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RESEARCH ARTICLE

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Re-Examination of Several *Elsinoë* Species Reported from Japan

Anysia Hedy Ujat[a], Tsuyoshi Ono[b], Yukako Hattori[c] and Chiharu Nakashima[a]

[a]Graduate School of Bioresources, Mie University, Tsu, Mie, Japan; [b]Tokyo Metropolitan Agriculture and Forestry Research Center, Tachikawa, Tokyo, Japan; [c]Department of Mushroom Science and Forest Microbiology, Forestry and Forest Product Research Institute, Tsukuba, Ibaraki, Japan

ABSTRACT

Elsinoë are plant pathogenic fungi that cause scabs, spotted anthracnose, and some morphological distortions on various plants, including woody plants, economically important crops, and ornamental plants. Taxonomical reexamination of *Elsinoë* species in Japan has not yet been conducted based on the modern species criteria. In this study, several Japanese isolates were reexamine based on the morphological and molecular-phylogenetic analysis of the internal transcribed spacer region (ITS), large subunit gene (LSU)m and protein-coding gene such as RNA polymerase II subunit (*rpb2)* and Translation elongation factor 1-alpha *(tef)*. Japanese isolates were divided into four clades and three new species, *Elsinoë hydrangeae*, *E. sumire,* and *E. tanashiensis* were proposed. One species, *Sphaceloma akebiae,* was transferred to the genus *Elsinoë*.

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1. Introduction

The genus *Elsinoë* (*Myriangiales*, *Ascomycota*) encompasses plant pathogenic fungi that cause 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 [1,2]. This genus was established by Raciborski [3] with the type species *Elsinoë canavaliae* Racib. Under the current code, the International Code of Nomenclature for Algae, Fungi, and Plants [4], 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 [5–8].

Scab is widely used as a disease name on leaves. Jenkins [9] 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 [2]. However, the symptoms caused by *Elsinoë* species is not limited to only necrotic lesion on leaves and stem. In some hosts, it distorts infected organs, such as twisting of infected stems of *Ipomea batatas* (L.) Lam. [10] and *Bidens* spp. [11], and elongation of the stem in *Manihot esculenta* Crantz [12]. 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 [13]. 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 (Figure 1(a,b)) and *Elsinoë australis* Bitanc. & Jenkins, and grape spot anthracnose by *Elsinoë ampelina* (de Bary) Shear (Figure 1(c)).

In the revision of *Elsinoë* taxonomy, a total of 79 species were accepted in the genus *Elsinoë* including new combinations transferred from the genus *Sphaceloma* [1,2,14]. In previous phylogenetic studies with multi genetic-loci, *Elsinoë* species appear to be host-specific fungus, as 77 out of 81 species are confined to only one host species or genus [2]. The identification of *Elsinoë* species is often difficult due to overlapping morphological characteristics, such as small conidia, similar conidiogenous cells, continuously expanding wide acervuli, and lacking fertile structure in nature [1]. Moreover, the establishment of pure culture was also challenging due to the slow growth of the isolates, and it is easily contaminated by other fungi [1]. On the other hand, 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 [1].

CONTACT Anysia Hedy Ujat adelia.anysia.hedy@gmail.com

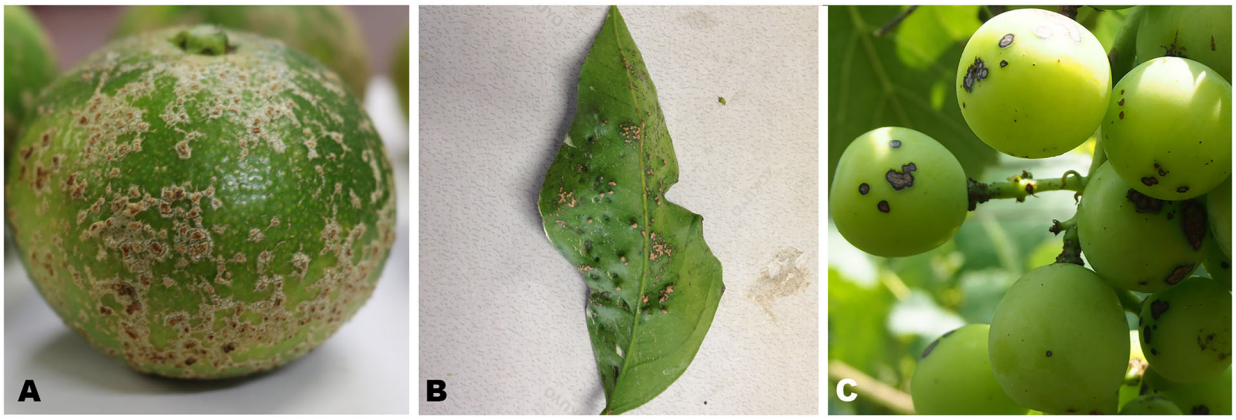

Figure 1. *Elsinoë* species causing citrus scab and anthracnose grape spot in Japan.

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 the Internal Transcriber Spacer (ITS) region of rDNA. As the advancement in molecular technique involves the usage of a polyphasic approach in species recognition by Consolidated Species Concept [15], a comprehensive study needs to be conducted to update the database of the Japanese isolates of *Elsinoë*. In this study, the isolates that were stored as *Elsinoë* and *Sphaceloma* in the culture collection of different research institutes in Japan were reexamined 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.

2. Materials and methods

2.1. Sample collection and morphological study

Specimens and culture were obtained from the National Agriculture and Food Research, Organization (MAFF, NARO), NITE Biological Resource Centre (NRBC), Herbarium of Forest Mycology and Pathology, Forestry and Forest Product Research Institute (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) and examined in the framework of morphological and molecular-phylogeny approaches. The isolates were obtained from symptomatic plants of various hosts (Table 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 °C for 21 days. Colony colors were described after 3 weeks according to the color chart by Rayner [16] Observation of specimens' collections (Table 2) were conducted by using the shear solution as a mounting medium.

2.2. 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 the manufacturer's instructions. 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 the final volume of 12.5 µL for all reaction was 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.

All PCR condition used in this study follows the previous study, where the PCR condition was as follows. For ITS and LSU [1]: Initial denaturation (95 °C, 2 min), 35 cycles of amplification (denaturation at 95 °C, 30 s; annealing at 48 °C, 1 min; extension at 72 °C, I minute), and final extension at 72 °C for 8 min; for *tef* [17]: Initial denaturation (95 °C, 2 min), 35 cycles of amplification (denaturation at 94 °C, 30 s; annealing at 60 °C, 1 min; extension at 72 °C, 2 min), and final extension at 72 °C for 10 min; for *rpb2*: Initial denaturation (95 °C, 5 min), touch-down amplification that consists of 5 cycles of 95 °C for 45 s, 56 °C for 45 s and 72 °C for 2 min, followed by 5 cycles of 95 °C for 45 s, 53 °C for 45 s and 72 °C for 2 min, and 30 cycles of 95 °C for 45 s, 50 °C for 45 s and 72 °C for 2 min, and final

Table 1. List of Japanese *Elsinoë* isolates used in this study.

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

elongation of 72 °C for 8 min. 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 Center (Tsu, Mie, Japan). Primer sets used in this study are summarized in Table 2.

2.2.1. Phylogenetic analyses

The resulting sequences were assembled and aligned with 81 sequences of *Elsinoë* retrieved from the previous study by Marin-Felix et al. [2]and Fan et al. [1] on MEGA X software package [18] (Table 3). This matrix was aligned by using MAFFT online version [19] and edited manually using AliView [20]. Maximum-likelihood (ML) and Bayesian inference (BI) analyses were used in this study to estimate the phylogenetic relationship of the samples. ModelTest-NG [21] was used to estimate the best substitution model for each gene for ML and BI analysis and ML analysis was performed by using RAxML-NG [22]. Tree strength was tested by bootstrap analysis of 1000 replication [23]. BI analysis was performed by using MrBayes 3.2 [24] to estimate the posterior probability (PP) of the tree topologies based on the Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) algorithm of four chains which run from a random tree for 150,000,000 generations with the 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 a burn-in phase of analysis and the PP was determined by the remaining tree. *Myriangium hispanicum* (CBS 327.33) was selected as an outgroup in all analyses and trees 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.

