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First report of *Diaporthe convolvuli* on field bindweed (*Convolvulus arvensis*) in Russia

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Abstract

Convolvulus arvensis (field bindweed) is a widely distributed and harmful weed in Russia. The fungal strains MF-SOR 61.20.1 and MF-SOR 61.20.2 were isolated from the visually healthy leaves of *C. arvensis* collected in northwest Russia (Saint Petersburg, Pushkin) and initially identified by morphological features as *Diaporthe* sp. The aim of this study was to identify these strains using molecular genetic, micromorphological, and cultural characteristics, and to assess their pathogenicity. Multilocus phylogenetic analysis, based on sequences of the internal transcribed spacer (ITS) of rDNA, as well as partial calmodulin (*cal*), histone 3 (*his3*), translation elongation factor 1α (*tef1*), and β-tubulin (*tub2*), allowed definitive identification of these strains as *Diaporthe convolvuli*. Pathogenicity assays demonstrated that strains were pathogenic to *C. arvensis*, causing necrosis on leaf segments under laboratory conditions. The pathogenicity was confirmed by reisolation of *D. convolvuli* from infected leaves. To our knowledge, this is the first finding of this fungus in Russia (Saint Petersburg, Pushkin). This study provides the first description, refinement, and comprehensive illustration of the fungus's cultural and micromorphological characteristics since 1988.

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Introduction

The genus *Diaporthe* Nitschke (*Sordariomycetes*, *Diaporthomycetidae*, *Diaporthales*, *Diaporthaceae*) was described in 1867[1] with the type species *D. eres* Nitschke. The genus *Phomopsis* (Sacc.) Bubák was described in 1905[2] with the type species *P. lactucae* (Sacc.) Bubák. *Diaporthe* species have a complex pleomorphic life cycle. Previously, *Phomopsis* species were considered to be the asexual stages of *Diaporthe* species. According to the International Code of Nomenclature for algae, fungi, and plants and the principle of "one fungus–one name", *Diaporthe*, being the older generic name, has priority over *Phomopsis*[3]. *Diaporthe* encompasses important plant pathogenic, endophytic, and saprobic species, with a diverse host range and a global distribution[4,5].

Species identification in the genus *Diaporthe* has evolved from host association and morphology to the widespread adoption of DNA sequencing[4−10]. The most taxonomically informative DNA loci more frequently used to reconstruction the molecular phylogeny of *Diaporthe* species are the internal transcribed spacer (ITS) of the ribosomal DNA and partial calmodulin (*cal*), histone 3 (*his3*), translation elongation factor 1α (*tef1*), and β-tubulin (*tub2*) genes[4,5,7,9,10]. A polyphasic approach for reliable species recognition should be used that relies on the consolidated species concept (CSC)[11], which involves the incorporation of morphological, biological, and phylogenetic characteristics[9,12,13]. Data on phylogenetic features should be obtained according to the genealogical concordance phylogenetic species recognition (GCPSR)[14] method using a multilocus phylogenetic approach for recognizing fungal species. Certain *Diaporthe* species are known to produce biologically active compounds with herbicidal activity[15−17]. For example, *Diaporthe convolvuli* (Ormeno-Nuñez, Reeleder & A.K. Watson) R.R. Gomes, Glienke & Crous, which is host-specific to *Convolvulus arvensis* L., produces secondary metabolites, namely convolvulanic acid A, convolvulanic acid B, convolvulol, and α-pyrone convolvulopyrone, which possess phytotoxic activity[15]. Fungi with this potential could be explored given their potential application in agriculture as promising candidates for the development of natural herbicides. Field bindweed *Convolvulus arvensis* is a harmful weed of major agricultural crops that is distributed almost worldwide[18]. In Russia, it is found in the European part, the Caucasus, western and eastern Siberia, and the Far East.

In July 2024, visually healthy leaves of *C. arvensis* were collected in northwest Russia (Pushkin, Saint Petersburg, Leningrad Region). Two *Diaporthe* sp. strains were isolated from these leaves. The aim of this study was to identify these strains according to the CSC by their phylogenetic, micromorphological, and cultural features and to assess their pathogenicity.

Materials and methods

Isolates

Leaves of *C. arvensis* were surface-sterilized by consistent rinsing with a 0.1 % $AgNO_3$ and sterile water and incubated on potato sucrose agar (PSA)[19]. The Petri dishes were incubated at 24 °C in the dark and were analyzed after 7–10 days of cultivation. The obtained strains MF-SOR 61.20.1 and MF-SOR 61.20.2 were stored in microtubes on PSA at 4 °C in the mycological collection of pure cultures at the A.A. Jaczewskii Laboratory of Mycology and Phytopathology, All-Russian Institute of Plant Protection (MF, VIZR, Saint Petersburg, Russia).

DNA isolation, polymerase chain reaction, and sequencing

Genomic DNA was extracted from mycelia obtained from the cultures incubated on PSA according to the standard cetyltrimethylammonium bromide (CTAB)/chloroform protocol[20].

Table 1. GenBank accession numbers of the *Diaporthe* section Sojae strains included in the study.

