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Diversity of caulicolous species of the genus *Diaporthe* on *Prunus sensu lato* in Japan

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Abstract: The genus *Diaporthe* Fuckel consists of a large number of species recorded as pathogens, saprobes, and endophytes. While more than 1200 species of *Diaporthe* have been recorded to date, only pathogenic strains of *Diaporthe* were reported from Japan, notably affecting economically important plants. This study successfully elucidated the diversity of endophytic *Diaporthe* spp. from *Prunus sensu lato* (*s.l.*), commonly known as the Sakura tree, along with the addition of two novel species of *Diaporthe* to the Japanese mycobiota. In this study, a polyphasic approach was employed, 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) genes. Additionally, morphological observation was also conducted by inducing sporulation of the isolates in artificial media. As a result, two novel species were introduced: *Diaporthe endoprunicola* A.H. Ujat & Y. Hattori and *Diaporthe pseudoamygdali* A.H. Ujat & Y. Hattori, along with confirmation of previously documented *Diaporthe* species in *Prunus s.l.*

Keywords: *Diaporthaceae, multi-locus phylogeny, new taxa, taxonomy, systematics*

INTRODUCTION

Species of the genus *Diaporthe* Fuckel (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]. Hongsanan et al., [2023] published an annotated list of *Diaporthe* species, which includes 832 species and information on 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, 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]. V. Guarnaccia and P.W. Crous [2017] revealed that, through inoculation tests, most *Citrus* L. species are susceptible to *Diaporthe* species, such as *D. baccae* L. Lombard, G. Polizzi & Crous and *D. novem* J.M. Santos, Vrandečić & A.J.L. Phillips. The abundance of *Diaporthe* species identified leads to the proposal of some species comprised into species complexes, such as *D. eres* Nitschke [Hilário et al., 2021a; Udayanga et al., 2014], *D. amygdali* (Delacr.) Udayanga, Crous & K.D. Hyde [Hilário et al., 2021b], *D. sojae* Lehman [Udayanga et al., 2015], and *D. areacae* (H.C. Srivast., Zakia & Govindar.) R.R. Gomes, Glienke & Crous [Pereira et al., 2023]. This taxonomical treatment prompts the recommendation of the 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 based on the phylogeny were synonymized under *D. eres* as the

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result of the re-examination of the taxonomical position of *Diaporthe* species.

Diaporthales are known to be the causal agent of branch dieback and trunk disease of *Prunus s.l.*, often reported as endophytes, saprobes or phytopathogens. This includes the genus *Diaporthe* [Abramczyk et al., 2022; Bien & Damm, 2020; Nekrasov et al., 2022] and *Cytospora* Ehrenb. [Fan et al., 2020]. According to V. 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* (L.) L.) and peach (*P. persica* (L.) Batsch). E.V. Nekrasov et al., [2022] also reported the occurrence of *D. eres* in *P. mandshurica* (Maxim.) Koehne. In addition, numerous *Diaporthe* species have been recorded on *Prunus s.l.*, such as *D. amygdali* on *P. dulcis* D.A.Webb [Guarnaccia et al., 2022] and on *P. persica* [Sessa et al., 2017], *D. foeniculina* (Sacc.) Udayanga & Castl. on *P. amygdalus* [Gomes et al., 2013] and *P. dulcis* [Guarnaccia et al., 2022], *D. hongkongensis* R.R. Gomes, Glienke & Crous on *P. persica* [Zhang et al., 2021], *D. jinxiu* X.H. Wang & G.P. Wang on *P. persica* [Wang et al., 2021], *D. mahothocarpus* (Y.H. Gao, W. Sun & L. Cai) Y.H. Gao & L. Cai on *P. avium* [Bien & Damm, 2020], *D. momicola* Dissan., J.Y. Yan, X.H. Li & K.D. Hyde on *P. persica* [Dissanayake et al., 2017b], *D. novem* on *P. dulcis* [Hongsanan et al., 2023], *D. oligocarpa* Nitschke on *P. spinosa* Walter [Hongsanan et al., 2023], *D. oxe* R.R. Gomes, Glienke & Crous on *P. persica* [Sessa et al., 2017] and *P. dulcis* [Gomes et al., 2013], *D. padicola* Petr. on *P. padus* L. [Petrak 1916], *D. paranensis* R.R. Gomes, Glienke & Crous on *P. persica* [Gomes et al., 2013], *D. pardalota* (Mont.) Nitschke ex Fuckel on *P. divaricata* Ledeb. [Hongsanan et al., 2023], *D. pennsylvanica* (Berk. & M.A. Curtis) Wehm. on *P. pensylvanica* L.f., *P. serotina* Ehrh. and *Prunus* sp. [Hongsanan et al., 2023], *D. perniciosa* Marchal & É.J. Marchal on *P. cerasus* L. [Hongsanan et al., 2023], *D. rudis* (Fr.) Nitschke on *P. avium* and *P. salicina* Lindl. [Guarnaccia et al., 2023], *D. taoicola* Dissan., J.Y. Yan, X.H. Li & K.D. Hyde 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). Hattori et al., [2022] in their recent study sampled 48 individual of *Prunus s.l.* from six prefectures in Japan and managed to isolate 377 endophytic fungi with *Diaporthe* making up 32% of the total isolates. This study describes the morphological and molecular characteristics and evaluates the diversity of *Diaporthe* species isolated from intact branches of *Prunus s.l.* in Japan.

MATERIAL AND METHODS

Fungal isolate collection and morphological observation. In 2022, field surveys were conducted to collect *Diaporthe* species associated with the plant genus *Prunus* spp. in Japan. 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 then 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, 1940] as the mounting medium. The colonies' colour was assessed using a colour chart by Rayner [1970], and the isolates were deposited into the Mie University Culture Collection (MUCC).