3. Results

3.1. 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, where the topologies of the generated tree from ML and BI analyses were congruent. Japanese isolates analyzed in this study formed four major clades with hitherto known or newly recognized species. These are clade 1 including *Elsinoë bidentis* (MUCC2983), clade 2 composed

Table 2. List of regions and primer set used in this study.

Region	Primer (forward)	Primer (reverse)	Annealing temperature
ITS	ITS5 (White et al. 1990)	ITS4 (White et al. 1990)	48 °C
LSU	LR0R (Rehner & Samuels, 1994)	LR5 (Vilgalys & Hester, 1990)	48 °C
RPB2	RPB2-5F (Sung et al. 2007)	fRPB2-7cR (Liu et al. 1999)	56 °C→ 53 °C→ 50 °C
TEF-1a	Elongation-1-F (Hyun et al. 2009)	Elongation-1-R (Hyun et al. 2009)	60 °C

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 genes 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 genes, LSU can give good resolution based on geographical distribution.

3.2. Taxonomy

Elsinoë akebiae (Kuros. & Katsuki) A.H. Ujat & C. Nakash., **comb. nov.,** Figure 3.

MycoBank no: MB847771

Basionym: *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 on long side, depressed in the center.

Asexual morph: Acervuli dark brown of 2–3 mm, 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. [25].

Cultural characteristic: On MA: colony cerebriform, straw to yellow, folded toward the center of the colony; reverse, pale luteous with folding toward the center is observable. On PDA: surface; covered with white short dense mycelia, a pale luteous margin that turns salmon over time; Reverse, pale luteous that turns olivaceous buff over time. On OMA: surface; olivaceous with short aerial mycelia with folding toward the center, agar color around the colony changed to olivaceous buff; Reverse, rust around the center with citrine growth margin with folding toward 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 [25]. The morphological characteristics of holotype specimen were 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 eliminate species of *E. akebiae* (MUCC2982), *E. bidentis* (MUCC2983), and *E. asclepiadis* (CPC 18544). A 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 4.

MycoBank no: MB847772

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

Table 3. Representative sequence used in this study.

Fungal species	Isolate number	Host	Country	Accession number			
				ITS	LSU	RPB2	TEF-1a
Elsinoë abutilonis	CBS 510.40	*Callianthe striata* (syn. *Abultilion striaum*)	Brazil	KX887185	KX886949	KX887068	KX886831
Elsinoë akebiae	**MUCC 2982**	***Akebia trifoliata***	**Japan**	**OQ504591**	**OQ504615**	**OQ472906**	**OQ472926**
Elsinoë ampelina	CBS 208.25	*Vitis vinifera*	Brazil	KX887186	KX886950	KX887069	KX886832
Elsinoë ampelina	**MUCC 2995**	***Vitis vinifera***	**Japan**	**OQ504587**	**OQ504611**	**OQ472902**	**OQ472922**
Elsinoë ampelina	**MUCC 2996**	***Vitis vinifera***	**Japan**	**OQ504588**	**OQ504612**	**OQ472903**	**OQ472923**
Elsinoë ampelina	**MUCC 2362**	***Vitis* sp.**	**Japan**	**OQ504589**	**OQ504613**	**OQ472904**	**OQ472924**
Elsinoë anacardia	CBS 470.62	*Anacardium occidentale*	India	KX887189	KX886953	KX887072	KX886835
Elsinoë annonae	CBS 228.64	*Annona* sp.	USA	KX887190	KX886954	KX887073	KX886836
Elsinoë arachidis	CBS 511.50	*Arachis hypogaea*	Brazil	KX887191	KX886955	KX887074	KX886837
Elsinoë araliae	**MUCC 2997**	***Aralia elata***	**Japan**	**OQ504590**	**OQ504614**	**OQ472905**	**OQ472925**
Elsinoë araliae	**MUCC 3490**	***Aralia cordata***	**Japan**	**OQ504586**	**OQ504610**	**OQ472901**	**OQ472921**
Elsinoë arrudai	CBS 220.50	*Tournefortia breviflora*	Brazil	KX887194	KX886958	KX887077	KX886840
Elsinoë asclepiadea	CPC 18544	*Asclepias mellodora* (syn. *A. curassavica*)	Brazil	KX887195	KX886959	KX887078	KX886841
Elsinoë australis	CBS 314.32	*Citrus aurantium*	Brazil	KX887198	KX886962	KX887081	KX886844
Elsinoë banksiicola	CBS 113734	*Banksia prionote*	Australia	KX887199	KX886963	KX887082	KX886845
Elsinoë barleriicola	CBS 471.62	*Barleria gibsonii*	India	KX887200	KX886964	KX887083	KX886846
Elsinoë bidentis	CBS 512.20	*Bidens pilosa*	Brazil	KX887201	KX886965	KX887084	KX886847
Elsinoë bidentis	**MUCC 2983**	***Bidens pilosa***	**Japan**	**OQ504595**	**OQ504619**	**OQ472910**	**OQ472930**
Elsinoë brasiliensis	CPC 18528	*Chamaesyce hyssopifolia*	Brazil	KX887204	N/A	KX887087	KX886850
Elsinoë caleae	CBS 221.50	*Calea pinnatifida*	Brazil	KX887205	KX886968	KX887088	KX886851
Elsinoë centrolobii	CBS 222.50	*Centrolobium robustum*	Brazil	KX887206	KX886969	KX887089	KX886852
Elsinoë citricola	CPC 18535 = RWB 1175	*Citrus limonia*	Brazil	KX887207	KX886970	KX887090	KX886853
Elsinoë corni	**MUCC 2998**	***Cornus florida***	**Japan**	**OQ504596**	**OQ504620**	**OQ472911**	**OQ472931**
Elsinoë coryli	CBS 275.76	*Corylus avellana*	France	KX887209	KX886972	KX887092	KX886855
Elsinoë diospyri	CBS 223.50	*Diospyros kaki*	Brazil	KX887210	KX886973	KX887093	KX886856
Elsinoë embeliae	CBS 472.62	*Embelia ribes*	India	KX887211	KX886974	N/A	KX886857
Elsinoë erythinae	CPC 18542	*Erythrina* sp.	Brazil	KX887214	KX886977	KX887096	KX886860
Elsinoë eucalypticola	CBS 124765	*Eucalyptus* sp.	Australia	KX887215	KX886978	KX887097	KX886861
Elsinoë eucalyptorum	CBS 120084	*Eucalyptus propinqua*	Australia	KX887216	KX886979	KX887098	KX886862
Elsinoë euphorbiae	CBS 401.63	*Euphorbia pariflora* (syn. *Euphorbia pilulifera*)	India	KX887217	KX886980	KX887099	KX886863
Elsinoë fagarae	CBS 514.50	*Fagara riedelianum*	Brazil	KX887218	KX886981	KX887100	KX886864
Elsinoë fawcettii	CBS 139.25	*Citrus* sp.	USA	KX887219	KX886982	KX887101	KX886885
Elsinoë fawcettii	**MUCC 2993**	***Citrus deliciosa* (syn. *Citrus unshiu*)**	**Japan**	**OQ504575**	**OQ504599**	**OQ472891**	**OQ472912**
Elsinoë fawcettii	**MUCC 2994**	***Citrus medica* (syn. *Citrus hassaku*)**	**Japan**	**OQ504577**	**OQ504601**	**OQ472893**	**OQ472914**
Elsinoë fawcettii	**MUCC 2999**	***Citrus deliciosa* (syn. *Citrus unshiu*)**	**Japan**	**OQ504578**	**OQ504602**	**OQ472894**	**OQ472915**
Elsinoë fawcettii	**MUCC 3000**	***Citrus deliciosa* (syn. *Citrus unshiu*)**	**Japan**	**OQ504576**	**OQ504600**	**OQ472892**	**OQ472913**
Elsinoë fici	CBS 515.50	*Ficus luschnathiana*	Brazil	KX887223	KX886986	KX887105	KX886869
Elsinoë fici-cariae	CBS 473.62	*Ficus caria*	India	KX887224	KX886987	KX887106	KX886870
Elsinoë flacourtiae	CBS 474.62	*Flacourtia sepiaria*	India	KX887225	KX886988	KX887107	KX886871
Elsinoë freyliniae	CBS 128204	*Freylinia lanceolata*	South Africa	KX887226	KX886989	KX887108	KX886872
Elsinoë genipae	CBS 342.39	*Genipa americana*	Brazil	KX887227	KX886990	KX887109	KX886873
Elsinoë genipae-americanae	CBS 516.50	*Genipa americana*	Brazil	KX887228	KX886991	KX887110	KX886874
Elsinoë glycines	CBS 389.64	*Glycine soja*	Japan	KX887229	KX886992	KX887111	KX886875
Elsinoë glycines	**MUCC 2984**	***Glycine soja***	**Japan**	**OQ504593**	**OQ504617**	**OQ472908**	**OQ472928**
Elsinoë glycines	**MUCC 2985**	***Glycine soja***	**Japan**	**OQ504594**	**OQ504618**	**OQ472909**	**OQ472929**
Elsinoë hederae	CBS 517.50	*Herdera helix*	Brazil	KX887231	KX886994	KX887113	KX886877
Elsinoë hydrangeae	**MUCC 2988**	***Hydrangea serrata***	**Japan**	**OQ504583**	**OQ504607**	**OQ472898**	N/A
Elsinoë ichnocarpi	CBS 475.62	*Ichnocarpus frustescencs*	India	KX887232	KX886995	KX887114	KX886878
Elsinoë jasminae	CBS 224.50	*Jasminum sambac*	Brazil	KX887233	KX886996	KX887115	KX886879
Elsinoë jasminicola	CBS 212.63	*Jasminum malabaricum*	India	KX887234	KX886997	N/A	KX886880
Elsinoë krugii	CPC 18531	*Euphorbia heterophylla*	Brazil	KX887235	KX886998	KX887116	KX886881
Elsinoë lagoa-santensis	CBS 518.50	*Brysonima coccolobifolia*	Brazil	KX887239	KX887002	KX887120	KX886885
Elsinoë ledi	CBS 167.33	*Rhododendron neoglandulosum (syn. Ledum glandulosum)*	USA	KX887240	KX887003	KX887121	KX886886
Elsinoë lepagei	CBS 225.50	*Manikara zapota* (syn. *Achras sapota*)	N/A	KX887241	KX887004	KX887122	N/A
Elsinoë leucospermi	CBS 111207	*Leucospermum* sp.	South Africa	KX887242	KX887005	KX887123	KX886887
Elsinoë lippiae	CBS 166.40	*Phyla lancelolata* (syn. *Lippia lanceolate*)	USA	KX887248	KX887011	KX887129	KX886893
Elsinoë mangiferae	CBS 226.50	*Mangifera foetida* (syn. *M. indica*)	Cuba	KX887249	KX887012	KX887130	KX886894
Elsinoë mattiroloanum	CBS 287.64	*Arbutus unedo*	Argentina	KX887250	KX887013	KX887131	KX886895
Elsinoë menthane	CBS 322.37	*Mentha piperita*	USA	KX887253	KX887016	KX887134	KX886898
Elsinoë mimosae	CPC 19478	*Mimosa invisa*	Brazil	KX887255	KX887018	KX887136	KX886900
Elsinoë oleae	CBS 227.59	*Olea europaea*	Italy	KX887256	KX887019	KX887137	KX886901
Elsinoë othonnae	CBS 139910	*Othonna quinquedentate*	South Africa	KR476726	N/A	MK540083	N/A
Elsinoë perseae	CBS 406.34	*Persea americana*	USA	KX887258	KX887021	KX887139	KX886903
Elsinoë phaseoli	CBS 165.31	*Phaseokus lunatus*	Cuba	KX887263	KX887026	KX887144	KX886908
Elsinoë piri	CBS 163.29	*Pyrus communis*	N/A	KX887267	KX887030	KX887148	KX886912
Elsinoë pitangae	CBS 227.50	*Eugenia pitanga*	Brazil	KX887269	KX887032	KX887150	KX886914
Elsinoë poinsettiae	CBS 109333	*Eugenia pulcherrima*	Guatemala	KX887270	KX887033	KX887151	KX886915
Elsinoë pongamiae	CBS 402.63	*Pongamia pinnata*	India	KX887272	KX887035	KX887153	KX886917
Elsinoë populi	CBS 298.64	*Populus deltoides* subsp. *deltoides* (syn. *P. serotine*)	Argentina	KX887273	KX887036	KX887154	KX886918
Elsinoë protearum	CBS 113618	*Protea* sp.	Zimbabwe	KX887275	KX887038	KX887156	KX886920
Elsinoë punicae	CPC 19968	*Punica granatum*	South Africa	KX887276	KX887039	KX887157	KX886921