Diaporthe species	Strain number	ITS	*cal*	*his3*	*tef1*	*tub2*
D. acaciarum	CBS 138862, t	NR_137939	–	KP004504	–	KP004509
D. alpiniae	CGMCC 3.28221, t	PQ321210	PQ336555	PQ336573	PQ336519	PQ336537
D. amaranthophila	MAFF: 246900, t	LC459575	LC459583	LC459581	LC459577	LC459579
D. ambigua	CBS 114015, t	KC343010	KC343252	KC343494	KC343736	KC343978
D. angelicae	CBS 111592; AR3724, t	KC343027	KC343269	KC343511	KC343753	KC343995
D. arctii	CBS 139280, t	KJ590736	KJ612133	KJ659218	KJ590776	KJ610891
D. arezzoensis	MFLU: 19-2880	MT185503	–	–	MT454019	MT454055
D. atlantica	CECT 21217; SP11, t	ON159893	ON364019	ON398810	ON398831	ON364040
D. batatas	CBS 122.21	KC343040	KC343282	KC343524	KC343766	KC344008
D. beilharziae	BRIP 54792	JX862529	–	–	JX862535	KF170921
D. berteroae	BRIP 57900a, t	OR019752	–	–	OR039642	OR039649
D. betae	HMPHU 3001; HHL19101101S2D1, t	MW882216	MW882219	MW882225	MW882222	MW882228
D. biguttulata	CFCC 52584, t	MH121519	MH121437	MH121477	MH121561	MH121598
D. brasiliensis	CBS 133183, t	KC343042	KC343284	KC343526	KC343768	KC344010
D. breyniae	CBS 148910, t	ON400846	ON409189	ON409187	ON409188	ON409186
D. brideliae	CBS 148911, t	OR348649	OR468837	OR468807	OR468817	OR468827
D. brumptoniae	BRIP 59403a, t	OM918702	–	–	OM960611	OM960629
D. butterlyi	BRIP 59194a, t	OR019753	–	–	OR039643	OR039650
D. caatingaensis	CBS 141542, t	KY085927	–	–	KY115603	KY115600
D. cameroonensis	CBS 148913, t	OR348650	OR468836	OR468806	OR468816	OR468826
D. camporesii	JZB320143	–	–	–	MN984254	MN561316
D. caryae	CFCC 52563, t	MH121498	MH121422	MH121458	MH121540	MH121580
D. cerradensis	CMRP 4331, t	MN173198	MW751655	MW751663	MT311685	MW751671
D. cha	SAUCC 1562, t	MT199863	MT366912	MT376714	MT376682	MT376652
D. chinensis	MFLUCC 19-0101, t	NR_172435	MW294199	–	MW205017	MW245013
D. chiangraiensis	MFLUCC 17-1669, t	MF190119	–	–	MF377598	–
D. chimonanthi	SCHM 3614, t	AY622993	–	–	–	–
D. cichorii	MFLUCC 17-1023, t	KY964220	KY964133	–	KY964176	KY964104
D. cinnamomi	CFCC 52569, t	MH121504	–	MH121464	MH121546	MH121586
D. citriasiana	ZJUD 30, t	JQ954645	KC357491	KJ490515	JQ954663	KC357459
D. convolvuli	CBS 124654, t	KC343054	KC343296	KC343538	KC343780	KC344022
D. convolvuli	FAU649	KJ590721	KJ612130	KJ659210	KJ590765	–
D. convolvuli	**MF-SOR 61.20.1**	**PX567840**	**PX585096**	**PX585101**	**PX585106**	**PX585111**
D. convolvuli	**MF-SOR 61.20.2**	**PX567841**	**PX585097**	**PX585102**	**PX585107**	**PX585112**
D. coracoralinae	FCCUFG 38; URM 8912, t	PP192078	PP408214	PP421133	PP430449	PP402241
D. cucurbitae	DAOM 42078, t	KM453210	–	KM453212	KM453211	KP118848
D. cuppatae	CBS 117499, t	KC343057	KC343299	KC343541	KC343783	KC344025
D. cyatheae	YMJ 1364, t	JX570889	KC465410	–	KC465406	KC465403
D. cylindriformispora	MFLU 18-2292, t	NR_191275	–	–	OR140382	OR189510
D. desmotis	CGMCC 3.28203, t	PQ321216	PQ336561	PQ336579	PQ336525	PQ336543
D. destruens	ZJUPD02	KP990617	–	KP990637	KP990647	KP990627
D. discoidspora	CGMCC 3.17255; ZJUD 89, t	KJ490622	–	KJ490564	KJ490501	KJ490443
D. elaeagni-confertae	SAUCC 194.47, t	MT822575	MT855656	MT855544	MT855888	MT855772
D. eleutherrhenae	01, t	OK017069	–	–	OK017070	OK017071
D. elizabethblackwelliae	BRIP 74769a, t	OR290128	–	–	OR335747	OR352596
D. fici-macrocarpae	SAUCC 0412, t	PQ321225	PQ336570	PQ336588	PQ336534	PQ336552
D. fici-septicae	MFLUCC 20-0178; KUMCC 19-0151, t	MW114348	–	–	MW192211	MW148268
D. fohaiensis	SAUCC 194.113, t	MT822641	MT855720	MT855608	MT855953	MT855838
D. foliorum	CMRP1321, t	MT576688	MT584341	MT584338	MT584310	MT584327
D. ganjae	CBS 180.91, t	KC343112	KC343354	KC343596	KC343838	KC344080
D. goulteri	BRIP 55657a, t	KJ197290	–	–	KJ197252	KJ197270
D. griceae	BRIP 67014a, t	OM918694	–	–	OM960603	OM960621
D. guangzhouensis	CFCC 58151, t	PP864725	PP938609	PP938613	PP938601	PP938605
D. gulyae	BRIP 54025, t	NR111615	–	–	JN645803	KJ197271
D. gulyae	MF-Ha17-043	MK024253	MZ671933	MZ671961	MK039421	MK033489
D. guttulata	CGMCC 3.20100, t	MT385950	MW022470	MW022491	MT424685	MT424705
D. hazeniae	BRIP 75103a, t	OR122283	–	–	OR135527	OR135528
D. helianthi	CBS 592.81, t	KC343115	KC343356	KC343598	KC343841	KC344083
D. hordei	CBS 481.92	KC343120	KC343362	KC343604	KC343846	KC344088
D. iberica	CECT 21218; TH77, t	ON159902	ON364028	ON398819	ON398841	ON364049
D. infecunda	CBS 133812, t	KC343126	KC343368	KC343610	KC343852	KC344094
D. infertilis	CBS 230.52, t	KC343052	KC343294	KC343536	KC343778	KC344020

(to be continued)

Table 1. (continued)