DNA extraction and PCR amplification. 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 unit 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 are listed in Table 1. Amplicons were analysed in both directions

Table 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]
TUB	T1 or Bt2a/Bt2b	55 °C	O'Donnell & Cigelnik [1997]

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.

Phylogenetic analysis. The resulting sequences were assembled and aligned with sequences retrieved from NCBI DNA GenBank, as indicated in Table 2 and the supplementary table. 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 the MAFFT online service [Katoh et al., 2018]. 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 the Metropolis Coupled Monte Carlo Markov Chain (MCMCMC) option. The convergence was judged when the Average Standard Deviation of

Table 2. List of reference sequence used in this study.

Species	Isolates	ITS	TUB	H3	TEF1-a	CAL
1	2	3	4	5	6	7
Diaporthe absenteum	LC 3924^T	KP267897	KP293477	KP293547	KP267971	NA
D. acaciigena	CBS 129521^T	KC343005	KC343973	KC343489	KC343731	KC343247
D. acerigena	CFCC 52554^T	MH121489	–	MH121449	MH121531	MH121413
D. actinidiae	ICMP 13683^T	KC145886	–	–	KC145941	–
D. aestuarium	BRIP 59930a^T	OM918686	OM960613	–	OM960595	–
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. 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**

1	2	3	4	5	6	7
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. araucanorum	CBS 145285^{T}	MN509711	MN509722	–	MN509733	MN974277
D. asheicola	CBS 136967^{T}	KJ160562	KJ160518	–	KJ160594	KJ160542
D. australafricana	CBS 111886^{T}	KC343038	KC344006	KC343522	KC343764	KC343280
D. batatas	CBS 122.21	KC343040	KC344008	KC343524	KC343766	KC343282
D. bauhiniae	CFCC 53071^{T}	MK432648	MK578051	MK442995	MK578124	MK442970
D. beckhausii	CBS 138.27	KC343041	KC344009	KC343525	KC343767	KC343283
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. betulina	CFCC 52560^{T}	MH121497	MH121579	MH121457	MH121539	MH121421
D. bicincta	CBS 121004^{T}	KC343134	KC344102	KC343618	KC343860	KC343376
D. breyniae	CBS 148910^{T}	ON400846	ON409186	ON409187	ON409188	ON409189
D. brumptoniae	BRIP 59403a^{T}	OM918702	OM960629	–	OM960611	–
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. charlesworthii	BRIP 54884m^{T}	KJ197288	KJ197268	–	KJ197250	–
D. chongqingensis	PSCG 435^{T}	MK626916	MK691321	MK726257	MK654866	MK691209
D. chromolaenae	MFLUCC 17-1422^{T}	MH094275	–	–	–	–
D. citri	CBS 135422^{T}	KC843311	KC843187	MF418281	KC843071	KC843157
D. collariana	MFLUCC 17-2770^{T}	MG806115	MG783041	–	MG783040	MG783042
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

1	2	3	4	5	6	7
D. crousii	CAA 823^{T}	MK792311	MK837932	MK871450	MK828081	MK883835
D. destruens	ZJUPD 06	MN708229	MN696537	–	MN696526	–
D. durionigena	VTCC 930005^{T}	MN453530	MT276159	–	MT276157	–
D. ellipsospora	GZCC 19-0231^{T}	MT385949	MT424704	MW022488	MT424684	MT424720
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. foikelawen	CBS 145189^{T}	MN509713	MN509724	–	MN509735	MN974278
D. fukushii	MAFF 625034	JQ807469	–	–	JQ807418	–
D. fusicola	CGMCC 3.17087^{T}	KF576281	KF576305	–	KF576256	KF576233
D. gardeniae	CBS 288.56	KC343113	KC344081	KC343597	KC343839	KC343355

1	2	3	4	5	6	7
D. gardeniae	CBS 288.56	KC343113	KC344081	KC343597	KC343839	KC343355
D. garethjonesii	MFLUCC 12-0542a^{T}	KT459423	KT459441	–	KT459457	KT459470
D. grandiflori	SAUCC 194.84^{T}	MT822612	MT855809	MT855580	MT855924	MT855691
D. guangdongensis	ZHKUCC 20-0014^{T}	MT355684	MT409292	–	MT409338	MT409314
D. guizhouensis	GZAAS 20-0338^{T}	OM060254	OL961762	–	OL961761	OL961763
D. helicis	CBS 138596^{T}	KJ210538	KJ420828	KJ420875	KJ210559	KJ435043
D. heliconiae	SAUCC 194.77^{T}	MT822605	MT855802	MT855573	MT855917	MT855684
D. heterophyllae	CPC 26215^{T}	MG600222	MG600226	MG600220	MG600224	MG600218
D. heterostemmatis	SAUCC 194.85^{T}	MT822613	MT855810	MT855581	MT855925	MT855692
D. hordei	CBS 481.92	KC343120	KC344088	KC343604	KC343846	KC343362
D. ilicicola	FPH 2015502^{T}	MH171064	MH171074	MH171084	–	–
D. incompleta	CGMCC 3.18288^{T}	KX986794	KX999226	KX999265	KX999186	KX999289
D. infertilis	CBS 230.52^{T}	KC343052	KC344020	KC343536	KC343778	KC343294
D. irregularis	CGMCC 3.20092^{T}	MT385951	MT424706	–	MT424686	MT424721
D. italiana	MFLUCC 18-0090^{T}	MH846237	MH853688	–	MH853686	MH853690
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. litchii	SAUCC 194.22^{T}	MT822550	MT855747	MT855519	MT855863	MT855635
D. longicolla	FAU 599^{T}	KJ590728	KJ610883	KJ659188	KJ590767	KJ612124
D. lonicerae	MFLUCC 17-0963^{T}	KY964190	KY964073	–	KY964146	KY964116
D. maritima	DAOM 695742^{T}	KU552025	KU574615	–	KU552023	–
D. masirevicii	BRIP 54256^{T}	KJ197276	KJ197257	–	KJ197239	–
D. mediterranea	DAL-34	MT007489	MT006686	MT007095	MT006989	MT006761
D. megalospora	CBS 143.27	KC343140	KC344108	KC343624	KC343866	KC343382
D. melonis	CBS 435.87^{T}	KC343142	KC344110	KC343626	KC343868	KC343384
D. miriciae	BRIP 54736j^{T}	KJ197283	KJ197263	–	KJ197245	–
D. moorei	BRIP 61500b^{T}	OR019755	OR039652	–	OR039645	–
D. moriniae	BRIP 60190a^{T}	OM918698	OM960625	–	OM960607	–
D. neilliae	CBS 144.27^{T}	KC343144	KC344112	KC343628	KC343870	KC343386
D. nobilis	CBS 587.79	KC343153	KC344121	KC343637	KC343879	KC343395