(*continued*)

Table 3. Continued.

Fungal species	Isolate number	Host	Country	Accession number			
				ITS	LSU	RPB2	TEF-1a
Elsinoë quercus-illcis	CBS 232.61	*Quercus ilex*	Italy	KX887277	KX887040	N/A	KX886922
Elsinoë randii	CBS 170.38	*Carya* sp.	Brazil	KX887278	KX887041	KX887158	KX886923
Elsinoë rhois	CBS 519.20	*Toxicodendron vernix* (syn. *Rhus vernix*)	Brazil	KX887280	KX887043	KX887160	KX886925
Elsinoë ricini	CBS 403.63 = ATCC15030	*Ricinus communis*	India	KX887281	KX887044	KX887161	KX886926
Elsinoë rosarum	CBS 212.33	*Rosa* sp.	USA	KX887283	KX887046	KX887163	KX886928
Elsinoë salicina	CPC 17824	*Salix* sp.	USA	KX887286	KX887049	KX887166	KX886931
Elsinoë samecarpi	CBS 477.62	*Melanichyla caesia* (syn. *Semecarpus anacardium*)	India	KX887287	KX887050	KX887167	KX886932
Elsinoë sesseae	CPC 18549	*Ceatrum laevigatum?*	Brazil	KX887288	KX887051	KX887168	KX886933
Elsinoë sicula	CBS 398.59	*Prunus amygdalus*	Italy	KX887289	KX887052	KX887169	KX886934
Elsinoë soludagnis	CBS 191.37	*Solidago fistulosa*	USA	KX887290	KX887053	KX887170	KX886935
Elsinoë sumire	**MUCC 2992**	***Viola* sp.**	**Japan**	**OQ504585**	**OQ504609**	**OQ472900**	**OQ472920**
***Elsinoë* sp.**	**MUCC 2986**	***Amphicarpaea edgeworthii***	**Japan**	**OQ504592**	**OQ504616**	**OQ472907**	**OQ472927**
Elsinoë tanashiensis	**MUCC 3463**	***Juglans* sp.**	**Japan**	**OQ504581**	**OQ504605**	N/A	N/A
Elsinoë tanashiensis	**MUCC 3466**	***Populus* sp.**	**Japan**	**OQ504582**	**OQ504606**	**OQ472897**	**OQ472918**
Elsinoë tectificae	CBS 124777 = CPC 14594		Australia	KX887292	KX887055	KX887172	KX886937
Elsinoë terminaliae	CBS 343.39	*Terminalia catappa*	Brazil	KX887293	KX887056	KX887173	N/A
Elsinoë theae	CBS 228.50	*Cemellia sinensis* (syn. *Thea sinensis*)	Brazil	KX887295	KX887058	KX887175	KX886939
Elsinoë tiliae	CBS 350.73 = ATCC 24510	*Tilia cordata*	New Zealand	KX887296	KX887059	KX887176	KX886940
Elsinoë tiliae	**MUCC 2989**	***Tilia playtphyllos***	**Japan**	**OQ504579**	**OQ504603**	**OQ472895**	**OQ472916**
Elsinoë tiliae	**MUCC 2990**	***Tilia europaea***	**Japan**	**OQ504580**	**OQ504604**	**OQ472896**	**OQ472917**
Elsinoë veneta	CBS 164.29 = ATCC 1833	*Rubus* sp.	N/A	KX887297	KX887060	KX887177	KX886941
Elsinoë verbenae	CPC 18561 = RWB 1232	*Verbena bonariensis*	Brazil	KX887298	KX887061	KX887178	KX886942
Elsinoë violae	CBS 336.35	*Viola* sp.	USA	KX887302	KX887065	KX887182	KX886946
Elsinoë zizyphi	CBS 378.62 = STCC 14656	*Zizyphus rugisa*	India	KX887303	KX887066	KX887183	KX886947
Sphaceloma tsujii	**MUCC 2991**	***Paulownia tomentosa***	**Japan**	**OQ504584**	**OQ504608**	**OQ472899**	**OQ472919**
Myriangium hispanicum	CBS 247.33	*Acer monspessulanum*	N/A	KX887304	KX887067	KX887184	KX886948

Isolates used in this study mark in boldface.

