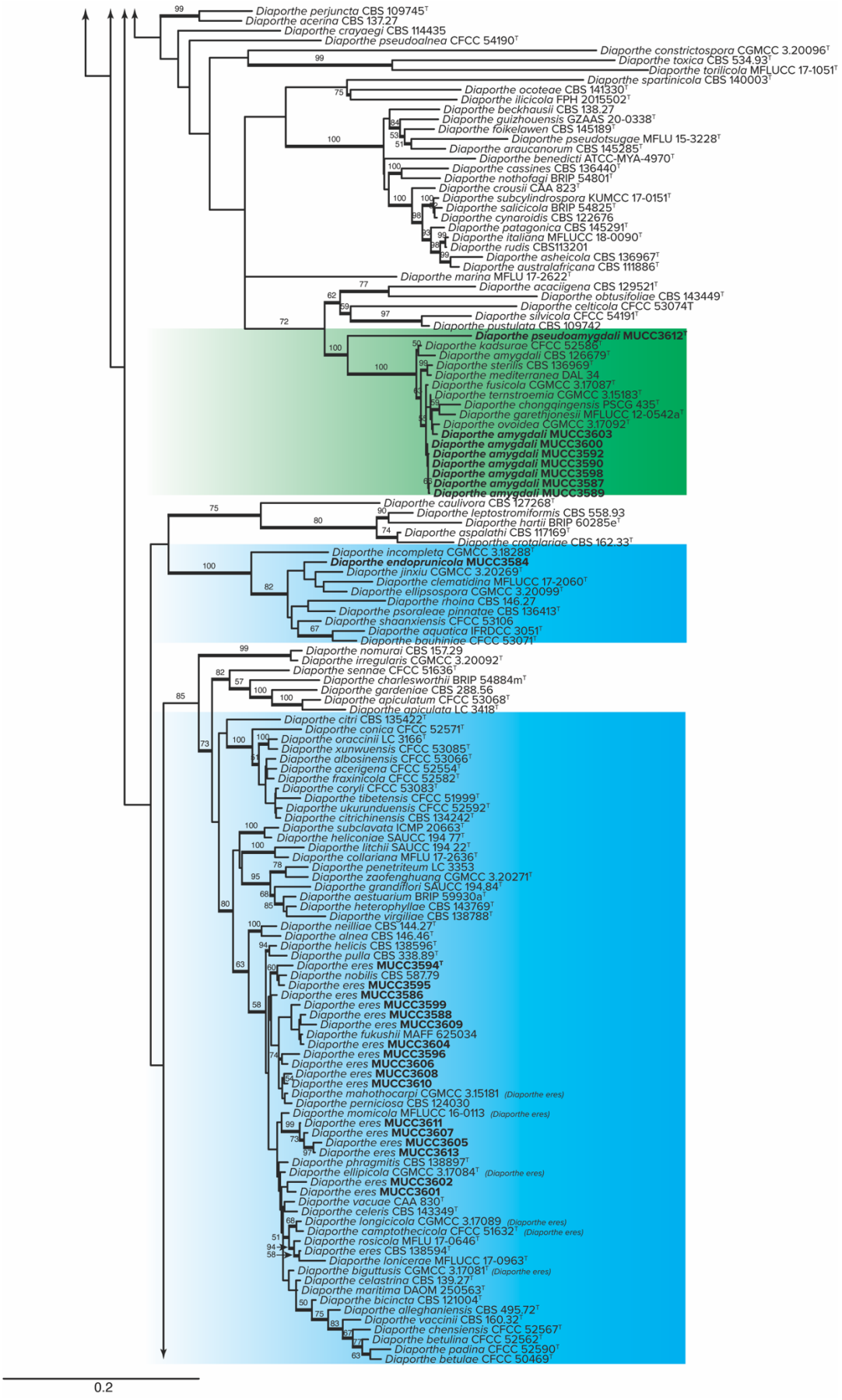

Figure 5.1: Continue

Figure 5.1: Continue

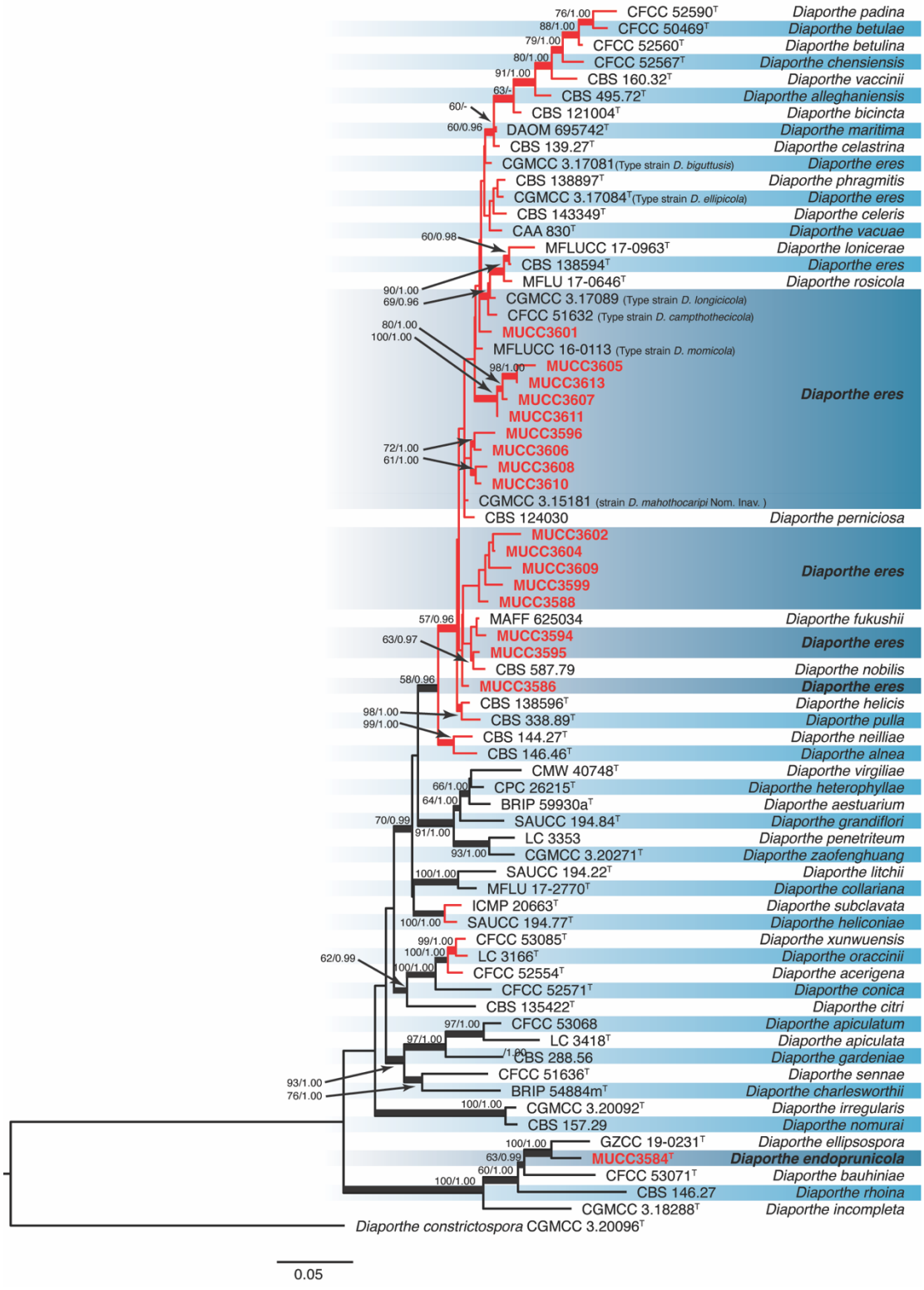

Figure 5.2: Maximum-likelihood (ML) phylogenetic tree consisting of *Diaporthe* eres species complex (DESC) constructed by using concatenated matrix of 5 loci. The bootstrap of ≥ 50 and posterior probability ≥ 0.95 are indicated near branch as BS/PP. *Diaporthe constrictopora* CGMCC 3.20096 were used as outgroup. Legend refers to nucleotide substitution per site. Red branch indicated coalescent/population process in PTP ML delimitation scheme.

