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Molecular phylogenetic analysis reveals seven new *Diaporthe* species from Italy

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Abstract

Seven new species of *Diaporthe*, *D. acericola* on *Acer negundo*, *D. cichorii* on *Cichorium intybus*, *D. dorycnii* on *Dorycnium hirsutum*, *D. lonicerae* on *Lonicera* sp., *Laurus nobilis* and *Torilis arvensis*, *D. pseudotsugae* on *Pseudotsuga menziesii*, *D. schoeni* on *Schoenus nigricans*, *Carduus* sp. and *Plantago* sp. and *D. torilicola* on *Torilis arvensis* from Italy are described and illustrated based on morphological characteristics and molecular analyses. In addition to the new species, eight known species of *Diaporthe*, *D. eres*, *D. foeniculina*, *D. gulyae*, *D. novem*, *D. ravennica*, *D. rhusicola*, *D. rudis* and *D. sterilis* were identified. Phylogenetic relationships of the new species with other *Diaporthe* species were revealed by DNA sequence analyses based on the internal transcribed spacer (ITS) region, translation elongation factor 1-alpha (TEF), partial regions of the β-tubulin (BT) and calmodulin (CAL). Among 44 isolates, *D. eres* was the dominant species, accounting for 27% of the frequency of occurrence. Our study revealed a high diversity of undescribed *Diaporthe* species from various hosts in Italy.

Key words – Diaporthales – Hosts – Morphology – Sordariomycetes – Taxonomy

Introduction

Diaporthe (including the *Phomopsis* asexual morph) belongs to family *Diaporthaceae*, order Diaporthales, and class Sordariomycetes (Hyde et al. 2014, Maharachchikumbura et al. 2015, 2016) and its species are found worldwide on a diverse range of host plants as endophytes, pathogens and saprobes (Gomes et al. 2013). Many *Diaporthe* species that are morphologically similar have proven to be genetically distinct (van Rensburg et al. 2006), and several isolates that were formerly identified based on their host, were shown to represent different taxa (Hyde et al. 2014). *Diaporthe* represents a highly complex genus containing numerous cryptic species. In recent studies, species of *Diaporthe* were distinguished mainly by their molecular phylogenies, and the best five gene regions to conduct a multi-gene phylogenetic analysis are ITS, TEF, ACT CAL and HIS (van Rensburg et al. 2006, Santos et al.

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2010, Udayanga et al. 2011, 2012, Gomes et al. 2013). Although ex-type/ex-epitype/ex-isotype/ex-neotype strains are available for 150 species of *Diaporthe* (Dissanayake et al. 2017b), only 13 have been reported associated with hosts in Italy (Table 1). During the last three years, a collection of *Diaporthe* isolates was obtained from branches and stems of various woody hosts in Arezzo, Forlì-Cesena and Ravenna Provinces in Italy. The aim of this study was to identify the species and reveal the distribution of species on the hosts. Isolates were characterized in terms of morphology and their phylogenetic position within *Diaporthe*.

Table 1 *Diaporthe* species associated with hosts in Italy

Species	Disease symptoms & hosts	References
Diaporthe alnea	Dieback of *Alnus glutinosa*	Moricca 2002
D. ambigua	Dieback of *Platanus acerifolia*	Gomes et al. 2013
D. eres	Cane blight of *Vitis vinifera*	Cinelli et al. 2016
D. foeniculina	Decline and mortality of *Eucalyptus camaldulensis*	Deidda et al. 2016
	Stem and shoot cankers on *Castanea sativa*	Annesi et al. 2016
	Branch cankers and stem-end rot of *Persea americana*	Guarnaccia et al. 2016
D. helianthi	Stem canker of *Helianthus annuus*	Pecchia et al. 2004
D. melonis	Black rot of *Cucumis melo*	Bertetti et al. 2011
D. ravennica	*Tamarix* sp.	Thambugala et al. 2017
D. sclerotioides	Black root rot of *Cucumis sativus*	Cappelli et al. 2004
Phomopsis endogena	Brown rot on nuts of *Castanea sativa*	Maresi et al. 2013
P. quercina	Endophyte in *Quercus* sp.	Ragazzi et al. 2003
	Endophyte in *Quercus robur*	Gonthier et al. 2006
Phomopsis sp.	Dieback of *Pinus nigra* seedlings	Nicosia et al. 2015
	Post-harvest fruit rot of *Actinidia* sp.	Luongo et al. 2011
	Symptomatic twigs of *Olea europaea*	Frisullo et al. 2015