Leaf spot pale yellow to purple slate with brick margin, circular, 2 mm in diameter on the leaves, along the leaves vein, expanded toward adaxial surface and coalescent in a chain-like pattern, becoming grayish white in the middle, petiole and stems crinkled, often observed on newly developed leaves. Brown lesions with pale yellow scabs 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.

Cultural characteristic: On MA: surface raised, cerebriform, erumpent, salmon to sienna; reverse, red with white margin. On PDA: surface; erumpent and folded toward the center, yellow to salmon, sparse white aerial mycelium, reverse: dark red and folded in toward 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. [26] 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 the 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. [26] by using rDNA ITS region shows that *E. hydrangeae* was closely related to *E. araliae*. As shown in Figure 1, although *E. hydrangeae* and *E. araliae* were placed in the same clade, the clade is 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.

Elsinoë sumire T. Ono, A. H. Ujat & C. Nakashima, **sp. nov.**, Figure 5.

MycoBank no: MB847783

Etymology: The name is derived from the generic name of *Viola* sp. in Japan, which means violet.

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.

Cultural characteristic: On MA: colony surface slightly raised, smooth, salmon with a white margin; aerial mycelia short and dense; reverse, red with a 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 toward the center. On OMA:

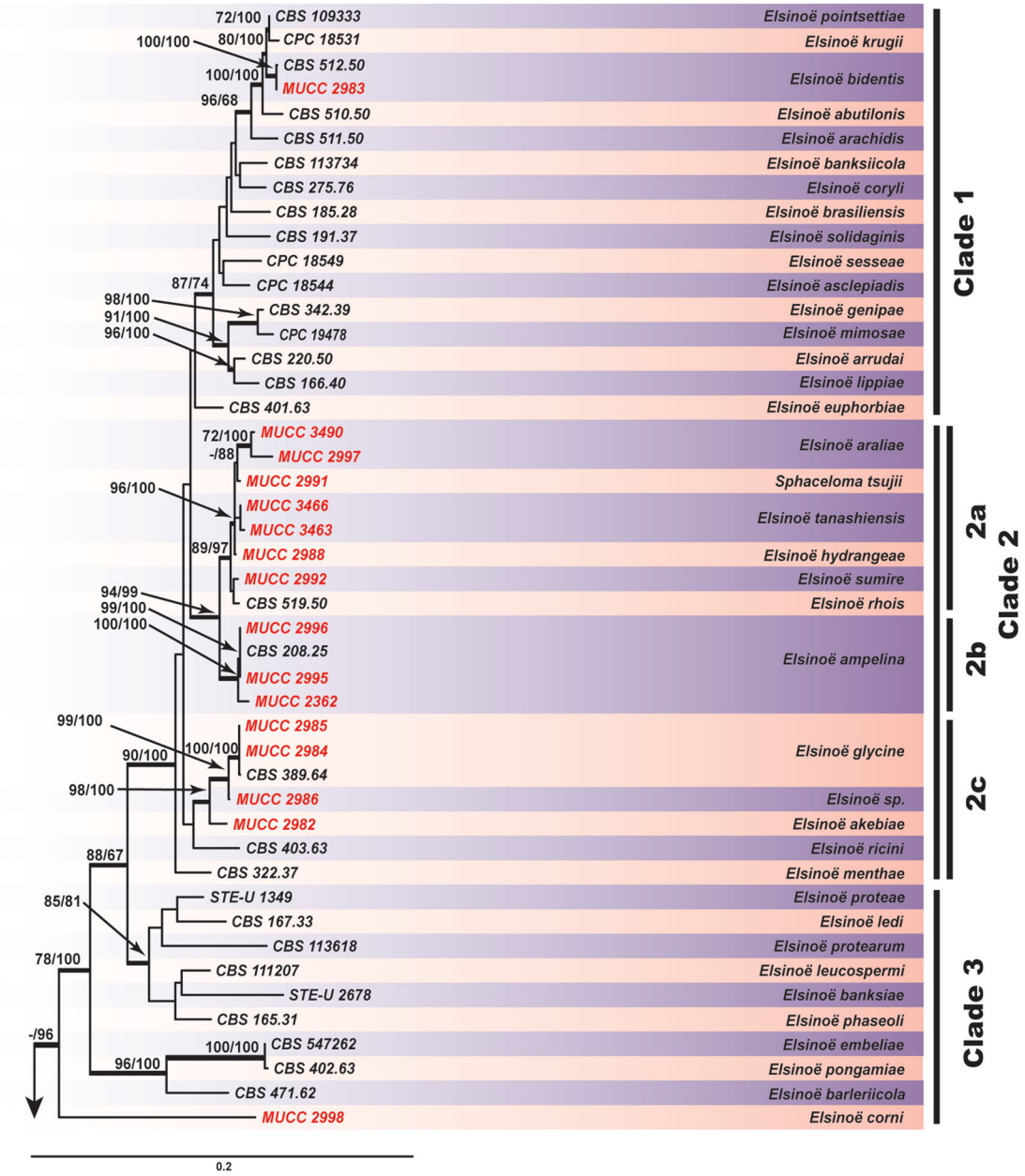

Figure 2. Phylogenetic tree of *Elsinoë* spp. constructed by ML using combined ITS, LSU, *rpb2*, and *tef* gene sequence datasets. ML bootstrap values and Bayesian PPs are given near branches (ML/PP).