1	2	3	4	5	6	7
D. nomurai	CBS 157.29	KC343154	KC344122	KC343638	KC343880	KC343396
D. nothofagi	BRIP 54801^{T}	JX862530	KF170922	–	JX862536	–
D. obtusifoliae	CBS 143449^{T}	MG386072	–	MG386137	–	–
D. ocoteae	CBS 141330^{T}	KX228293	KX228388	–	–	–
D. oraccinii	LC 3166^{T}	KP267863	KP293443	KP293517	KP267937	–
D. ovalispora	ICMP 20659^{T}	KJ490628	KJ490449	KJ490570	KJ490507	–
D. ovoidea	CGMCC 3.17093	KF576265	KF576289	–	KF576240	KF576223
D. ovoidea	CGMCC 3.17092^{T}	KF576264	KF576288	–	KF576239	KF576222
D. padina	CFCC 52590^{T}	MH121525	MH121604	MH121483	MH121567	MH121443
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. perniciosa	CBS 124030	KC343149	KC344117	KC343633	KC343875	KC343391
D. phragmitis	CBS 138897^{T}	KP004445	KP004507	KP004503	–	–
D. pseudoamygdali	**MUCC 3612^{T}**	**OR897104**	**OR913164**	**OR913195**	**OR913226**	**OR913254**
D. pseudotsugae	MFLU 15-3228^{T}	KY964225	KY964108	–	KY964181	KY964138
D. pulla	CBS 338.89^{T}	KC343152	KC344120	KC343636	KC343878	KC343394
D. pustulata	CBS 109742	KC343185	KC344153	KC343669	KC343911	KC343427
D. racemosae	CBS 143770^{T}	MG600223	MG600227	MG600221	MG600225	MG600219
D. rhoina	CBS 146.27	KC343189	KC344157	KC343673	KC343915	KC343431
D. rosae	MFLUCC 17-2658^{T}	MG828894	MG843878	–	–	MG829273
D. rosiphthora	COAD 2913^{T}	MT311196	–	–	MT313692	MT313690
D. rudis	CBS 113201	KC343234	KC344202	KC343718	KC343960	KC343476
D. schini	CBS 133181^{T}	KC343191	KC344159	KC343675	KC343917	KC343433
D. sennae	CFCC 51636^{T}	KY203724	KY228891	–	KY228885	KY228875
D. silvicola	CFCC 54191^{T}	MZ727041	MZ753491	MZ753481	MZ816347	MZ753472
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**

1	2	3	4	5	6	7
***Diaporthe* sp. 3**	**MUCC 3583**	**OR897075**	**OR913135**	**OR913166**	**OR913197**	**OR913228**
D. sterilis	CBS 136969^{T}	KJ160579	KJ160528	MF418350	KJ160611	KJ160548
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. tecomae	CBS 100547	KC343215	KC344183	KC343699	KC343941	KC343457
D. tectonendophytica	MFLUCC 13-0471^{T}	KU712439	KU743986	–	KU749367	KU749354
D. terebinthifolii	CBS 133180^{T}	KC343216	KC344184	KC343700	KC343942	KC343458
D. ternstroemia	CGMCC 3.15183^{T}	KC153098	–	–	KC153089	–
D. thunbergiicola	MFLUCC 12-0033^{T}	KP715097	–	–	KP715098	–
D. trevorrowii	BRIP 70737a^{T}	OM918703	OM960630	–	OM960612	–
D. ueckerae	CBS 132527^{T}	JX069860	KY435674	KY435654	KY435633	KY435664
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. vexans	CBS 127.14	KC343229	KC344197	KC343713	KC343955	KC343471
D. virgiliae	CMW 40748^{T}	KP247573	KP247582	–	–	–
D. vochysiae	LGMF 1583^{T}	MG976391	MK007527	MK033323	MK007526	MK007528
D. xunwuensis	CFCC 53085^{T}	MK432663	MK578063	MK443008	MK578137	MK442983
D. zaofenghuang	CGMCC 3.20271^{T}	MW477883	MW480875	–	MW480871	MW480867

Note: [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*; [T] ex-type material. Boldface type font indicate isolate used in this study.

Split Frequencies was below 0.01, and the posterior probability (PP) was determined using the remaining tree. *Diaporthella corylina* Lar. N. Vassiljeva was used as an outgroup. After evaluation of the inferred tree, the alignment was split into three sections (Fig. 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 10,000,000 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), which accommodate different degrees of intraspecific genetic diversity within the phylogeny [Zhang et al., 2013]. Non-annotated Newick format restricted analysis of ML trees was used for PTP analysis performed with

Figure 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 ≥ 50 are indicated along the branch with thicken. Legend refers to nucleotide substitution per site.