Materials & methods

Sample collection, specimen examination and isolations

During 2014 to 2016, 44 isolates were collected from woody branches and stems of 42 hosts belonging to 26 host families (*Adoxaceae*, *Apiaceae*, *Asteraceae*, *Betulaceae*, *Brassicaceae*, *Caprifoliaceae*, *Caprifoliaceae*, *Cornaceae*, *Cupressaceae*, *Cyperaceae*, *Dioscoreaceae*, *Fabaceae*, *Hemerocallidoideae*, *Juglandaceae*, *Lamiaceae*, *Lauraceae*, *Pinaceae*, *Plantaginaceae*, *Platanaceae*, *Poaceae*, *Rhamnaceae*, *Rosaceae*, *Rubiaceae*, *Salicaceae*, *Sapindaceae*, *Simaroubaceae*) from three provinces of Italy: Arezzo, Forlì-Cesena and Ravenna (Fig. 1). Specimens were observed and examined with a Motic SMZ 168 stereomicroscope. Micro-morphological characters were determined with a Nikon ECLIPSE 80i compound microscope and images were captured with a Canon EOS 550D digital camera. Observations and photographs were made from materials mounted in water. Measurements were made with the Tarosoft (R) Image Frame Work and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0. Single spore isolations were prepared following the method of Chomnunti et al. (2014). Spore germination on water agar (WA) was examined after 24 h and germinating spores were transferred to potato dextrose agar (PDA) media. Cultures were incubated at 18°C in the dark and colony color was examined according to Rayner (1970) after 15 d of growth on PDA at 25 °C in the dark. Herbarium specimens are deposited in Mae Fah Luang University Herbarium (MFLU) while, ex-type living cultures are deposited at the Mae Fah Luang University Culture Collection (MFLUCC) in Thailand (Table 2).

Table 2 *Diaporthe* species studied in this study (Fig. 2). Details of ex-type species introduced in this study are in bold.