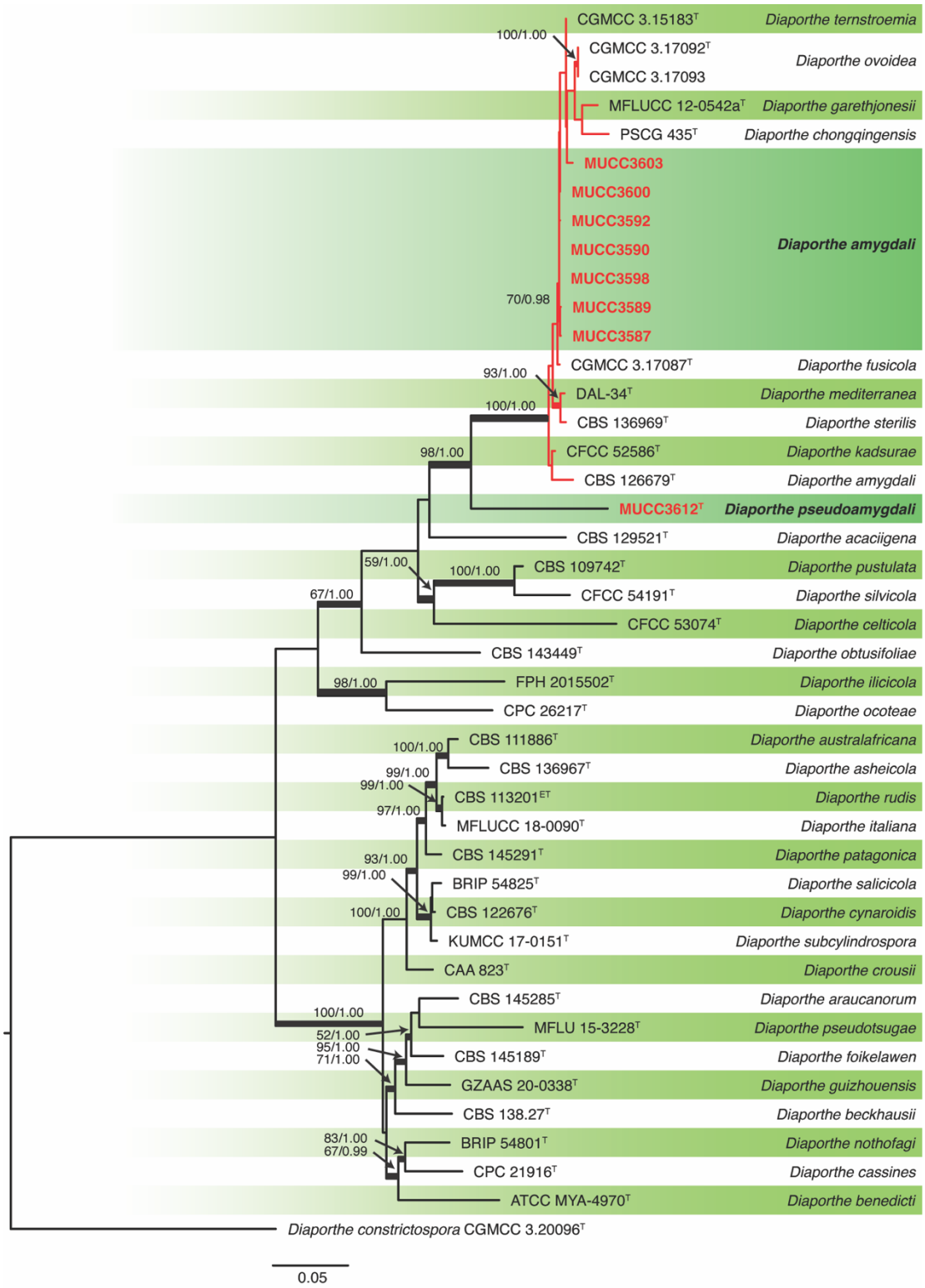

Figure 5.3: Maximum-likelihood (ML) phylogenetic tree consisting of *Diaporthe amygdali* species complex (DASC) constructed by using concatenated matrix of 5 loci. The bootstrap of ≥ 50 and posterior probability ≥ 0.95 are indicated near branch as BS/PP. *Diaporthe constrictopora* CGMCC 3.20096 were used as outgroup. Legend refers to nucleotide substitution per site. Red branch indicated coalescent/population process in PTP ML delimitation scheme.