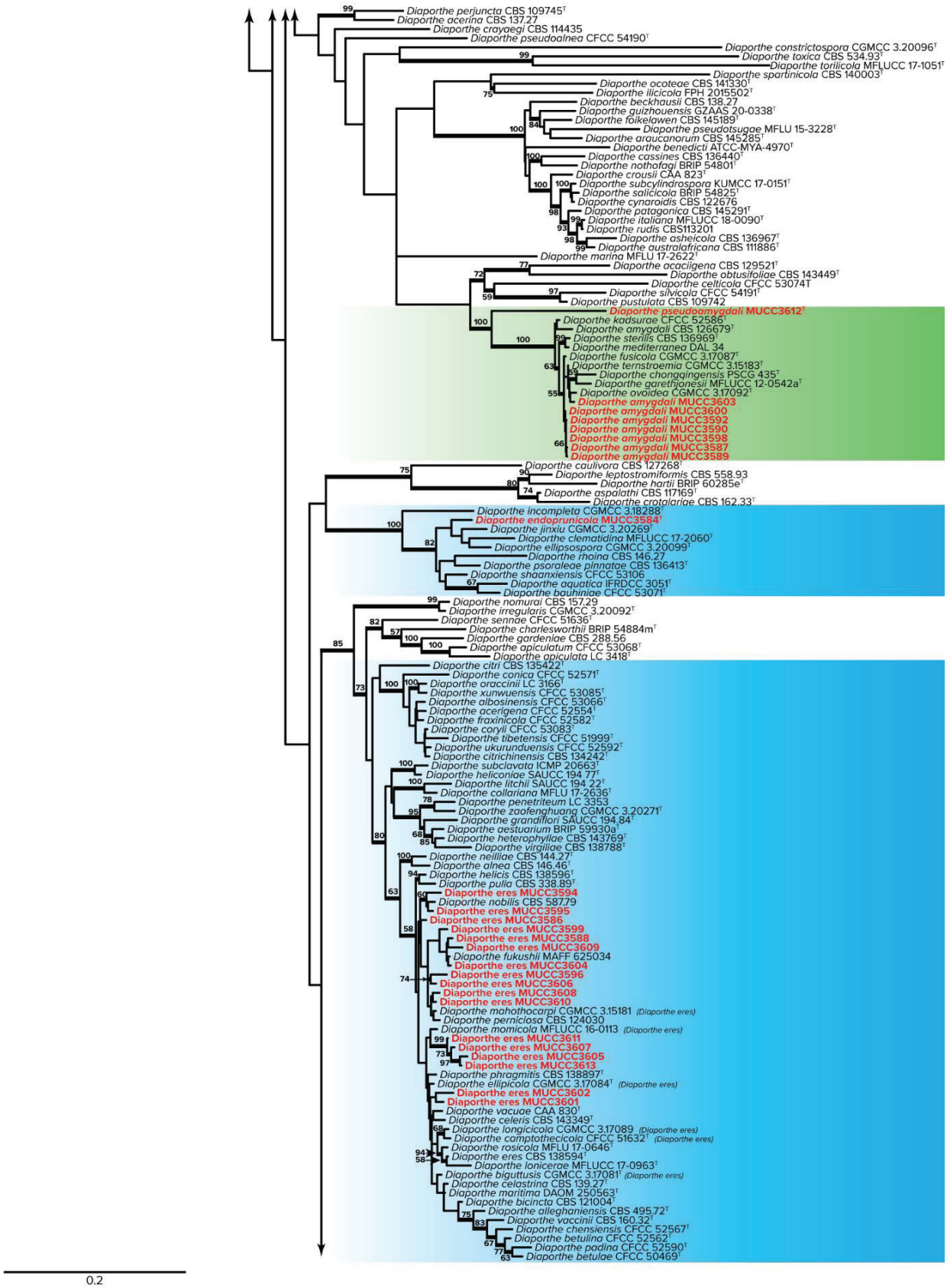

Figure 1. Continued.

Figure 1. Continued.

500,000 MCMC generations, 100 sets of thinning, and 10% burn-in on the webserver for PTP [https://species.h-its.org/ptp].

RESULTS

Isolation and phylogenetic analysis. A total of 31 isolates were obtained from the sample in this study (Tab. 3) and the morphological characteristics were described in the taxonomy sections. Preliminary analysis of the combined matrix consisting 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 1 shows the phylogenetic trees generated from a combined dataset, revealing isolates obtained from *Prunus* spp.

Table 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

were located in three major clades; the first subclade contains isolates of *D. eres* species complex (DESC), the second subclade contains *D. amygdali* species complex (DASC), and the third subclade consists of *D. 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. The matrix was split into three sections according to the species complex, realigned and reanalysed for taxonomical position in the phylogenetic tree. The reference taxa retrieved from GenBank were reextracted for more detailed analyses.

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) (Fig. 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 (Fig. 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) (Fig. 4). The tree topologies of split trees showed congruency between ML and BI, where the 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.

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 examined: JAPAN, Ibaraki, Tsukuba, endophyte in *Prunus yedoensis* Matsum, 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 examined: 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* C.K.Schneid., 12 May 2022, collected by Y. Hattori, culture MUCC3594, MUCC 3595; Kita-ibaraki, endophyte in *P. jamasakura* Siebold ex Koidz., 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* F.Schmidt, 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 maximowiczii* (Rupr.) Masam. & S.Suzuki, 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* L. from Poland [Abramczyk et al., 2022] in *P. mandshuria* from Russia [Nekrasov et al., 2022], and it occurs as pathogen on *P. davidiana* Franch. 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)