Diaporthe species	Strain number	ITS	*cal*	*his3*	*tef1*	*tub2*
D. jazanensis	PPDU28R, t	OQ586408	–	–	OR137582	OR137586
D. juglandigena	GUCC 422.16, t	OP581229	–	–	OP688534	OP688559
D. kyushuensis	ch-D-1, t	AB302250	–	–	–	–
D. leucospermi	CBS 111980	JN712460	KY435663	KY435653	KY435632	KY435673
D. longicolla	FAU 599, t	KJ590728	KJ612124	KJ659188	KJ590767	KJ610883
D. longispora	CBS 194.36, t	KC343135	KC343377	KC343619	KC343861	KC344103
D. lusitanicae	CBS 123212	KC343136	KC343378	KC343620	KC343862	KC344104
D. machili	SAUCC 194.111, t	MT822639	MT855718	MT855606	MT855951	MT855836
D. mayteni	CBS 133185, t	KC343139	KC343381	KC343623	KC343865	KC344107
D. megalospora	CBS 143.27	KC343140	KC343382	KC343624	KC343866	KC344108
D. melongenicola	CGMCC 3.27978, t	PQ321221	PQ336566	PQ336584	PQ336530	PQ336548
D. melonis	CBS 507.78, t	KC343142	KC343384	KC343626	KC343868	KC344110
D. middletonii	BRIP 54884e, t	KJ197286	–	–	KJ197248	KJ197266
D. minusculata	CGMCC 3.20098, t	MT385957	MW022475	MW022499	MT424692	MT424712
D. monetii	MF-Ha18-049, t	MW008494	MZ671939	MZ671965	MW008516	MW008505
D. moorei	BRIP 61500b, t	OR019755	–	–	OR039645	OR039652
D. morindendophytica	ZHKUCC 22-0069, t	ON322897	–	ON315027	ON315054	ON315087
D. moriniae	BRIP 60190a, t	OM918698	–	–	OM960607	OM960625
D. myracrodruonis	URM 7972, t	NR_163320	MK205290	–	MK213408	MK205291
D. neoarctii	CBS 109490, t	KC343145	KC343387	KC343629	KC343871	KC344113
D. novem	CBS 127270, t	KC343156	KC343398	KC343640	KC343882	KC344124
D. nepheliicola	MFLU 23-0067, t	PP915805	PP911610	–	PP911611	PP911608
D. orixiae	KUNCC 21–10714; HKAS 121465, t	OK283041	OK484485	OK484486	OK432279	OK432278
D. ovalispora	CGMCC 3.17256; ZJUD 93	KJ490628	–	KJ490570	KJ490507	KJ490449
D. oxe	CBS 133186, t	KC343164	KC343406	KC343648	KC343890	KC344132
D. pachirae	CDA 728, t	MG559537	MG559535	–	MG559539	MG559541
D. paranensis	CBS 133184, t	KC343171	KC343413	KC343655	KC343897	KC344139
D. passiflorae	GZCC 22-0014	OP056703	–	OP150773	OP150542	OP150620
D. passiflorae	CBS 132527, t	JX069860	–	KY435654	–	–
D. pedratalhadensis	FCCUFG 48; URM 8304, t	PP192073	–	PP421129	PP430438	PP402232
D. phaseolorum	AR4203; CBS 139281, t	KJ590738	KJ612135	KJ659220	KJ590739	KJ610893
D. pseudobiguttulata	CGMCC 3.17248; ZJUD 47, t	KJ490582	–	KJ490524	KJ490461	KJ490403
D. pseudofoliicola	HNCM045, t	OR647680	–	OR671932	OR671940	OR671948
D. pygmaeae	CDP 1370, t	PP577992	PP579348	–	PP579317	PP579332
D. quercicola	CSUFTCC104, t	ON076567	ON081670	ON081667	ON081659	–
D. racemosae	CPC 26646	MG600223	MG600219	MG600221	MG600225	MG600227
D. raonikayaporum	CBS 133182, t	KC343188	KC343430	KC343672	KC343914	KC344156
D. rizhaoensis	CFCC 57562, t	OP955930	OP959782	OP959785	OP959767	OP959773
D. rosae	MFLUCC 17-2658, t	NR_172401	MG829273	–	–	MG843878
D. rosiphthora	COAD 2914	MT311197	MT313691	–	MT313693	–
D. sackstonii	BRIP 54669b, t	KJ197287	–	–	KJ197249	KJ197267
D. schini	CBS 133181, t	KC343191	KC343433	KC343675	KC343917	KC344159
D. schoeni	MFLU 15-1279, t	KY964226	KY964139	–	KY964182	KY964109
D. sclerotioides	CBS 296.67, t	KC343193	KC343435	KC343677	KC343919	KC344161
D. serafiniae	BRIP 54136, t	KJ197273	–	–	KJ197235	KJ197253
D. shawiae	BRIP 64534a, t	OM918701	–	–	OM960610	OM960628
D. siamensis	MFLUCC 10_0573a	JQ619879	–	–	JX275393	JX275429
D. sojae	FAU635; CBS 139282, t	KJ590719	KJ612116	KJ659208	KJ590762	KJ610875
D. stewartii	CBS 193.36	FJ889448	JX197415	–	GQ250324	JX275421
D. submersa	CGMCC 3.24297; GZCC 19-0129, t	OP056717	OP150710	OP150786	OP150556	OP150633
D. subordinaria	CBS 464.90, t	KC343214	KC343456	KC343698	KC343940	KC344182
D. talong	MCC-MNH 2727, t	OQ123545	–	–	OR943637	OR943674
D. tarchonanthi	CBS 146073	MT223794	–	MT223759	–	MT223733
D. tecomae	CBS 100547	KC343215	KC343457	KC343699	KC343941	KC344183
D. tectonendophytica	MFLUCC 13-0471	KU712439	KU749354	–	KU749367	KU743986
D. terebinthifolii	CBS 133180, t	KC343216	KC343458	KC343700	KC343942	KC344184
D. thunbergiicola	MFLUCC 12–0033, t	KP715097	–	–	KP715098	–
D. trevorrowii	BRIP 70737a, t	OM918703	–	–	OM960612	OM960630
D. tulliensis	BRIP 62248a, t	KR936130	–	–	KR936133	KR936132
D. ueckerae	FAU 656, t	KJ590726	KJ612122	KJ659215	KJ590747	KJ610881
D. unshiuensis	ZJUD50, t	KJ490585	–	KJ490527	KJ490464	KJ490406
D. vangoghii	MF-Ha18-046, t	MW008492	MZ671937	MZ671963	MW008514	MW008503

(to be continued)

Table 1. (continued)

Diaporthe species	Strain number	ITS	*cal*	*his3*	*tef1*	*tub2*
D. vargemgrandensis	FCCUFG 58; URM 8784, t	PP192069	PP421068	PP421135	PP430456	PP421092
D. vexans	CBS 127.14, t	KC343229	KC343471	KC343713	KC343955	KC344197
D. vochysiae	LGMF1583, t	MG976391	MK007528	MK033323	MK007526	MK007527
D. yunnanensis	CGMCC 3.18289; LC6168, t	KX986796	KX999290	KX999267	KX999188	KX999228
Diaporthella corylina	CBS 121124, t	KC343004	KC343246	KC343488	KC343730	KC343972