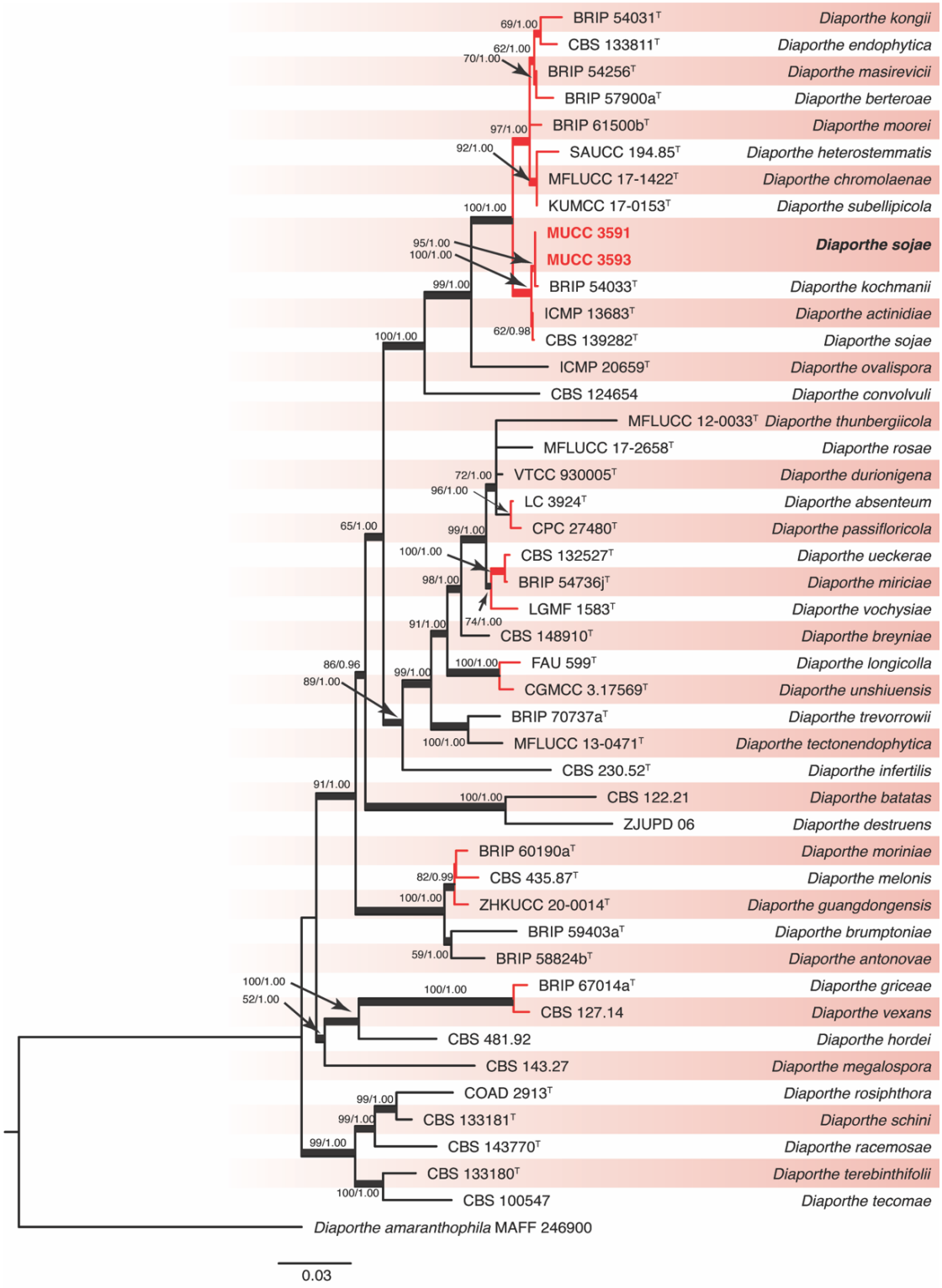

Figure 5.4: Maximum-likelihood (ML) phylogenetic tree consisting of *Diaporthe sojae* species complex (DSSC) constructed by using concatenated matrix of 5 loci. The bootstrap of ≥ 50 and posterior probability ≥ 0.95 are indicated near branch as BS/PP. *Diaporthe amaranthophila* MAFF 246900 were used as outgroup. Legend refers to nucleotide substitution per site. Red branch indicated coalescent/population process in PTP ML delimitation scheme.