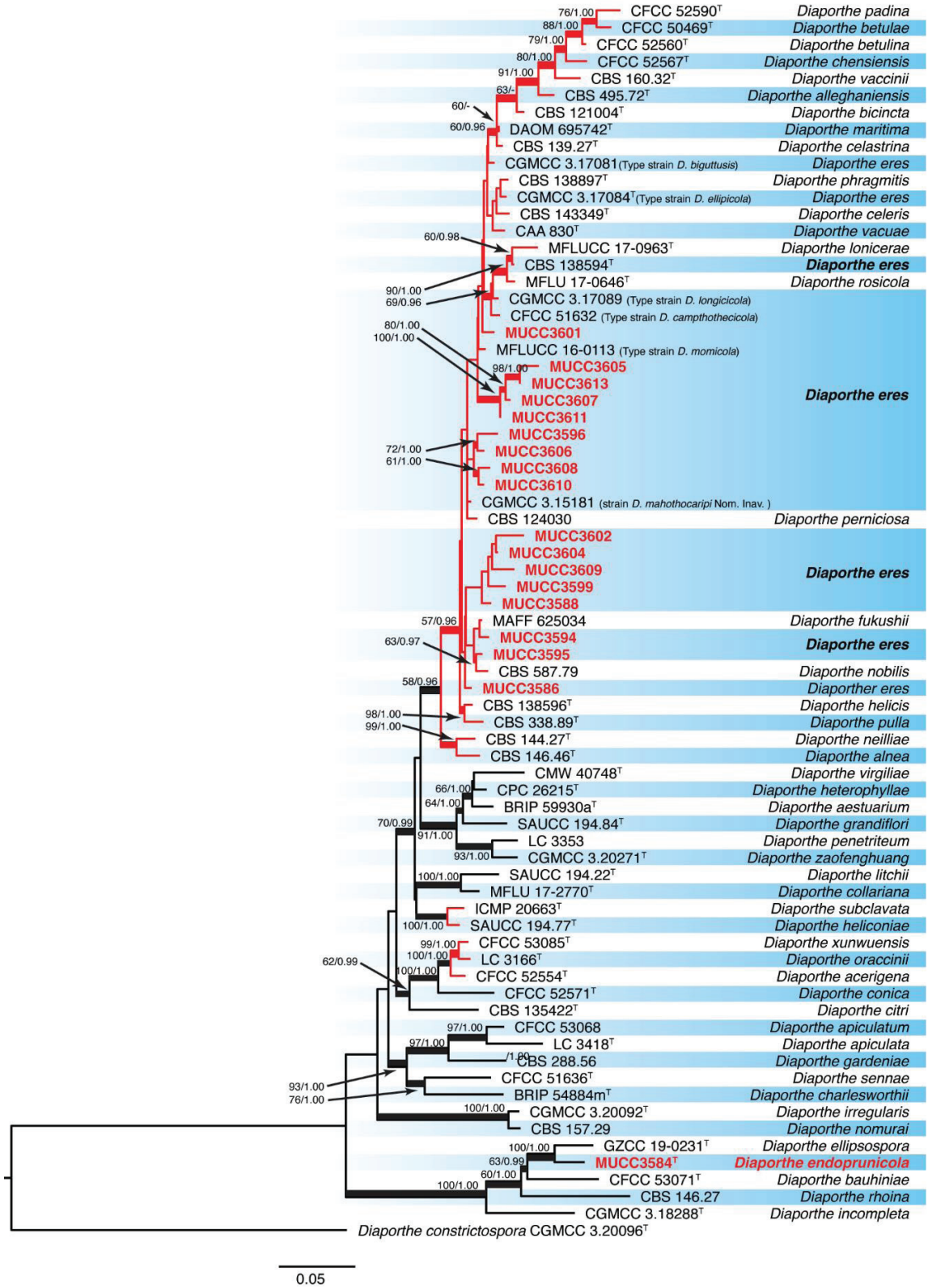

Figure 2. Maximum-likelihood (ML) phylogenetic tree consisting of *Diaporthe eres* species complex (DESC) constructed by using concatenated matrix of 5 loci. The bootstrap of ≥ 50 and posterior probability ≥ 0.95 are indicated near branch as BS/PP. *Diaporthe constrictopora* CGMCC 3.20096 were used as outgroup. Legend refers to nucleotide substitution per site. Red branch indicated coalescent/population process in PTP ML delimitation scheme.