The studied *Diaporthe* strains are indicated in bold. t, the ex-type strain. Acronyms of culture collection: AR: isolates in culture collection of Systematic Mycology and Microbiology Laboratory; BRIP: Plant Pathology Herbarium, Department of Employment, Economic, Development and Innovation, Queensland, Australia; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CDP: culture collection of D.S. Pereira, housed at the Lab Bugworkers | M&B-BioISI | Tec Labs – Innovation Centre, Faculty of Sciences, University of Lisbon, Lisbon, Portugal; CECT: Spanish Type Culture Collection at University of Valencia, Valencia, Spain; CFCC: China Forestry Culture Collection Center, Beijing, China; CMRP: Microbiological Collections of Paraná Network, Paraná, Brazil; COAD: Coleção Octávio Almeida Drummond, Universidade Ferderal de Viçosa, Viçosa, Brazil; CPC : Culture collection of Pedro Crous in Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CSUFTCC: Central South University of Forestry and Technology Culture Collection, Hunan, China; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; FAU: Florida Atlantic University, Biological Sciences Department, Davie, Florida, USA; FCCUFG: Coleção de Culturas de Fungos da Universidade Federal de Goiás, Laboratório de Micologia, Instituto de Patologia Tropical e Saúde Pública (IPTSP), Universidade Federal de Goiás, Goiânia, Brazil; GUCC: Culture Collection at the Department of Plant Pathology, Agriculture College, Guizhou University, China; GZCC: Guizhou culture collection, Guiyang, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; HMPHU: Herbarium of Microbiology and Phytopathology, Heilongjiang University, Harbin, China; HUMCC: Heilongjiang University Microbiological Culture Collection Centre, Harbin, China; JZB: Beijing Academy of Agriculture and Forestry Sciences Culture Collection, China; KUNCC: Kunming Institute of Botany Culture Collection, Kunming, China; LC: Personal Culture Collection Lei Cai, State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, China; LGMF: Culture collection of Laboratory of Genetics of Microorganisms, Federal University of Parana, Curitiba, Brazil; MAFF: MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MF: collection of pure cultures of A. A. Jaczewskii Laboratory of mycology and phytopathology of All-Russian Institute of Plant Protection, Saint Petersburg, Russia; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MFLU: Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; SAUCC: Shandong Agricultural University Culture Collection, Shandong, China; SCHM: Mycological Herbarium of South China Agricultural University, Guangzhou, China; ZHKUCC: culture collection of Zhongkai University of Agriculture and Engineering, Guangzhou, China; ZJUD, ZJUPD: Diaporthe strains deposited in Zhejiang University, Zhejiang, China.

The ITS region of the rDNA and partial *cal*, *his3*, *tef1*, and *tub2* genes were amplified and sequenced for the strains studied. The primers ITS1 and ITS4[21], CAL-228F and CAL-737R[22], CYLH3F[23] and H3-1b[24], EF1-728F and EF1-986R[22], and βtub2Fw and βtub4Rd[25] or T1 and Bt2b[26], were used to amplify the ITS region and the partial *cal*, *his3*, *tef1*, and *tub2* genes, respectively. The polymerase chain reaction (PCR) conditions were as follows: 95 °C for 5 min; followed by 35 cycles of 92 °C for 50 s; 55 °C for 40 s, (ITS1/ITS4), 52 °C for 40 s (βtub2Fw/βtub4Rd and T1/Bt2b), 56 °C for 40 s (CAL-228F/CAL-737R), or 58 °C for 40 s (CYLH3F/H31-b and EF1-728F/EF1-986R); 72 °C for 75 s; and a final elongation for 5 min at 72 °C.

Single-strand DNA of amplicons were sequenced by Sanger's method on an ABI Prism 3500 analyzer (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) with a Bigdye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's instructions. The nucleotide sequences of the ITS, *cal*, *his3*, *tef1*, and *tub2* genes were deposited in the GenBank database with the corresponding accession numbers (Table1).

Phylogenetic analysis

Sequences were assembled using Vector NTI advance v. 11.0 (Invitrogen, Thermo Fisher Scientific) and aligned with ClustalX 1.8[27]. The alignments were concatenated using Sequence Matrix[28]. To implement GCPSR and estimate the possibility of combining the set of five loci, single locus trees were generated and compared to detect conflict (data not shown). A multilocus phylogenetic analysis was based on the combined ITS, *cal*, *his3*, *tef1* and *tub2* sequences for all strains studied and all species currently accepted in the *Diaporthe* section Sojae sensu Dissanayake et al.[4] and Zhang et al.[5], 89 species in total. Additionally, sequences for 34 species published in 2023–2025 that were omitted by these authors were included. The tree was rooted using the ex-type *Diaporthella corylina* Lar.N. Vassiljeva (CBS 121124). Sequences of representative *Diaporthe* strains and type species were obtained from GenBank (Table 1).

Phylogenetic analysis of the combined aligned data consisted of maximum likelihood (ML) and Bayesian inference. ML analysis was implemented in the IQ-TREE[29]. Modeltest in IQ-TREE determined the most suitable nucleotide substitution model for the nucleotide dataset according to the Bayesian information criterion (BIC) model TIM2e+F+R5. Bootstrap values with 100,000 replicates were calculated for tree branches. Bayesian inference was performed by MrBayes v. 3.2.1. in ARMADILLO v. 1.1[30] using a Markov chain Monte Carlo (MCMC) sampling method. The general time-reversible model of evolution, including estimation of invariable sites and assuming a gamma distribution with six rate categories was used for Bayesian inference analyses. Four MCMC chains were run simultaneously, starting from random trees for 10^7 generations and sampled every 1,000 generations. The first 25% of the generations were discarded as burn-in, and Bayesian posterior probabilities (BPP) were calculated from the remaining trees.

Morphology

Pure cultures of the studied strains were incubated on PSA, oatmeal agar (OA)[31], and Czapek agar (CZ)[19]. Petri dishes (90 mm in diameter) were placed for 10 days under 12 h near-ultraviolet light/12 h dark to stimulate sporulation. Colony morphology and diameter were examined after 10 days. Observations and measurements of 100 conidia for each strain were conducted with an Olympus SZX16 stereomicroscope (Olympus, Tokyo, Japan) and an Olympus BX53 microscope. Images were captured with a PROKYON camera (Jenoptik, Jena, Germany) with Nomarski differential interference contrast.