Taxonomy

Diaporthe amygdali (Delacr.) Udayanga, Crous, & K.D Hyde, Fungal Divers. 56-166 (2012)

Synonym: listed in Hilário et al. 2021b

Description. See Udayanga et al. 2012.

Isolates examine: JAPAN, Ibaraki, Tsukuba, endophyte in *Prunus yedoensis*, 11 Mar. 2022, collected by Y. Hattori, culture MUCC 3587; ibid, endophyte in *P. lannesiana* cv *Sekiyama*, 11 Mar. 2022, collected by Y. Hattori, culture MUCC 3589, MUCC 3592; ibid, endophyte in *P. lannesiana* cv *Hisakura*, 11 March 2022, collected by Y. Hattori, culture MUCC 3590; Tokyo, Hachioji, endophyte in *Cerasus* Sato-zakura Group '*Grandiflora*' A.Wagner, 16 May 2022, collected by Y. Hattori & H. Masuya, culture MUCC 3598; Gunma, Shimonita, endophyte in *P. lannesiana* '*alborosea*', 05 Jul. 2022, collected by Y. Hattori & H. Masuya, culture MUCC 3603; Iwate, Morioka, endophyte in branch of *Prunus* sp., 16 May 2022, collected by Y. Hattori & H. Masuya, culture MUCC 3600.

Diaporthe eres Nitschke, Pyrenomyc. Germ. 2:245 (1870)

Synonym: listed in Hilário et al. 2021a.

Description. See Udayanga et al. 2014.

Isolates examine: JAPAN, Ibaraki, Tsukuba, endophyte in *Prunus yedoensis*, 11 Mar. 2022, collected by Y. Hattori, culture MUCC 3586; ibid, endophyte in *P. pendula* cv. *Pleno-rosea*, 22 Apr. 2022, collected by Y. Hattori, culture MUCC 3588; ibid, endophyte in *Padus grayana*, 12 May 2022, collected by Y. Hattori, culture MUCC3594, MUCC 3595; Kita-ibaraki, endophyte in *P. jamasakura*, 27 Jun. 2022, collected by Y. Hattori & H. Masuya, culture MUCC 3602; Aichi, Seto, endophyte in *Prunus* sp., 08 May 2022, collected by Y. Hattori, culture MUCC 3596; Tokyo, Hachioji, endophyte in *Cerasus* Sato-zakura Group '*Nobilis*' Miyashi, 16 May 2022, collected by Y. Hattori & H. Masuya, culture MUCC 3599; Iwate, Morioka, endophyte in *P. ampanulate* '*Yoko*' , 23 Jun. 2022, collected by Y. Hattori & H. Masuya , culture MUCC 3601; Gunma, Shimonita, endophyte

in *Prunus* sp., 5 Jul. 2022, collected by Y. Hattori & H. Masuya, culture MUCC 3604; Hokkaido, Sapporo, endophyte in *P. ssiori*, 21 Jul. 2022, collected by Y. Hattori & H. Masuya, culture MUCC 3605, MUCC3607, MUCC 3611, MUCC 3613; Sapporo, endophyte in *Cerasus* × *yedoensis* cv *Yedoensis*, 21 Jul. 2022, collected by Y. Hattori & H. Masuya, culture MUCC3608, MUCC3609, MUCC3610; Sapporo, endophyte in *Cerasus maximowwiczii,* 21 Jul. 2022, collected by Y. Hattori, culture MUCC 3606.

Notes. On *Prunus* s.l., *D. eres* was described as an endophyte in *P. domestica* from Poland (Abramczyk et al., 2022) in *P. mandshuria* from Russia (Nekrasov et al., 2022), and it occurs as pathogen on *P. davidiana* from China (H. Zhu et al., 2019), on *P. persica* from Greece (Thomidis & Michailides, 2009) and Italy (Prencipe et al., 2017), *P. salicina* from China (Bai et al., 2023).

Diaporthe sojae Lehman, Ann Missouri Bot. Gard. 10: 128 (1923)

Remark. Synonyms are listed in Udayanga et al. (2015)

Description. Udayanga et al., (2015)

Isolates examined: JAPAN, Ibaraki, Tsukuba, endophytic in *Prunus yedoensis*, 22 Apr. 2022, collected by Y. Hattori, culture MUCC3591, MUCC 3593

Notes. The isolates MUCC 3591 and MUCC 3593 are placed in the *Diaporthe sojae* species complex, closely related to *Diaporthe kochmanii*. In the report by Udayanga et al. (2015), *D. kochmanii* are synonymize with *D. sojae*.

Diaporthe endoprunicola A.H. Ujat & Y. Hattori, **sp. nov.** Mycobank MB 851350; Figure 5.5.