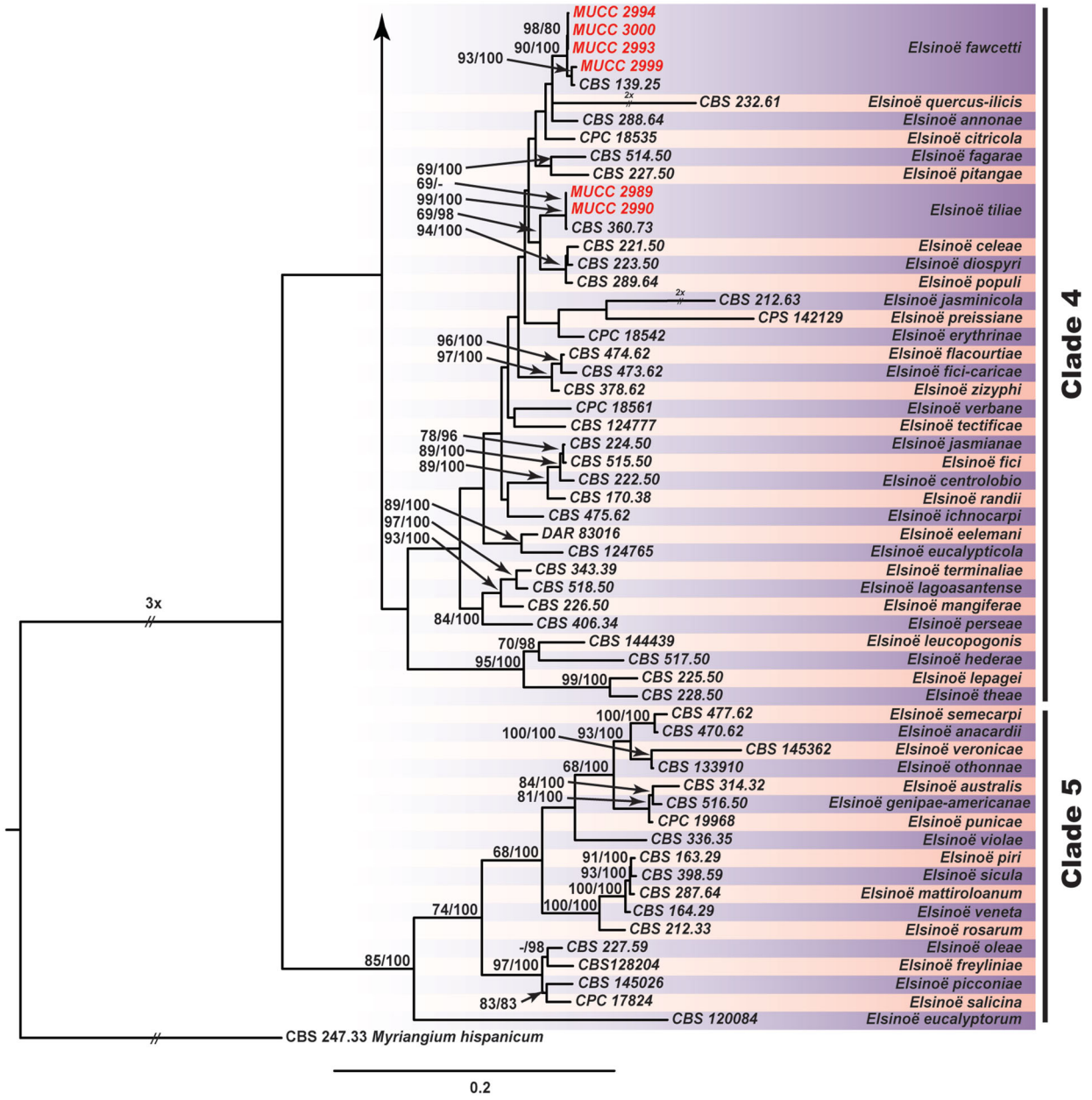

Figure 2. Continued.

surface; smooth, bay to rust, and covered by white arial mycelia; reverse, red and folded with cracks forming toward 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, at 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, [27] 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 with 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 [28], the species habitats were mainly from North America and Europe, and on more than 15 *Viola* species. On the

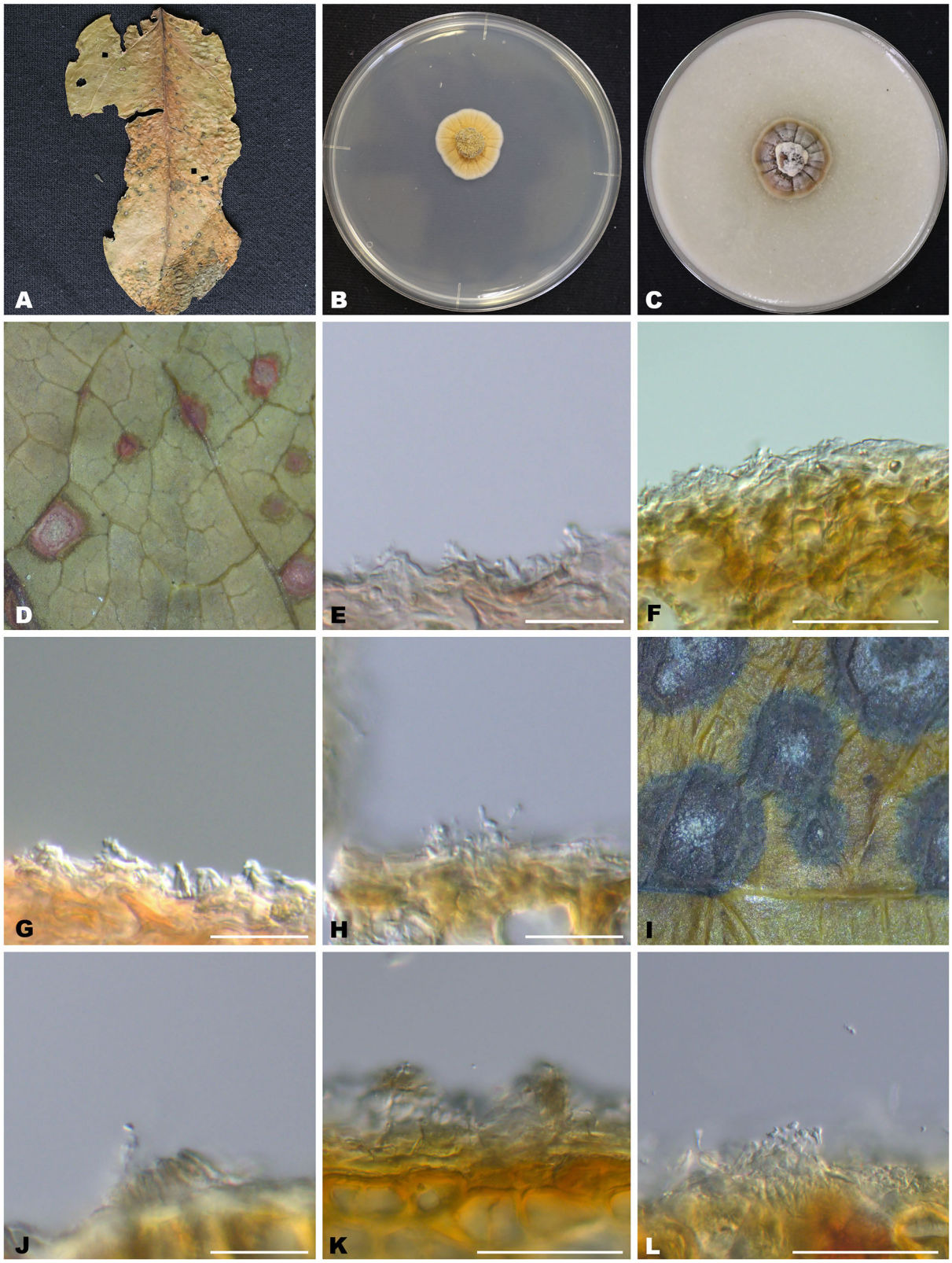

Figure 3. Morphological feature of *Elsinoë akebiae* [A, D, F–H: TNS-F-99468; B–C, E: TSU-MUMH11977 (MUCC2982); I-L: TNS-F-99471]. (A) Specimen TNS-F-99468. (B–C) Isolate MUCC2982 on MA (B) and OMA (C). (D) Symptom of scab forming on the leaf of *Akebia trifoliata*. (E–G) Acervuli. (H) Conidia. (I) Symptom of scab forming on the leaf of *Stauntonia hexaphylla*. (J–K) Acervuli. (L) Conidia. Scale bars, 25 μm (F–H) and 50 μm (J–L).

other hand, Kurosawa and Katsuki [25] 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), *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