Pathogenicity test

For the pathogenicity tests, leaf segments of *C. arvensis* were inoculated with all the studied strains. The mycelial suspension was used as the inoculum. For inoculation, strains were grown in a liquid soybean (*Glycine max*) nutrient medium with the following composition: KH_2PO_4 (0.2%), $(NH_4)_2SO_4$ (0.1%), $MgSO_4$ (0.1%), glucose (2%), and soy flour (1%). In total, 50 mL of the medium in 250-mL flasks was inoculated with three 5-mm mycelial discs cut from 2-week-old colonies grown on PSA and kept on an orbital shaker (200 rpm) for four days at 24 °C. The mycelium was separated from the liquid

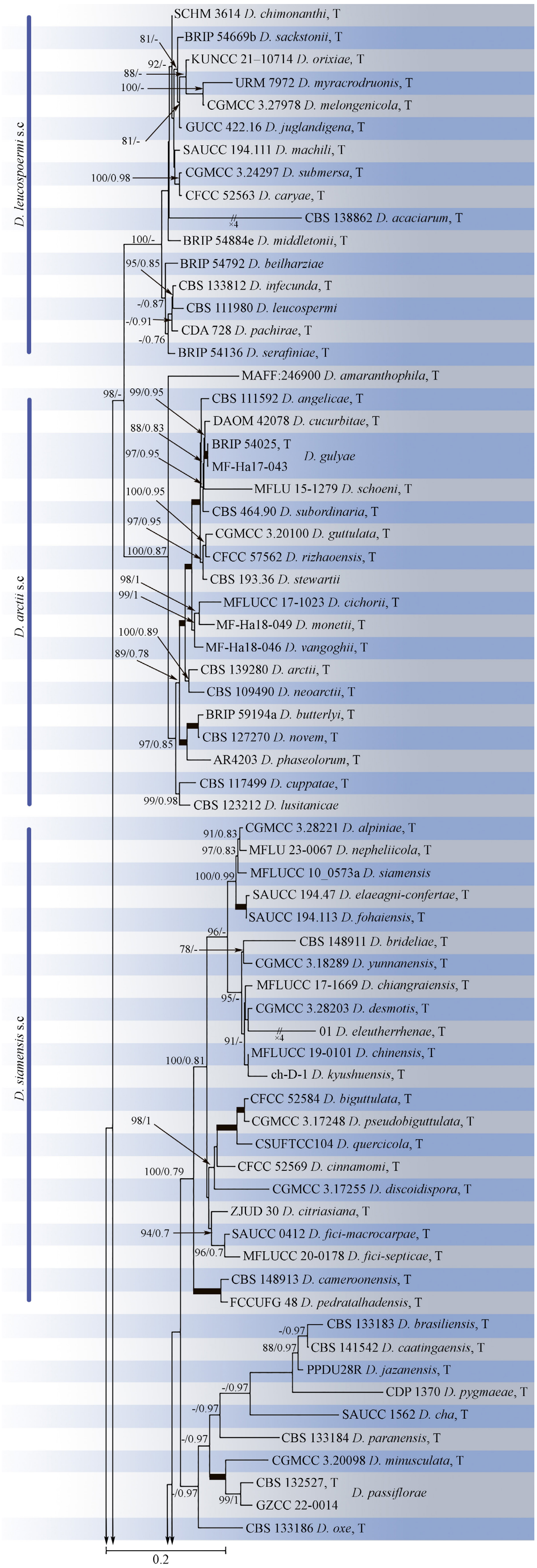

Fig. 1 to be continued

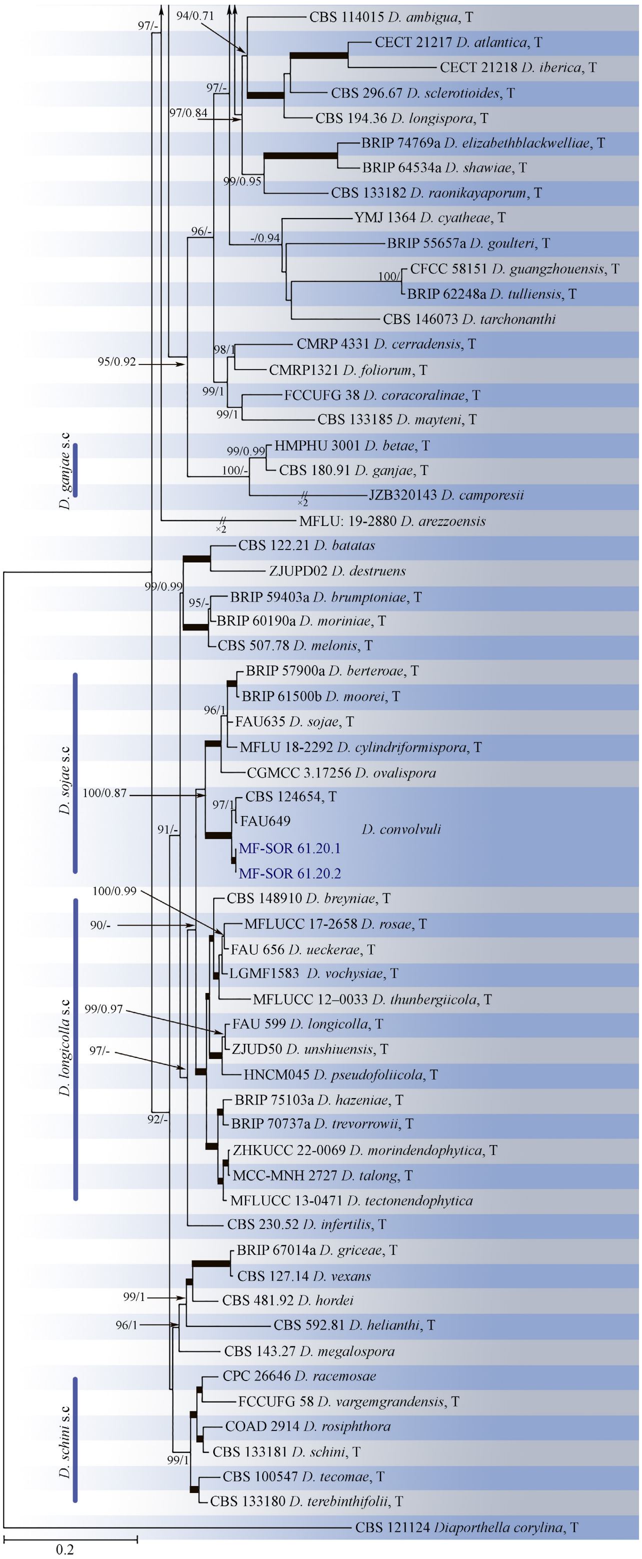

Fig. 1 Phylogenetic tree of *Diaporthe* section Sojae inferred from a ML analysis based on a concatenated alignment of the ITS region, and partial *cal*, *his3*, *tef1*, and *tub2* genes. The ML bootstrap support values (MLBS ≥ 70%) and Bayesian posterior probabilities (BPP ≥ 0.70) are given at the nodes (MLBS/BPP). The studied strains are given in blue. The ex-type strains are marked with T. Purple lines indicate the boundaries of species complexes.