Etymology: Named after its endophytic nature in *Prunus yedoensis*

On PCA, Sexual morph not observed. Conidiomata pycnidial, gregarious, brown, 200–450 µm in diam. *Pycnidia*, brown, globose, immersed, producing pale yellow conidial masses as a droplet*; pycnidia cell wall* 2-4 layer, composed of *textura angularis,* 2–2.5 µm, with an ostiole*,* 200–250 µm in diam*. Phialides* hyaline, elongated, sporulating enteroblastically, 1-septate, cylindrical, slightly wide at the base with tapered apex, smooth, unbranched, 5.0–13.0 µm*. Alpha conidia* hyaline, aseptate, ellipsoidal, rounded at the apex, truncated at the base, non-guttulate, 5.0–6.0 × 1.5–2.1 µm*. Beta conidia* hyaline, aseptate, filiform, or straight, rounded at the apex, tapered at the base. 30–36 × 1.0–1.5 µm*. Gamma* conidia not observed*.*

Culture characteristics. On PCA, conidiomata observed, immersed and semi-immersed in medium, producing droplet containing conidial masses from the top of semi-immersed conidiomata; covered with aerial mycelia*. On PDA,* mycelia flat, pale yellow to brown in colour, forming conidiomata, semi-immeresed, without droplets on conidiomata*.*

Type: JAPAN, Ibaraki, Tsukuba, endophyte in branch of *Cerasus × yedoensis*, 11 March 2022, collected by Y. Hattori (TSU-MUMH 12005, dried culture of MUCC 3584).

Isolate examined: Japan, Ibaraki, Tsukuba, endophyte in branch of *Cerasus × yedoensis*, 11 March 2022, by Y. Hattori (MUCC3584, original isolate of TSU-MUMH 12005, ex-holotype).

Note: The phylogenetic position of MUCC 3584 is in the sister clade of the *D. eres* species complex (BS/PP of 100/1.00). The closest species to the present species is *D. ellipsospora*, described as saprobes on decaying wood in China (Dissanayake et al., 2020)

Diaporthe pseudoamygdali A.H. Ujat & Y. Hattori, **sp. nov.** Mycobank MB851351; Figure 5.5.

Etymology: Named for its close relationship with *D. amygdali*

On PCA, *Sexual morph not observed*. *Conidiomata* pycnidial, gregarious, dark brown to black, up to 300 µm. *Pycnidia* globose, or irregular, immersed, solitary or aggregated, 150–160 µm, releasing pale yellow to pale flesh droplets containing conidial masses, pycnidia cell wall 1–3 layer, composed of *textura angularis*, 2–2.5 µm, with an ostiole, 50–85 um in diam. *Phialides* hyaline, smooth, elongated, sporulating enteroblastically, unbranched, aseptate, ampulliform, tapered towards the end, 1.5–2 × 5.2–11.7 µm. *Alpha conidia* elipsoidal, aseptate, multi-guttulated, hyaline, rounded at the apex, tapered at the base, 6.4–8.5 × 2.0–2.5µm. Beta conidia and gamma conidia not observed.

Culture characteristics. On PCA, conidiomata observed, brown to black, immersed in medium, forming white to pale pink droplets containing conidial masses on top on conidiomata, without aerial mycelium.

Specimens examine: See ex-type.

Type: JAPAN, Hokkaido, Sapporo, 22 Jul. 2022, collected by Y. Hattori, endophyte in branch of *Cerasus × yedoensis* (TSU-MUMH 12006, dried culture of MUCC 3612).

Isolate examined: Japan, Hokkaido, Sapporo, endophyte in twig of *Cerasus × yedoensis,* 22 July 2022, by Y. Hattori (MUCC 3612, original isolate of TSU-MUMH 12006, ex-holotype).

Notes: This species was placed in the same clade with *Diaporthe amygdali*, but form an independent branch highly supported by BS/PP (98/1.00). The present species differs with *D. amygdali* in conidiogenous cell as the conidiogenous cell of *D. amygdali* are either branch or septated or both, but *D. pseudoamygdali* are unbranch and aseptate.

Diaporthe sp. 1

Culture characteristics. On PCA, colony white, sparse at the edge, with dense aerial mycelia at the centre. Conidiomata not observed. On PDA, colony white, thick mycelial mat.

Isolate examined: Japan, Ibaraki, Tsukuba, endophyte in twig of *Cerasus yedoensis*, 11 Mar. 2022, by Y. Hattori (MUCC 3585).

Note: MUCC 3585 placed next sister taxon *D. orixae* forming a well-supported clade (ML BS:55) (Fig. 5.1). However, it is not formally described as an independent species because the culture is sterile. Therefore, we don't describe species formally.

Diaporthe sp. 2

Culture characteristics. On PCA, colony pale yellow to pale brown, with dense aerial mycelia, filling the petri dish. Conidiomata not observed. On PDA, colony thick, white mycelial mat.

Isolate examined: Japan, Aichi, Seto, endophyte in *Prunus* sp., 8 May 2022, by Y. Hattori (MUCC 3597).

Note: The isolate distinctly placed a lineage on the phylogenetic tree within a well-supported clade with *D. howardiae* (ML BS: 67) (Fig. 5.1). However, it is not formally described as an independent species because the culture is sterile. Phylogenetically, it is placed next to sister taxon of *D. howardiae* isolated from leaf spot of *Agave* sp.

Diaporthe sp. 3

Culture characteristics. On PCA, colony white, with sparse aerial mycelia. Conidiomata not observed. On PDA, colony dense, pale-yellow, with short aerial mycelia.