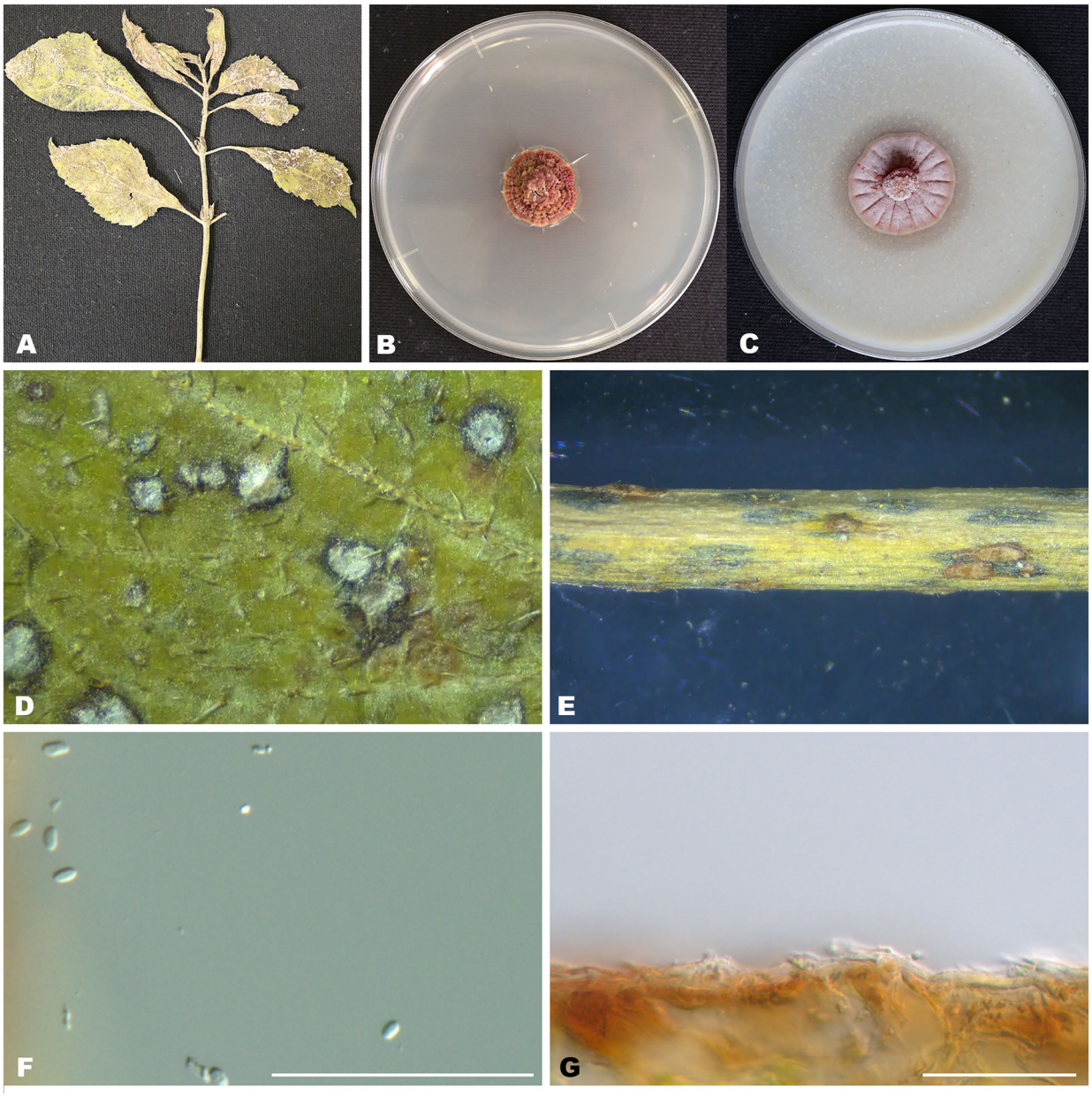

Figure 4. Morphological feature of *Elsinoë hydrangeae* [A–G: TSU-MUMH11976 (MUCC2988)]. (A, D–E) Specimen TSU-MUMH11976. (B–C) Isolate MUCC2988 on MA (B) and OMA (C). (F) Conidia. (G) Acervuli. Scale bars, 50 µm.

symptoms on the host plant and the micromorphology varies greatly.

Elsinoë tanashiensis A.H. Ujat & C. Nakashima, **sp. nov.**, Figure 6.

MycoBank no: MB847784

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

On the leaf's surface, lesions are observed as both black and white spots. Black spots, scattered, circular, depressed, and turned into grayish brown at the center, or developed as white spots and raised, scabbed in numerous on the 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 were observed on the lesion with grayish brown scab under humid conditions, and pale orange to brown mucilaginous masses observed on the 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, 70–200 mm. Conidia hyaline, aseptate, globular, ellipsoid to irregular, 4–5.5 × 1.5 µm.

Cultural characteristic: On MA: colony surface with short dense aerial mycelia, salmon, folded toward center of the 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

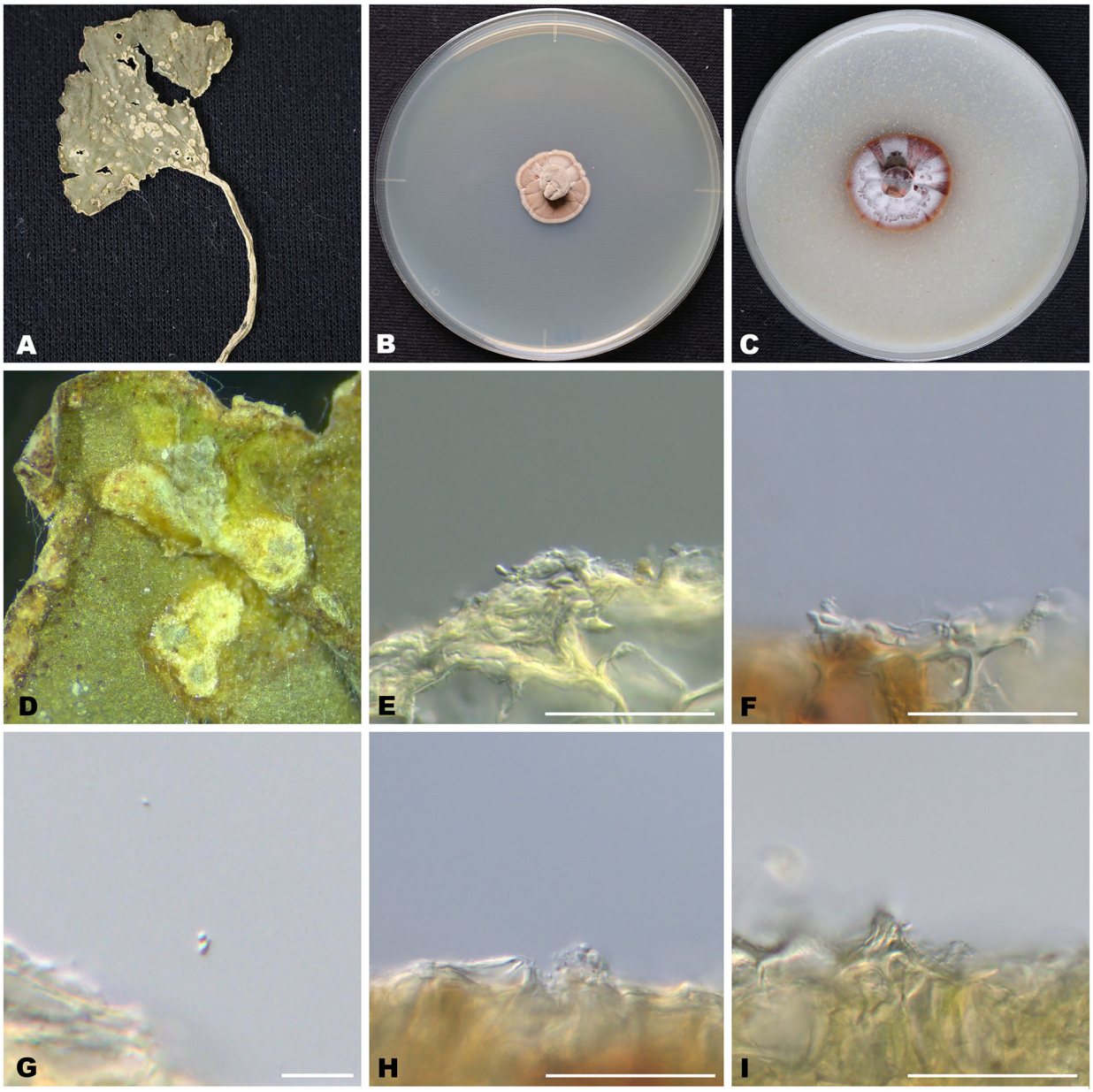

Figure 5. Morphological feature of *Elsinoë sumire* [A–I: TSU-MUMH11977 (MUCC2992)]. (A, D) Specimen TSU-MUMH11976. (B–C) Isolate MUCC2992 on MA (B) and OMA (C). (E–F) Conidiogenous cell. (G) Conidia. (H–I) Acervuli. Scale bars, 100 µm (E–F, H–I) and 10 µm (G).

mycelia with rings, agar around the colony changed to amber; Reverse, amber to umber with folding toward 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)

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* [29]

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 [29] as the morphological description is different from *E. populi* described by Jenkins [30]. ITS region of MUCC3466 is identical with MUCC3463.

Elsinoë tanashiensis isolated from *Populus deltoides* from Japan is closely related to *Elsinoë* isolated from *Juglans* spp. Based on the phylogenetic analysis, ITS region is identical, while LSU gene are 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* sp. on herbarium specimens are identical, whereby the size and shape of the conidiogenous cell is also same. Due to the similarity of the conidiogenous cell and conidia size, these two species could not be determined as different species although they infect different host plants.

In this study, novel species of *Elsinoë 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 [1] and Europe, [30], the first record of occurrence in Japan was only recorded as "*Sphaceloma* sp." on *Populus* [29]. It was mentioned in the previous study by Chiba and Kobayashi [29] that this fungus could not be identified as *Sphaceloma populi* due to the difference 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 the *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 [31] and *Juglans* sp. in North and South America [32] and on *Juglans regia* L. and *Juglans mandshurica* Maxim. [33], this study shows that *E. randii* are placed in a different clade compared to *E. tanashiensis*.