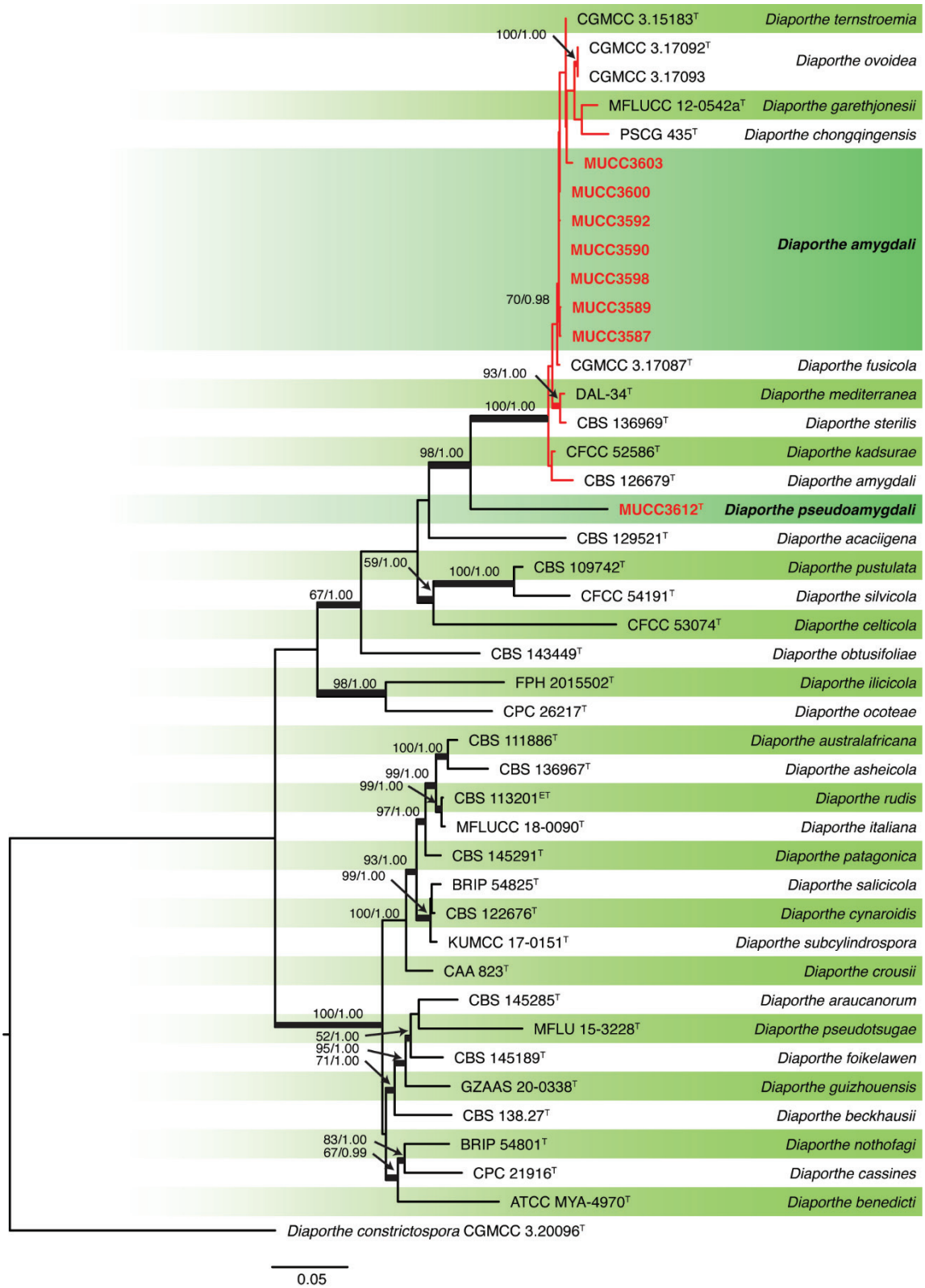

Figure 3. Maximum-likelihood (ML) phylogenetic tree consisting of *Diaporthe amygdali* species complex (DASC) constructed by using concatenated matrix of 5 loci. The bootstrap of ≥ 50 and posterior probability ≥ 0.95 are indicated near branch as BS/PP. *Diaporthe constrictopora* CGMCC 3.20096 were used as outgroup. Legend refers to nucleotide substitution per site. Red branch indicated coalescent/population process in PTP ML delimitation scheme.