Material examined. Japan, Ibaraki, Tsukuba, endophyte in *Cerasus × yedoensis*, 11 Mar. 2022, by Y. Hattori (MUCC 3583)

Note: MUCC 3583 placed an independent lineage next to *D. hunanensis* and *D. liquidambaris* (Fig. 5.1). However, the sterility of the culture, this species is not formally described.

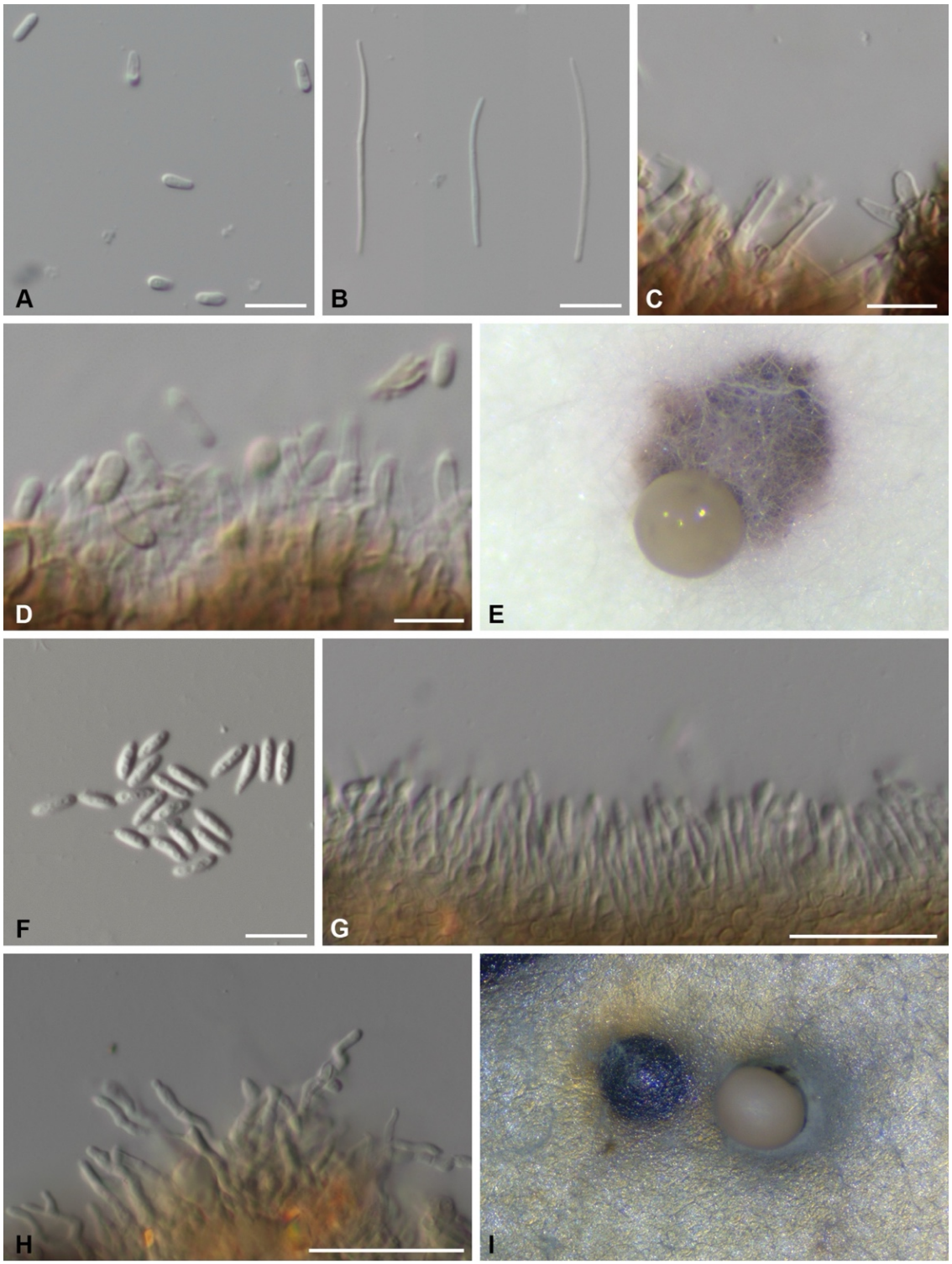

Figure 5.5: A-E. *Diaporthe endoprunicola* A. Alpha conidia. B. Beta conidia, C–D. Philiade. E. Pale yellow conidial mass produce on top of conidiomata in PCA. F-I *Diaporthe pseudoamygdali* F. Alpha conidia, G–H, Philiade. I. White conidial masses produce on top of conidiomata in PCA. Scalebar = 10 µm (A–F); 20 µm (G–H)

DISCUSSION

This study described two new *Diaporthe* species, *D. endoprunicola* and *D. pseudoamygdali*, along with three hitherto known species of *Diaporthe* (*D. eres*, *D. amygdali,* and *D. sojae*), and three sterile *Diaporthe* spp. from the 31 isolates obtained from *Prunus* s.l. from Japan. This result indicates that the *Diaporthe* species living endophytically in *Prunus* s.l. without symptoms is rich in diversity. As the taxonomical re-examination of the genus *Diaporthe* is still underway, Gao et al. (2017) showed the paraphyly of the genus *Diaporthe* using multi-loci analyses and divergent morphological characteristics. However, they stated that splitting the genera based on the monophyly is premature.