***Elsinoë* sp.,** Figure 7.

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

Asexual morph: Acervuli hyaline, coalescing, compact, up to 100 µm wide. Conidiogenous cells hyaline, erumpent, cylindrical to spindle-shape, monophialidic, sporulating enteroblastically, integrated, 15–20 mm. 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 [34]. 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 the 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*.

Sphaceloma tsujii Hara, Figure 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 grayish red, slightly concave, eventually perforated. The abaxial symptoms were 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 color, but later turn grey and fall into a blotch [35].

Asexual morph: Mycelium branched with septate, colorless, 3–4 um width, acervuli scattered, subcuticular subsequently erumpent, 30–60 µm in diameter. Conidiogenous cell cylindrical, hyaline, monophialidic, 5–13.2 µm [35].

Cultural characteristic: On MA: colony surface cerebriform, salmon; reverse, bay to flesh. On PDA: surface; cerebriform, folded toward center, rust with buff margin; reverse; brick to cinnamon, folded toward center. On OMA: surface; amber, covered by white short dense arial mycelia folded toward center; Reverse, straw with folding toward 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 *Sphaceloma tsujii* (MUCC2991) and *Elsinoë tanashiensis* (MUCC3466). Initially described as *Gloeosporium sp.* on *Paulownia sp*. [36], *Sphaceloma tsujii* was proposed as a new species by Hara [35] 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.

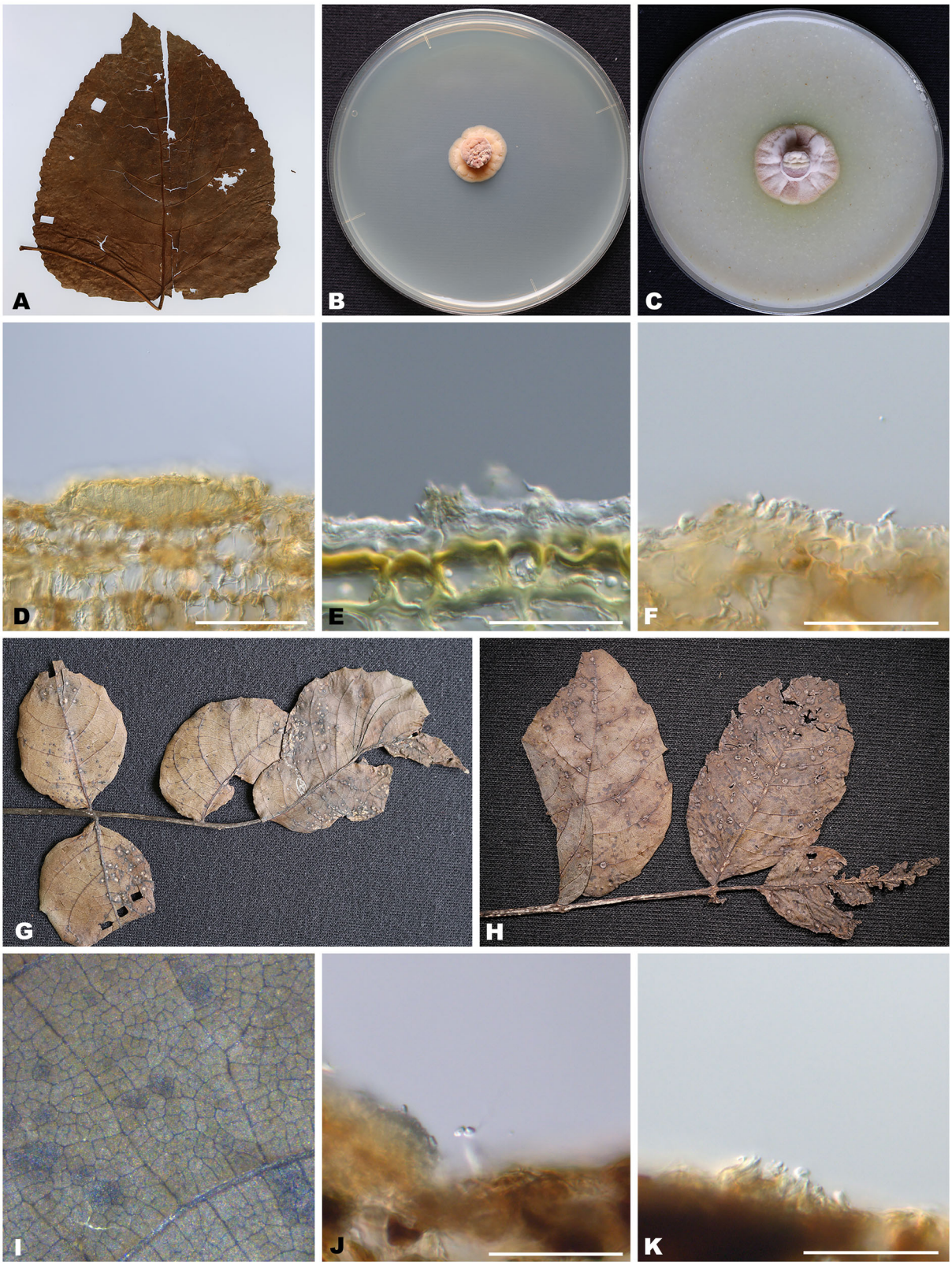

Figure 6. Morphological feature of *Elsinoë tanashiensis* on *Populus deltoides* [A–F: TFM:FPH-01697 (MUCC3466)] and *Juglans* sp. [G, J–K: TFM:FPH-0330; H–I: TNS-F-185379]. (A) Specimen TFM:FPH-01697. (B–C) Isolate MUCC3466 on MA (B) and OMA (C). (D–E) Acervuli. (F) Conidiogenous cell. (G) Specimen 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).

3.3. Taxonomical treatment based on the herbarium specimens

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

Basionym: *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).