culture by filtering and then dried with filter paper and ground in sterile water to prepare a mycelial suspension of 100 mg/mL. Visually healthy *C. arvensis* leaves were collected from their natural habitat and cut into segments (1.5 cm × 1.5 cm) then placed in Petri dishes. Six inoculated leaf segments (replicates) were used per strain. To create a moisture chamber effect, filter paper premoistened with sterile water was added to each Petri dish. A drop of the mycelial suspension (10 μL) was applied to the center of leaves which had previously been wounded with a needle (diameter of damage: 0.5 mm) or intact leaves on the abaxial surface of the leaf. In the control, the wounded and nonwounded leaf segments were treated with 10 μL of sterile distilled water. After inoculation, closed Petri dishes with the leaf segments were kept at room temperature and evaluated for the presence of symptoms at 3, 5, and 7 d post treatment (dpt). Subsequent reisolation of the fungus from the leaf segments and identification were carried out to fulfill the Koch postulates.

Results

Phylogeny

A multilocus phylogenetic analysis based on the ITS, *cal*, *his3*, *tef1*, and *tub2* sequences inferred intraspecific relationships within all species (123) currently accepted in the *Diaporthe* section Sojae for which nucleotide sequences are available. The total analysis included 129 strains, 2 of which were our strains and 127 were references. The total combined matrix consisted of 2,900 characters with gaps (520 bp, ITS region; 560 bp, *cal*; 498 bp, *his3*; 452 bp, *tef1*; and 870 bp, *tub2*), among which 616 (21%) characters were conserved, 1,480 (51%) were informative, and 804 (28%) were uninformative.

The analysis of the single-gene phylogenies revealed no conflicts. The individual gene trees, as well as the combined tree (Fig. 1), showed that the studied strains MF-SOR 61.20.1 and MF-SOR 61.20.2 with the ex-type CBS 124654 and representative Florida Atlantic University (FAU) 649 strains clustered in distinct monophyletic clade corresponding to *D. convolvuli* species.

Morphology

On all tested nutrient media, the colonies of the strains reached the edge of the Petri dish within 10 days. Colonies on PSA (Fig. 2a) had sparse, silvery-white aerial mycelia generally at the colony periphery. On OA (Fig. 2b), aerial mycelia were absent. On CZ (Fig. 2c), aerial mycelia that were abundant, silvery-white, and floccose covered the entire colony, forming dense cushions. Conidiomata were pycnidial, abundant on all media, and the colonies on PSA and OA appeared mottled because of the presence of numerous pycnidia. Pycnidia were distributed evenly across the colony on all media and formed concentric rings at the periphery on PSA, and were superficial and immersed, solitary or within large conglomerates (Fig. 2d, e), often arranged in long (5–10 pycnidia on PSA and OA; Fig. 2f) or short (on CZ 3–5 pycnidia) chains. Pycnidia (Fig. 2g, h)

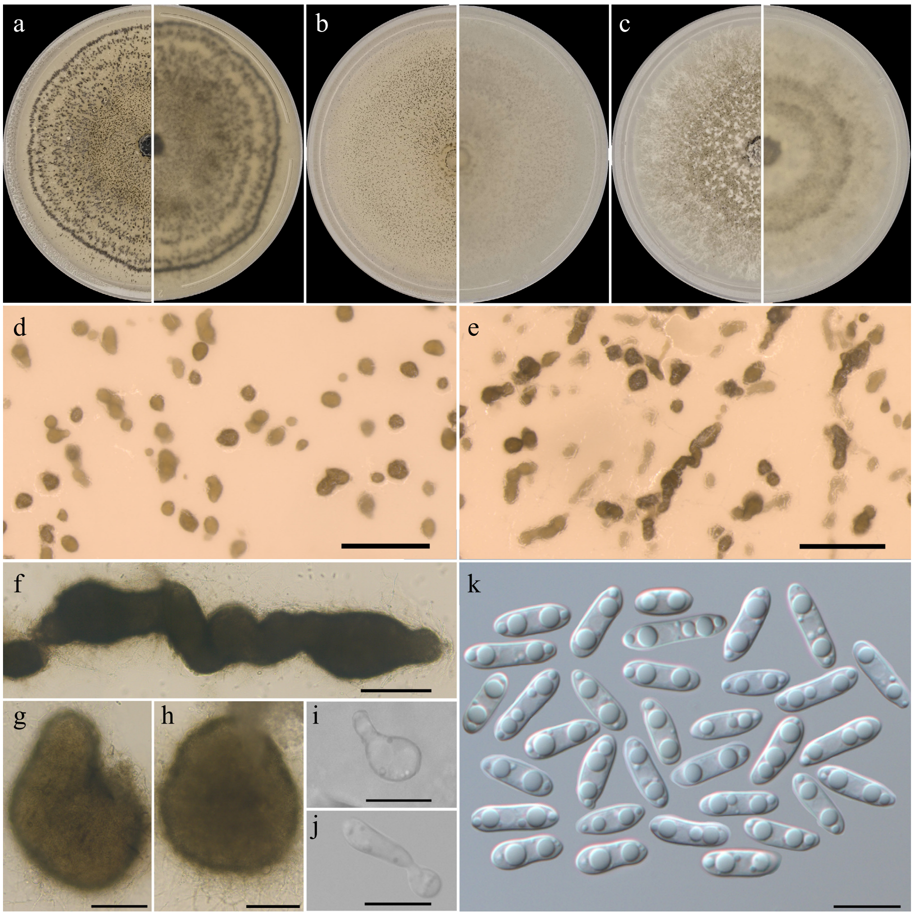

Fig. 2 *Diaporthe convolvuli* strain MF-SOR 61.20.1 at 10 days of growth. (a)–(c) Pure cultures: left half, front; right, reverse. (a) PSA. (b) OA. (c) CZ. (d), (e), (g), (h) Pycnidia on OA. (f) Pycnidia on OA aggregated in chains. (i) Conidiogenous cell. (j) Conidia on a conidiogenous cell. (k) Conidia. Scale bars: (d), (e) = 1 mm; (f) = 200 μm; (g)–(h) = 100 μm; and (i)–(k) = 10 μm.