On the other hand, the concept of species complex in *Diaporthe* is being continuously discussed. Udayanga et al. (2014) attempt to resolve the complexity of *D. eres* by constructing a phylogenetic tree consisting of 7 loci. Although their robust phylogeny using muti-loci phylogeny is applied to the identification of the species, the identification of species complex remains challenging. Hilário et al. (2021a, b) examine the DASC and DESC, species complexes of *D. amygdali* and *D.* eres, by employing the Genealogical Concordance Phylogenetic Species Recognition principle (GCPSR) (Taylor et al., 2000), General Mixed Yule-Coalescent (GMYC) (Pons et al., 2006), and Coalescent-based model Poisson Tree Processes (PTP) (Zhang et al., 2013) for discussing the species delimitation of both species complexes, proposing that each species complex should be treated as a single species. They proposed to synonymise 31 names under *D. eres* of the DESC and 11 names under *D. amygdali* of DASC. Additionally, Norphanphoun et al., (2022) revised the species complexes based on the phylogeny with a five-loci combined matrix and expanded the range of complexes. In their study, *D. amygdali* and *D. eres* belonged to *Diaporthe pustulata* species complex and *Diaporthe alnea* species complex, respectively. However, Bai et al., (2023) proposed using *D. eres* for the complex name instead of *D. alnea* because the name was easily recognised as a representative species. This study employs the broad sense of *D. eres* and *D. amygdali*. Considering the methodology, result, and conclusion of previous research regarding *D. eres*, this study applied the results of the PTP to carefully establish a new species, *D. endoprunicola,* in the sister clade of the *D. eres* species complex.

Seven isolates identified as *D. amygdali* were clustered next to *D. fusicola*, which had been synonymized as *D. amygdali* by Hilário et al. (2021b). *Diaporthe pseudoamygdali* are located at the basal clade of the *D. amygdali* species complex, forming an independent lineage. Although *D. pseudoamygdali* showed overlapping morphology with *D. amygdali,* the PTP analysis suggested distinctiveness from other species. Hilário et al. (2021a, b) showed high genetic heterogenicity in DASC and DESC even though species belonging to these species complexes share the host plants, such as *Prunus* s.l. In fact, about 50% of *Diaporthe* isolates examined in this study were *D. eres.* This nature calls for a cautious introduction of new species (Lambert et al., 2023).

Current study shows in two of the regions where samples are taken the most, in Ibaraki prefecture, the diversity of *Diaporthe* species is highest where it includes *D. amygdali*, *D. endoprunicola*, *D. eres*, *D. sojae* and two species of sterile *Diaporthe*. While in Hokkaido region, *D. eres* make up most of the isolates with only one isolates of *D. pseudoamygdali*. *Diaporthe* species in other regions are made up of *D. eres* and *D. amygdali.* This shows that *D. eres* and *D. amygdali* are widely distributed in *Prunus* s.l. in Japan as endophytic fungi. Zhu et al., (2023) in their study of *Diaporthe* in citrus shows that there are several *Diaporthe* species that are weakly aggressive or non-pathogenic which could be of endophytic or latent pathogens. This shows that these *Diaporthe* species found in *Prunus* s.l. are potential as disease causing pathogens.

CONCLUSION

This study has successfully elucidated the diversity of *Diaporthe* spp. in Prunus s.l. as endophytes. Characterisation by multi-locus phylogeny and morphological observation was followed up with the coalescent method had, confirmed the new species of *Diaporthe* in this study. As more than molecular data is needed to describe new species, species with no observable morphological characteristics are refrained from being discussed as new species as the nature of Diaporthe spp. itself is paraphyletic (Gao et al., 2017). This is to prevent further destabilisation in taxonomy of *Diaporthe* spp. Hilário et al., (2021a, b) showed in their study that in-depth phylogenetic analysis is needed by employment of Genealogical Concordance Phylogenetic Species Recognition (GCPSR) in their study of *D. amygdali* species complex and *D. eres* species complex. A recent study by Hongsanan et al., (2023) provided taxonomic updates to the work by Dissanayake et al., (2017a), providing an insight to describe species and references formally. The addition of an integrative taxonomic approach should also be considered in aiding the delimitation of *Diaporthe* spp. (Hilário et al., 2021a, b; Pereira et al., 2023).

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Chapter 6 – General Discussion

In this study, the taxonomy of Japanese collection of Coelomycetes was analysed based on their morphological and culture characteristic as well as molecular phylogenetic relationships. A total of four genus of fungi from three families was subjected to polyphasic analysis which include the *Elsinoë* (Elsinoaceae), *Sphaerulina* and *Septoria* (Mycosphaerellaceae) and *Diaporthe* (Diaporthaceae). The result of the analysis includes proposal of three new species and six new combinations of *Elsinoë*, seven new species and two new combinations of *Sphaerulina*, one new species of *Septoria* and two new species of *Diaporthe*. The result revealed that most of the Japanese collection of coelomycetes are distinctive enough morphologically and molecularly from other part of the world. Taxonomic revisions in this study are also made based on the concept of "one fungus one name", following the International Code of Nomenclature for algae, fungi, and plants (Shenzen Code).

From the result and discussion of the previous chapter, Japan is a country with a diverse range of plant pathogenic Coelomycetes. Although most of it was previously identified based on morphological characteristics only, most of the fungus are identified and classified into the correct genus based on morphological characteristics at the time of the specimen's collection.