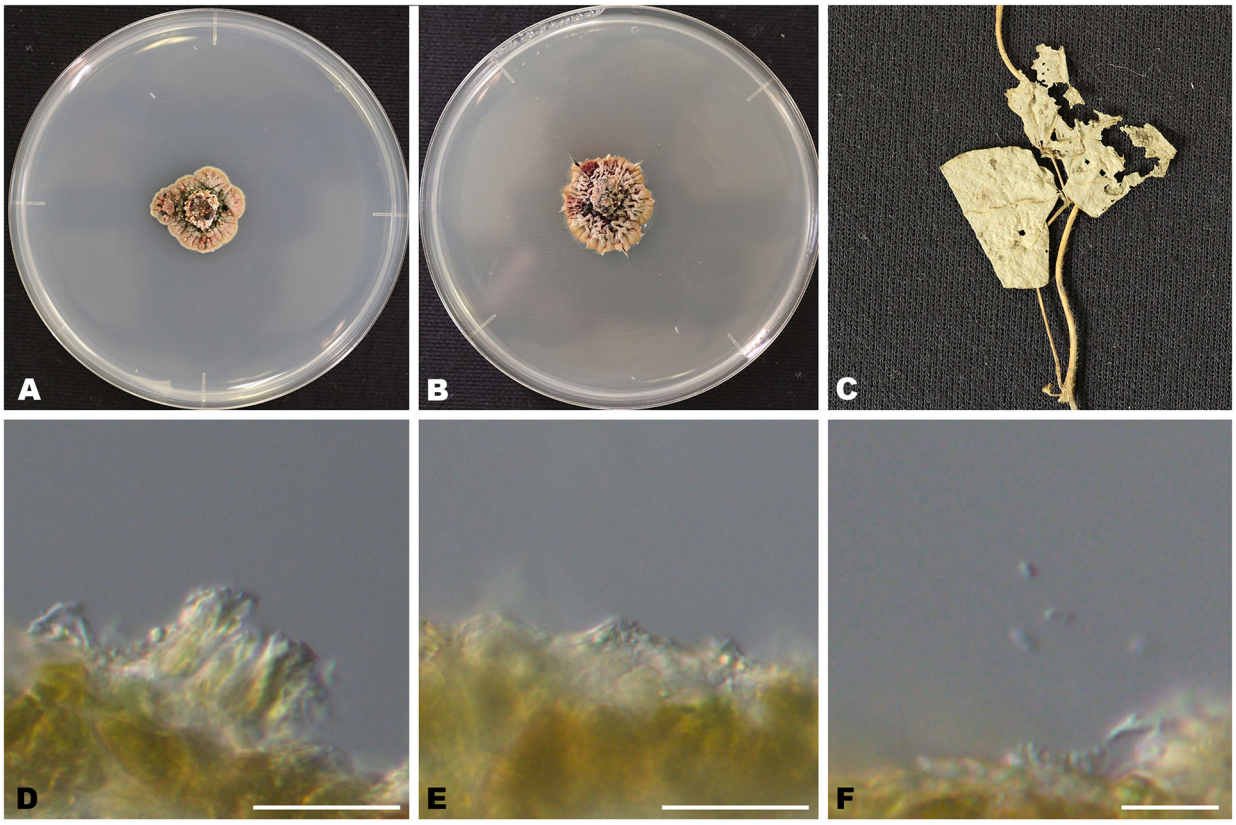

Figure 7. Morphological feature of *Elsinoë* sp. on *Amphicarpaea edgeworthii* [A–B: MUCC2986; C–F: TNS-F-185385] (A–B) Isolate MUCC2986 on MA (A) and PDA (B). (C) Specimen TNS-F-185385. (D) Conidiogenous cell. (E) Acervuli. (F) Conidia. (I) Acervuli. Scale bars, 50 µm (D–E) and 10 µm (F).

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 [33].

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

Basionym: *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 [25].

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

Basionym: *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 [37].

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

Basionym: *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 [33].

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

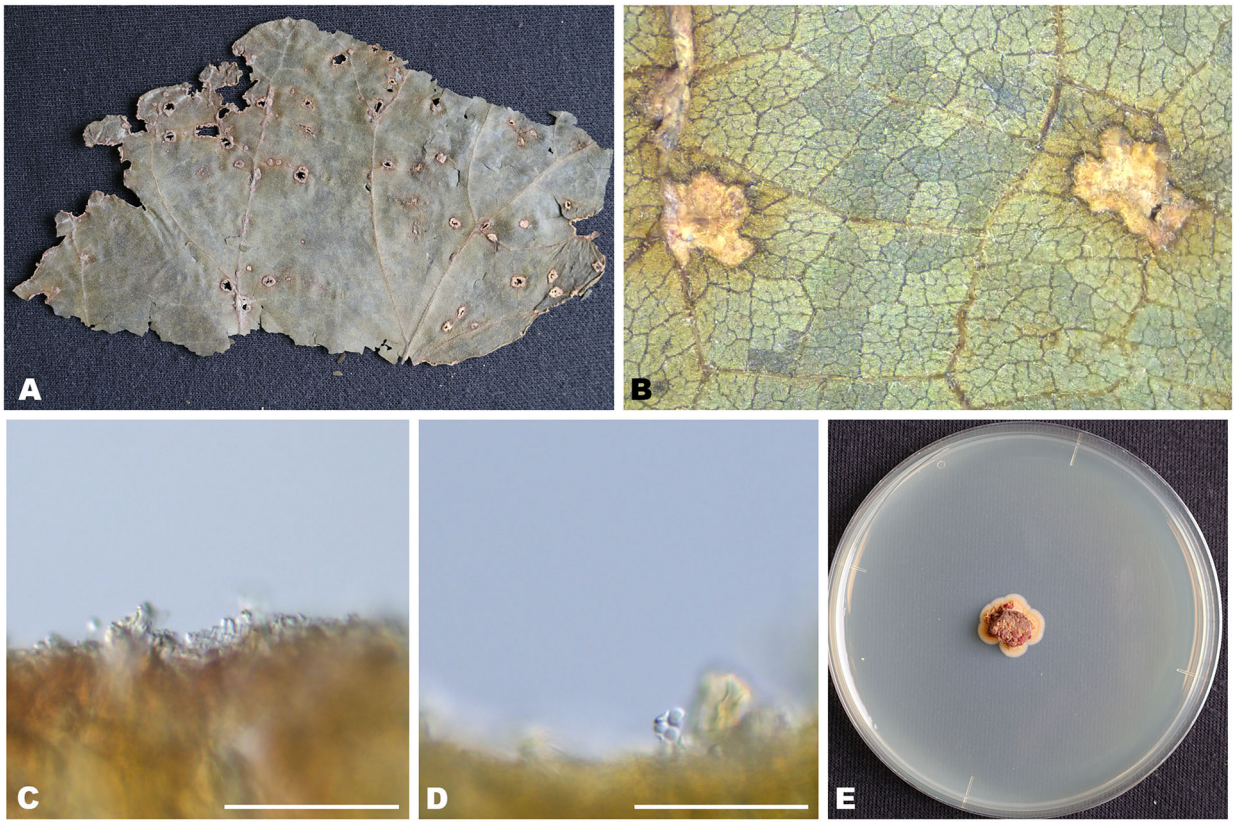

Figure 8. Morphological feature of *Sphaceloma tsujii* on *Paulownia tomentosa* [A–E: TFM:FPH-0448. (A) Specimen TFM:FPH-01697. (B) Conidiomata on leaf of *Paulownia tomentosa*. (D) Acervuli. (E) Conidia. (F) Isolate MUCC2991 on MA. Scale bars, 50 µm.

Basionym: *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 [33].

4. 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* [1,2]. 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) (Figure 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 hypothesized 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 placed in different subclade, even though they have a common host plant of genus *Populus*. Similar examples 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 obtained the host plant at different places independently. *Elsinoë corni*, isolated from *Cornus florida* in Japan are placed in Clade 3 as an independent species. In this study, morphological characteristic and the phylogenetic analysis comparison could not be conducted as there are no specimens linked to the isolate used in this study (MUCC2998) and no phylogenetic analysis were conducted on the holotypus material by Jenkins & Bitancourt [38].

Conversely, several species, widely distributed worldwide and having similar sequences on those generic loci regardless of the quite far geographical origin, were known in the previous study [1,2,17], and some are reconfirmed in this study, too. These are *E. ampelina*, *E. bidentis* (Bitanc. & Jenkins) Fan & Crous, *E. fawcettii* Bitanc. & Jenkins and *E. tiliae* Creelman. On *Elsinoë* species infecting Japanese citrus cultivar, only *E. fawcetti* was identified and not *E. australis* or *E. citricola*. Although *E. australis* is a phytopathogens of citrus, it remains restricted to Australia, Bolivia, Brazil, and Ethiopia [1]. *E. citricola*, on the other hand, was initially identified as *Sphaceloma fawcettii,* however it can be distinguished based on molecular data on *rpb2* and *tef* region even though ITS and LSU region failed to distinguish between *E. fawcetti* and *E. citricola* [1]. 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 cropped or distributed around the world. From this situation, it is suggested that a specific strain of *Elsinoë* species has been spreading with the migration of the host plant.

Generally, each *Elsinoë* species has a narrow host range, which occurs on only one host species or genus [1,2]. 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. [1] 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. On the other hand, our results suggested that *E. sumire* is a closely related species of *E. rhois* (Bitanc. & Jenkins) X.L. Fan & Crous on *Rhus vernix* L. (syn. *Toxicodendron vernix* Kuntze). According to Fan et al. [1], *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 cites changes of *tef* region sequence. In this study, the criteria of species delimitation of the genus *Elsinoë* were comprehensively judged based on the host plants, morphological characteristics, and phylogenetic relationship and did not synonymize the hitherto known species. Future discussion about *Elsinoë* species delimitation based on experimental host range or new barcode region sequence of this genus is needed.

In conclusion, the result of this study shows that many of the Japanese isolates are confined into one main clade which shows that Japanese isolates of *Elsinoë* could be endemic to East Asia. This study concurs with the previous study by Fan et al. [1] mentioning that *Elsinoë* species appear to be host specific. Although in our study there are lack of fresh specimens and isolates, type material and isolates designated from the previous study provide to be helpful in creating a workable taxonomy for Japanese isolates of *Elsinoë*.

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ORCID

Anysia Hedy Ujat http://orcid.org/0000-0002-1236-7622
Tsuyoshi Ono http://orcid.org/0000-0003-4560-1902
Yukako Hattori http://orcid.org/0000-0002-1908-4182
Chiharu Nakashima http://orcid.org/0000-0002-7626-5289

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