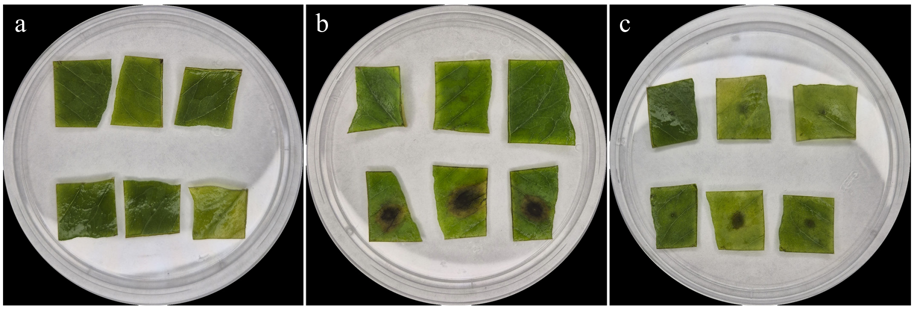

Fig. 3 Pathogenicity of *Diaporthe convolvuli* on leaf segments of *Convolvulus arvensis* at 5 dpt. The first line in each set (Petri dish) contains the inoculated intact abaxial part of the leaf segments; the second line is the abaxial part wounded with a needle. Inoculation variants: (a) Inoculation with sterile water; (b) mycelial suspension of MF-SOR 61.20.1; (c) mycelial suspension of MF-SOR 61.20.2.

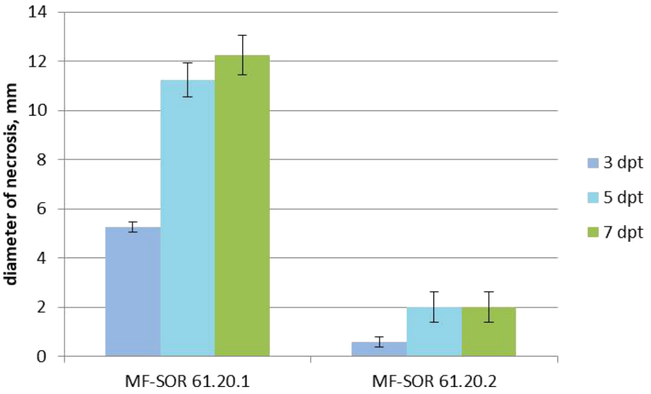

Fig. 4 Diameter of necrosis (mm) caused by *Diaporthe convolvuli* strains on *Convolvulus arvensis* leaf segments at 3, 5, and 7 dpt. Error bars represent standard errors of the means.

were subglobose or globose, oval or pyriform, glabrous, ostiolate, and pappilate or with a short neck. On PSA, they were 210–386 (292 ± 14) × 140–359 (249 ± 14) µm; on OA, they were 88–364 (273 ± 17) × 95–277 (200 ± 12) µm; on CZ, they were 230–454 (343 ± 13) × 218–499 (312 ± 17) µm. The pycnidial wall was pseudoparenchymatous, composed of isodiametric cells, with the outer layers pigmented. Conidiophores reduced to phialidic conidiogenous cells (Fig. 2i, j) formed from the inner cells of the pycnidial wall, and were hyaline, smooth, and bottle-shaped, measuring 5.64–15.89 (9.69 ± 0.87) × 3.69–8.97 (5.65 ± 0.47) µm. Only alpha conidia were observed. Alpha conidia (Fig. 2k) were smooth, hyaline, guttulate, and ellipsoidal. On PSA, they were 8.1–16.2 (12.3 ± 0.1) × 3.2–4.7 (3.8) µm; on OA, they were 8.8–14 (11.5 ± 0.1) × 3.2–4.5 (3.8) µm; on CZ, they were 7.5–16.2 (11.8 ± 0.2) × 3.3–5 (4.1) µm. Ascomata were absent.

Pathogenicity

The pathogenicity of *D. convolvuli* was tested using *C. arvensis* leaf segments (Fig. 3). No distinct necrosis was observed on the intact sides of the segments. The first significant symptoms, namely necrosis, on the wounded abaxial surfaces of the leaf segments were observed at 3 dpt. At 3 dpt, inoculation with the strain MF-SOR 61.20.2 led to the development of tan to brown necrotic lesions measuring 0.6 ± 0.3 mm in diameter on leaf segments. At 5 dpt, the lesions measured 2.0 ± 0.5 mm. By 7 dpt, lesion size did not increase. In contrast, the strain MF-SOR 61.20.1 induced brown to black necrotic lesions. At 3 dpt, the lesions measured 5.2 ± 0.4 mm in diameter. A marked increase was observed by 5 dpt, with the lesions reaching 11.9 ± 1.3 mm. Subsequently, lesion expansion slowed, and at 7 dpt, the lesions measured 12.25 ± 0.81 mm (Fig. 4). To confirm Koch's postulates, fungal cultures were reisolated in all cases from the necrosis formed on the leaf segments. These isolates had morphological characteristics identical to those of all the strains tested.

Discussion

Two studied strains isolated from *C. arvensis*, MF-SOR 61.20.1 and MF-SOR 61.20.2, were definitively identified as *D. convolvuli*. Phylogenetically, these strains were closely related to the ex-type (CBS 124654) and representative (FAU 649) *D. convolvuli* strains, and their morphological features were consistent with that of *D. convolvuli*[32]. *Diaporthe convolvuli* was originally described as *Phomopsis convolvuli* Ormeno-Nunez, Reeleder & A.K. Watson in 1988[32] as the causal agent of leaf spot and anthracnose in field bindweed in Montreal (Canada). To our knowledge, this is the first finding of this fungus in Russia (Saint Petersburg, Pushkin) and the third in the world. The first report from Canada[32] was of a strain that was later patented for the biological control of *C. arvensis*[33]. The second was from Turkey[34]. Furthermore, this study provides the first description, refinement, and comprehensive illustration of the fungus's cultural and micromorphological characteristics since 1988.

Both strains exhibited pathogenicity to field bindweed leaves under laboratory conditions. This observation suggests that they might be worth investigating further as potential candidates for biological weed control. The fact that these strains were isolated from visually healthy leaves but demonstrated pathogenicity under laboratory conditions indicates a latent infection in the original plant material.

Author contributions

The authors confirm their contributions to the paper as follows: study conception and design, analysis and interpretation of results: Gomzhina MM, Gasich EL; data collection, manuscript review and editing: Gasich EL; draft manuscript preparation: Gomzhina MM. Both authors reviewed the results and approved the final version of the manuscript.