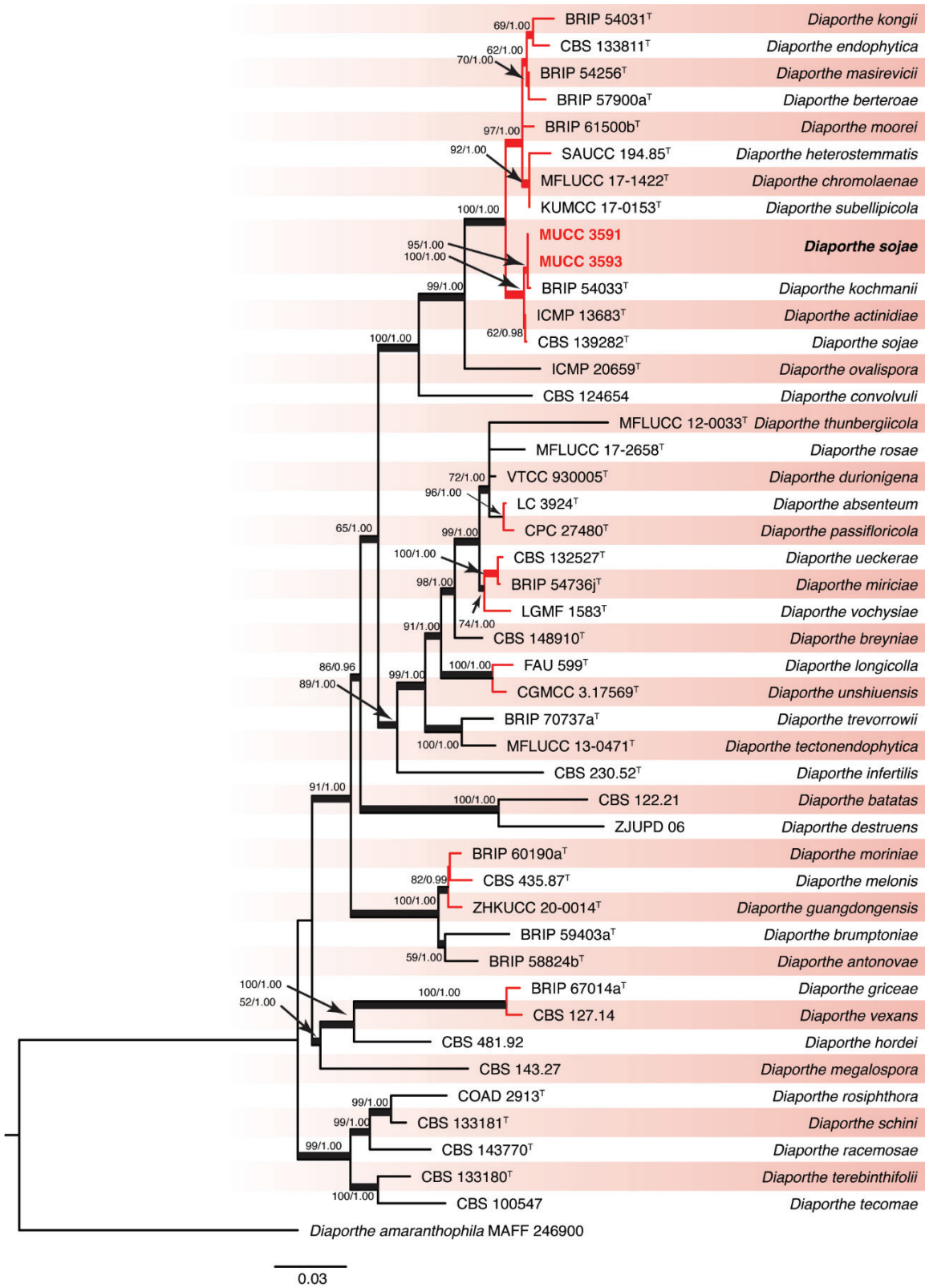

Figure 4. Maximum-likelihood (ML) phylogenetic tree consisting of *Diaporthe sojae* species complex (DSSC) constructed by using concatenated matrix of 5 loci. The bootstrap of ≥ 50 and posterior probability ≥ 0.95 are indicated near branch as BS/PP. *Diaporthe amaranthophila* MAFF 246900 were used as outgroup. Legend refers to nucleotide substitution per site. Red branch indicated coalescent/population process in PTP ML delimitation scheme.

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* R.G. Shivas, S.M. Thompson & A.J. Young. 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.

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 droplets containing conidial masses from the top of semi-immersed conidiomata; covered with aerial mycelia. On PDA, mycelia flat, pale yellow to brown, forming conidiomata, semi-immersed, without droplets on conidiomata.

Type: JAPAN, Ibaraki, Tsukuba, endophyte in branch of *Cerasus* × *yedoensis* (Matsum.) A.N.Vassiljeva, 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* Y.Y. Chen, Dissan. & Jian K. Liu, 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.

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 ellipsoidal, 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 of conidiomata, without aerial mycelium.

Specimens examined: 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 as *Diaporthe amygdali*, but forms an independent branch highly supported by BS/PP (98/1.00). The present species differs from *D. amygdali* in conidiogenous cells as the conidiogenous cells 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 was placed next to sister taxon *D. orixae* Q.T. Lu & Zhen Zhang forming a well-supported