Predate the molecular biology era, the anamorphic and teleomorphic stage are separated and often have different name. While some genera of fungi are clearly related, for example *Elsinoë* and *Sphaceloma* (Fan et al., 2017) or *Diaporthe* and *Phomopsis* (Udayanga et al., 2012), there are also fungus that are difficult to distinguished morphologically, such as *Septoria* and *Sphaerulina* which the teleomorph was recorded as *Mycospharella* (Quaedvlieg et al., 2013; Verkley et al., 2013). Polyphasic approach which includes molecular analysis, morphological observation of fungi in natural condition and culture characteristic observation had shown clear distinction between anamorphic and teleomorphic stages of fungi(Crous et al., 2013b). This is notably valuable in family with similar anamorphic morphology. A clear example of this is, molecular method had successfully demarcated *Septoria* from *Sphaerulina* and unlinking both to *Mycospharella* (Quaedvlieg et al., 2013; Verkley et al., 2013).

This study also carries the hypothesises of host jumping, rather than co-evolution of plant pathogens with host plant. In the study of *Elsinoë*, Japanese isolates forms a clade whereby it clustered several different species with different host plant together very closely. Similar instances could be seen in the study of *Sphaerulina* and Septoria. Fan et al., (2017) in their study of *Elsinoë* mention that there are probability of host jumping occurring in *Elsinoë* infecting Eucalyptus plant, while in the study of Septoria and *Sphaerulina*, Verkley et al., (2013) mention that the theory of co-evolution was rejected as there are no evidence supporting co-evolution with host plant. Most of the coelomycetes in Japan are collected and discussed as pathogenic agents to plant. However, some of the fungi previously reported as plant pathogens were discovered as having endophytic lifestyle. Previously *Diaporthe* spp. was collected and studies in Japan as a causal agents of tree diseases. However, this study had identified a diverse group of *Diaporthe* spp. colonizing Sakura tree as endophytic fungi, exhibiting no symptom of disease on the host plant.

Although this study had managed to demonstrate the diversity of coelomycetes in Japan by the introducing new species and providing taxonomical updates by new combination, the introduction of new species should be carefully made to avoid destabilization of taxonomy. It is necessary to collect fresh specimens for comparison with type material to clarify on morphological characteristics, pathogenicity, geographical distribution, host range and lifestyle before the introduction of a new species.

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Conclusion

This study sheds light on the diversity of Japanese coelomycetes, focusing on four genera – *Elsinoë*, *Sphaerulina*, *Septoria*, and *Diaporthe*. The investigation highlights the significance of a polyphasic approach that integrates morphological and cultural characteristics, morphological analysis, and the habitat of the host plant within these fungal groups, providing taxonomic updates. The adoption of a polyphasic approach not only proposed novel species and new combinations but also revised the taxonomic classification of Japanese coelomycetes in line with the "one fungus, one name" principle.

The re-examination of *Elsinoë*, a fungus known for causing scab anthracnose on various plants, reveals the presence of three novel species (*Elsinoë hydrangeae*, *Elsinoë tanashiensis*, and *Elsinoë sumire*) and proposes six new combinations (*Elsinoë akebiae, Elsinoë catalpa, Elsinoë japonicum, Elsinoë paderiae, Elsinoë peucedani,* and *Elsinoë zelkovae*). Similarly, in the identification of the leaf spot causal pathogen *Sphaerulina*, seven novel species (*Sphaerulina farfugii, Sphaerulina hydrangeicola, Sphaerulina idesiae, Sphaerulina lapsanastri, Sphaerulina miurae, Sphaerulina styracis,* and *Sphaerulina viburnicola*) were identified, while two species (*Sphaerulina duchesneae* and *Sphaerulina namubana*) were proposed as new combinations. The study on *Septoria* highlighted that multiple species of pathogenic fungi from same genus, could exist in same species of host plant, proposing a novel species, *Septoria cannabicola*, isolated from hemp and found to be distinct from its American counterpart. Lastly, the investigation of the genus *Diaporthe* in asymptomatic Sakura trees resulted in the identification of two novel species, *D. endoprunicola* and *D. pseudoamygdali*.

As highlighted in this study, Japanese coelomycetes are rich and diverse, existing as pathogens or living endophytically within host plants. While the study enriches our understanding of Japanese coelomycetes diversity, caution is urged in introducing new species to prevent taxonomic destabilization. This study integrates traditional morphological observations in the identification of fungi along with molecular data to reveal the diversity of coelomycetes in Japan.

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List of publications

1. Anysia Hedy Ujat, Tsuyoshi Ono, Yukako Hattori, Chiharu Nakashima (2023) Re-Examination of Several *Elsinoë* Species Reported from Japan. Mycobiology, 51(3): 122-138.
2. Anysia Hedy Ujat, Yukako Hattori, Hayato Masuya, Abd Hadi Kamil Fatin Farhana, Chiharu Nakashima (2024) Diversity of Caulicolous species of the genus *Diaporthe* on *Prunus* sensu lato in Japan. Plant and Fungal Research. (Accepted on 19th December 2023).
3. Anysia Hedy Ujat, Shinju Konishi, Yurina Kato, Hana Tonami, Chiharu Nakashima. *Septoria cannabicola*, a new species on *Cannabis sativa* from Japan. Mycoscience. (Accepted on 17th January 2024)

The contents of Chapters 3 were submitted as follows.

1. Anysia Hedy Ujat, Chiharu Nakashima. Piecing Together The Taxonomic Puzzle of *Sphaerulina*. Fungal Systematics and Evolution. (Submitted on 11th October 2023)