Data availability

The data generated and analyzed during this study are available in this article. DNA sequence data are available in the GenBank database, and the accession numbers are provided in Table 1. The *Diaporthe convolvuli* MF-SOR 61.20.1 and MF-SOR 61.20.2 strains have been deposited in the the mycological collection of pure cultures at the A.A. Jaczewskii Laboratory of Mycology and Phytopathology, All-Russian Institute of Plant Protection (MF, VIZR, Saint Petersburg, Russia).

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Conflict of interest

The authors declare that they have no conflict of interest.

Dates

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References

[1] Nitschke TRJ. 1870. *Pyrenomycetes Germanici*. Breslau: Eduard Trewendt. 320 pp. https://catalog.hathitrust.org/Record/001997930

[2] Saccardo PA, Roumeguère C. 1884. Reliquiae mycologicae Libertianae (Series IV). *Revue Mycologique Toulouse* 6:26−39

[3] Rossman AY, Adams GC, Cannon PF, Castlebury LA, Crous PW, et al. 2015. Recommendations of generic names in *Diaporthales* competing for protection or use. *IMA Fungus* 6:145−154

[4] Dissanayake AJ, Zhu JT, Chen YY, Maharachchikumbura SSN, Hyde KD, et al. 2024. A re-evaluation of *Diaporthe*: refining the boundaries of species and species complexes. *Fungal Diversity* 126:1−125

[5] Zhang ZX, Shang YX, Liu QY, Li DH, Yin CZ, et al. 2025. Deciphering the evolutionary and taxonomic complexity of *Diaporthales* (*Sordariomycetes, Ascomycota*) through integrated phylogenomic and divergence time estimation. *Fungal Diversity* 132:1−125

[6] Udayanga D, Liu X, Crous PW, McKenzie EHC, Chukeatirote E, et al. 2012. A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). *Fungal Diversity* 56:157−171

[7] Gomes RR, Glienke C, Videira SIR, Lombard L, Groenewald JZ, et al. 2013. *Diaporthe*: a genus of endophytic, saprobic and plant pathogenic fungi. *Persoonia - Molecular Phylogeny and Evolution of Fungi* 31:1−41

[8] Santos L, Alves A, Alves R. 2017. Evaluating multi-locus phylogenies for species boundaries determination in the genus *Diaporthe*. *PeerJ* 5:e3120

[9] Hilário S, Gonçalves MFM, Alves A. 2021. Using genealogical concordance and coalescent-based species delimitation to assess species boundaries in the *Diaporthe eres* complex. *Journal of Fungi* 7:507

[10] Norphanphoun C, Gentekaki E, Hongsanan S, Jayawardena R, Senanayake IC, et al. 2022. *Diaporthe*: formalizing the species-group concept. *Mycosphere* 13:752−819

[11] Crous PW, Hawksworth DL, Wingfield MJ. 2015. Identifying and naming plant-pathogenic fungi: past, present, and future. *Annual Review of Phytopathology* 53:247−267

[12] Gomzhina MM, Gannibal PB. 2022. *Diaporthe* species infecting sunflower (*Helianthus annuus*) in Russia, with the description of two new species. *Mycologia* 114:556−574

[13] Ferro LO, Bezerra JDP, da Silva TM, de Oliveira CS, dos S Nascimento S, et al. 2024. Endophytic *Diaporthe* species from Brazil. *Fungal Systematics and Evolution* 14:251−270

[14] Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, et al. 2000. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* 31:21−32

[15] Isaka M, Jaturapat A, Rukseree K, Danwisetkanjana K, Tanticharoen M, et al. 2001. Phomoxanthones A and B, novel xanthone dimers from the endophytic fungus *Phomopsis* species. *Journal of Natural Products* 64:1015−1018

[16] Kaul S, Gupta S, Ahmed M, Dhar MK. 2012. Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. *Phytochemistry Reviews* 11:487−505

[17] Hilário S, Gonçalves MFM. 2022. Endophytic *Diaporthe* as promising leads for the development of biopesticides and biofertilizers for a sustainable agriculture. *Microorganisms* 10:2453

[18] POWO. 2025. *Kew Plants Database*. https://powo.science.kew.org (Accessed on 19 November 2025)

[19] Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O. 2002. *Introduction to food- and airborne fungi*. 6th Edition. Utrecht, Netherlands: Centraal Bureau Voor Schimmel Cultures. 389 pp.

[20] Doyle JJ, Doyle JL. 1990. Isolation of DNA from small amounts of plant tissues. *BRL Focus* 12:15

[21] White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*, eds. Innis MA, Gelfand DH, Sninsky JJ, White TJ. New York, USA: Academic Press. pp. 315−322 doi: 10.1016/b978-0-12-372180-8.50042-1

[22] Carbone I, Kohn LM. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91:553−556

[23] Crous PW, Groenewald JZ, Risède JM, Simoneau P, Hyde KD. 2006. *Calonectria* species and their *Cylindrocladium* anamorphs: species with clavate vesicles. *Studies in Mycology* 55:213−226

[24] Glass NL, Donaldson GC. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61:1323−1330

[25] Aveskamp MM, Verkley GJM, de Gruyter J, Murace MA, Perelló A, et al. 2009. DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties. *Mycologia* 101:363−382

[26] O'Donnell K, Cigelnik E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* 7:103−116

[27] Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25:4876−4882

[28] Vaidya G, Lohman DJ, Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27:171−180

[29] Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, et al. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 35:1530−1534

[30] Lord E, Leclercq M, Boc A, Diallo AB, Makarenkov V. 2012. Armadillo 1.1: an original workflow platform for designing and conducting phylogenetic analysis and simulations. *PLoS One* 7:e29903

[31] Boerema GH, de Gruyter J, Noordeloos ME, Hamers MEC. 2004. Phoma *identification Manual. Differentiation of specific and infra-specific taxa in culture*. UK: CABI Publishing. 470 pp. doi: 10.1079/9780851997438.0000

[32] Ormeno-Nunez J, Reeleder RD, Watson AK. 1988. A new species of *Phomopsis* recovered from field bindweed (*Convolvulus arvensis*). *Canadian Journal of Botany* 66:2228−2233

[33] Watson AK, Reeleder RD, Ormeno-Nunez J. 1993. *Fungal herbicides*. U.S. Patent 5212086. 6 pp. www.freepatentsonline.com/5212086.pdf

[34] Kuleci E, Tunali B, Berner DK, Cavin CA, Castlebury LA. 2009. First report of leaf anthracnose caused by *Phomopsis convolvuli* on field bindweed in Turkey. *Plant Disease* 93:847