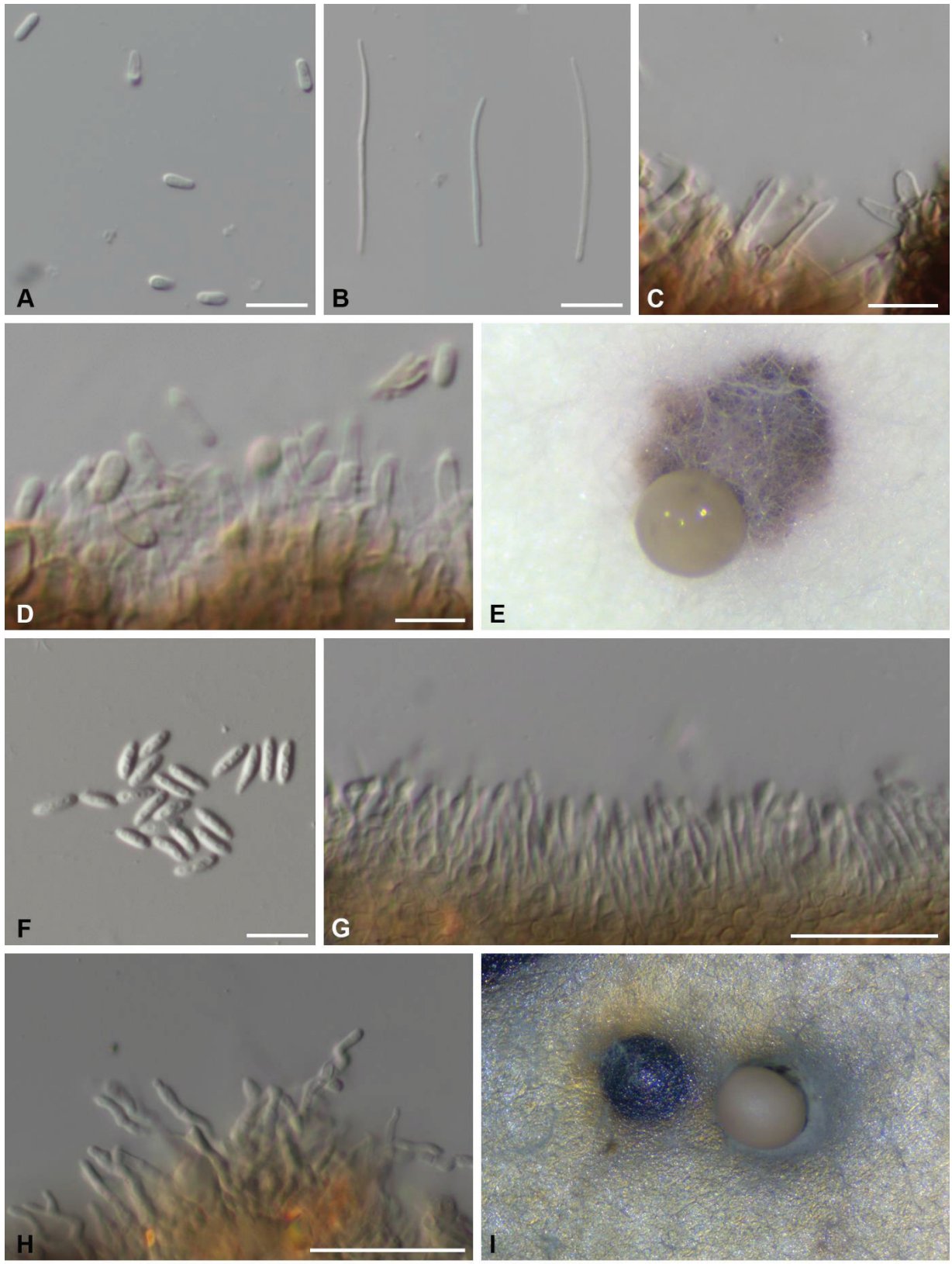

Figure 5. A-E. *Diaporthe endoprunicola* A. Alpha conidia. B. Beta conidia, C–D. Philiade. E. Pale yellow conidial mass produce on top of conidiomata in PCA. F-I *Diaporthe pseudoamygdali* F. Alpha conidia, G–H, Philiade. I. White conidial masses produce on top of conidiomata in PCA. Scalebar = 10 µm (A–F); 20 µm (G–H).

clade (ML BS: 65) (Fig. 1). However, it is not formally described as an independent species because the culture is sterile. Therefore, the species was not described 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* Y.P. Tan & R.G. Shivas (ML BS: 70) (Fig. 1). However, it is not formally described as an independent species because the culture is sterile. Phylogenetically, it is placed next to the sister taxon of *D. howardiae* isolated from the 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* Q. Yang and *D. liquidambaris* (C.Q. Chang, Z.D. Jiang & P.K. Chi) Udayanga & Castl (Fig. 1). However, due to the sterility of the culture, this species is not formally described.

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, Y. 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. D. 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. S. 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 the DASC. Additionally, C. 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 the *Diaporthe pustulata* Sacc. species complex and *Diaporthe alnea* Fuckel species complex, respectively. However, Y. 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 S. 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. S. 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.* 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].

The 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*. In the Hokkaido region, *D. eres* makes up most of the isolates with only one isolate 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. Y.Q. Zhu et al., [2023] in their study of *Diaporthe* in citrus showed that several *Diaporthe* species are weakly aggressive or non-pathogenic which could be endophytic or latent pathogens. This shows that these *Diaporthe* species found in *Prunus s.l.* are potentially disease-causing pathogens.

CONCLUSIONS

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 the taxonomy of *Diaporthe* species. S. 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 S. Hongsanan et al., [2023] provided taxonomic updates to the work by A.J. 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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Yaponiyada *Prunus sensu lato* gövdəsində *Diaporthe* cinsinin növlərinin müxtəlifliyi

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Diaporthe Fuckel cinsi patogenlər, saproblar və endofitlər kimi çoxlu sayda qeydə alınan növdən ibarətdir. Bu günə qədər 1200-dən çox *Diaporthe* növü qeydə alınsa da, Yaponiyada yalnız *Diaporthe* cinsinin patogen ştammları müəyyən edilmişdir ki,

onlar da xüsusilə iqtisadi əhəmiyyətli bitkilərə təsir göstərir. Bu tədqiqat adətən Sakura ağacı kimi tanınan *Prunus sensu lato* (*s.l.*) üzərində endofit *Diaporthe* spp. müxtəlifliyini uğurla aydınlaşdırmaqla yanaşı, Yaponiya mikobiotasına iki yeni *Diaporthe* növünü əlavə etmişdir. Tədqiqatda daxili transkripsiya sahəsi (ITS) bölgəsi, translyasiyanın elonqasiya faktoru 1-alfa (TEF), beta-tubulin (TUB), histon H3 (HIS) və kalmodulin (CAL) genlərinin qismən ardıcıllığı daxil olmaqla, 5 lokusun multilokus analizindən istifadə edərək çoxfazalı bir yanaşma istifadə edilmişdir. Bundan başqa, süni mühitdə kulturalarda spor əmələgəlməni təhrik etməklə morfoloji müşahidələrdə aparılmışdır. Nəticədə *Prunus s.l.* cinsində əvvəllər qeydə alınmış *Diaporthe* növlərinin təsdiqi ilə yanaşı, iki yeni növ təsvir edilmişdir: *Diaporthe endoprunicola* A.H. Ujat & Y. Hattori və *Diaporthe pseudoamygdali* A.H. Ujat & Y. Hattori.

Açar sözlər: *Diaporthaceae, çoxlokuslu filogeniya, yeni taksonlar, taksonomiya, sistematika*

Разнообразие видов рода *Diaporthe* на стеблях *Prunus sensu lato* в Японии колючий

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Род *Diaporthe* Fuckel содержит большое количество видов, зарегистрированных как патогены, сапробы и эндофиты. Хотя к настоящему времени зарегистрировано более 1200 видов *Diaporthe*, в Японии зарегистрированы только патогенные штаммы рода *Diaporthe*, которые особенно поражают экономически важные растения. Это исследование успешно выявило разнообразие эндофитных видов *Diaporthe* на *Prunus sensu lato* (*s.l.*), широко известном как дерево сакуры, и добавило два новых вида *Diaporthe* к японской микобиоте. В исследовании использовался мультилокусный мультилокусный анализ 5 локусов, включая частичные последовательности области внутреннего транскрибируемого сайта (ITS), фактора элонгации трансляции 1-альфа (TEF), бета-тубулина (TUB), гистона H3 (HIS) и кальмодулина (CAL) генов. Кроме того, морфологический наблюдение также проводилось путем индуцирования спорообразования изолятов в искусственных средах. В результате *Prunus s.l.* Наряду с подтверждением ранее отмеченных в роде видов *Diaporthe* интродуцированы два новых вида: *Diaporthe endoprunicola* А.Н. Уджат и Ю. Хаттори и *Diaporthe pseudoamygdali* А.Н. Уджат и Ю. Хаттори.

Ключевые слова: *Diaporthaceae, мультилокусная филогения, новые таксоны, систематика, систематика*