Species	Strain	Host	Habit	Locality	Collector	Colle. date	GenBank Accession numbers			
							ITS	TEF	BT	CAL
D. acericola	**MFLUCC 17-0956**	***Acer negundo (Sapindaceae)***	**Dead branch, samaras**	**Forlì-Cesena, Italy**	**E. Camporesi**	**22.01.2015**	**KY964224**	**KY964180**	**KY964074**	**KY964137**
D. cichorii	**MFLUCC 17-1023**	***Cichorium intybus (Asteraceae)***	**Dead aerial stem**	**Forlì-Cesena, Italy**	**E. Camporesi**	**17.07.2016**	**KY964220**	**KY964176**	**KY964104**	**KY964133**
D. dorycnii	**MFLUCC 17-1015**	***Dorycnium hirsutum (Fabaceae)***	**Dead aerial stem**	**Forlì-Cesena, Italy**	**E. Camporesi**	**02.05.2016**	**KY964215**	**KY964171**	**KY964099**	**No**
D. eres	MFLUCC 17-0957	*Sambucus nigra (Adoxaceae)*	Dead aerial branch	Forlì-Cesena, Italy	E. Camporesi	07.02.2015	KY964187	KY964143	KY964070	KY964114
D. eres	MFLUCC 17-0965	*Lonicera sp. (Caprifoliaceae)*	Dead aerial branch	Forlì-Cesena, Italy	E. Camporesi	28.02.2015	KY964189	KY964145	KY964072	KY964115
D. eres	MFLUCC 17-0964	*Sonchus oleraceus (Asteraceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	06.05.2015	KY964192	KY964148	KY964076	KY964117
D. eres	MFLUCC 17-0971	*Salix caprea (Salicaceae)*	Dead aerial branch	Arezzo, Italy	E. Camporesi	19.06.2015	KY964194	KY964150	KY964078	KY964119
D. eres	MFLUCC 17-0993	*Picea excels (Pinaceae)*	Dead land cone	Forlì-Cesena, Italy	E. Camporesi	18.01.2016	KY964200	KY964156	KY964084	KY964123
D. eres	MFLUCC 17-0997	*Juglans regia (Juglandaceae)*	Dead land branch	Forlì-Cesena, Italy	E. Camporesi	22.02.2016	KY964202	KY964158	KY964086	KY964124
D. eres	MFLUCC 17-0999	*Populus nigra (Salicaceae)*	Dead aerial branch	Forlì-Cesena, Italy	-	-	KY964203	KY964159	KY964087	KY964125
D. eres	MFLUCC 17-1012	*Sanguisorba minor (Rosaceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	11.04.2016	KY964213	KY964169	KY964097	KY964128
D. eres	MFLUCC 17-1016	*Pinus pinaster (Pinaceae)*	Dead land cone	Forlì-Cesena, Italy	E. Camporesi	03.05.2016	KY964216	KY964172	KY964100	KY964129
D. eres	MFLUCC 17-1017	*Ostrya carpinifolia (Betulaceae)*	Dead aerial branch	Forlì-Cesena, Italy	E. Camporesi	07.05.2016	KY964217	KY964173	KY964101	KY964130
D. eres	MFLUCC 17-1021	*Galega officinalis (Fabaceae)*	Dead aerial stem	Arezzo, Italy	E. Camporesi	07.07.2016	KY964219	KY964175	KY964103	KY964132
D. eres	MFLUCC 17-1025	*Rhamnus alpinus (Rhamnaceae)*	Dead aerial branch	Forlì-Cesena, Italy	E. Camporesi	14.08.2016	KY964221	KY964177	KY964105	KY964134
D. foeniculina	MFLUCC 17-1068	*Ailanthus altissima (Simaroubaceae)*	Dead land stem-leaf	Forlì-Cesena, Italy	E. Camporesi	07.02.2015	KY964188	KY964144	KY964071	-
D. foeniculina	MFLUCC 17-0974	*Melilotus officinalis (Fabaceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	07.09.2015	KY964196	KY964152	KY964080	-
D. foeniculina	MFLUCC 17-0995	*Hemerocallis fulva (Hemerocallidoideae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	10.02.2016	KY964201	KY964157	KY964085	-
D. foeniculina	MFLUCC 17-1003	*Achillea millefolium (Asteraceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	16.03.2016	KY964205	KY964161	KY964089	-
D. foeniculina	MFLUCC 17-1005	*Arctium minus (Asteraceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	08.03.2016	KY964207	KY964163	KY964091	-
D. foeniculina	MFLUCC 17-1006	*Wisteria sinensis (Fabaceae)*	Dead aerial stems	Forlì-Cesena, Italy	E. Camporesi	09.03.2016	KY964208	KY964164	KY964092	-
D. foeniculina	MFLUCC 17-1008	*Lunaria rediviva (Brassicaceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	16.03.2016	KY964209	KY964165	KY964093	-
D. foeniculina	MFLUCC 17-1009	*Cupressus sepervirens (Cupressaceae)*	Dead land cone	Forlì-Cesena, Italy	E. Camporesi	21.03.2016	KY964210	KY964166	KY964094	-
D. foeniculina	MFLUCC 17-1020	*Vicia sp. (Fabaceae)*	Dead aerial stem	Arezzo, Italy	E. Camporesi	19.06.2016	KY964218	KY964174	KY964102	KY964131
D. gulyae	MFLUCC 17-1026	*Heracleum sphondylium (Apiaceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	28.08.2016	KY964223	KY964179	KY964107	KY964136
D. lonicerae	**MFLUCC 17-0963**	***Lonicera sp. (Caprifoliaceae)***	**Dead aerial branch**	**Forlì-Cesena, Italy**	**E. Camporesi**	**28.02.2015**	KY964190	**KY964146**	**KY964073**	**KY964116**
D. lonicerae	MFLUCC 17-0976	*Laurus nobilis (Lauraceae)*	Dead aerial branch	Forlì-Cesena, Italy	E. Camporesi	15.09.2015	KY964197	KY964153	KY964081	KY964121
D. lonicerae	MFLUCC 17-0978	*Torilis arvensis (Apiaceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	23.09.2015	KY964198	KY964154	KY964082	KY964122
D. novem	MFLUCC 17-1028	*Galium sp. (Rubiaceae)*	Dead aerial stem	Arezzo, Italy	E. Camporesi	19.06.2015	KY964195	KY964151	KY964079	KY964120
D. pseudotsugae	**MFLU 15-3228**	***Pseudotsuga menziesii (Pinaceae)***	**Dead land cones**	**Forlì-Cesena, Italy**	**E. Camporesi**	**10.04.2015**	**KY964225**	**KY964181**	**KY964108**	**KY964138**
D. ravennica	MFLUCC 17-1029	*Salvia sp. (Lamiaceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	21.04.2015	KY964191	KY964147	KY964075	-
D. rhusicola	MFLUCC 17-0987	*Amorpha fruticosa (Fabaceae)*	Dead aerial branch	Forlì-Cesena, Italy	E. Camporesi	17.11.2015	KY964199	KY964155	KY964083	-
D. rhusicola	MFLUCC 17-1001	*Angelica sylvestris (Apiaceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	29.02.2016	KY964204	KY964160	KY964088	-
D. rhusicola	MFLUCC 17-1004	*Rubus sp. (Rosaceae)*	Dead aerial branch	Forlì-Cesena, Italy	E. Camporesi	14.03.2016	KY964206	KY964162	KY964090	-
D. rhusicola	MFLUCC 17-1014	*Platanus hybrida (Platanaceae)*	Dead aerial branch	Forlì-Cesena, Italy	E. Camporesi	27.04.2016	KY964214	KY964170	KY964098	-
D. rudis	MFLUCC 17-1030	*Cornus sp. (Cornaceae)*	Dead aerial branch	Forlì-Cesena, Italy	E. Camporesi	10.11.2014	KY964186	KY964142	KY964069	KY964113
D. rudis	MFLU 15-1264	*Anthoxanthum odoratum (Poaceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	21.05.2015	KY964227	KY964183	KY964110	KY964140
D. rudis	MFLUCC 17-0969	*Carlina vulgaris (Asteraceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	22.04.2015	KY964193	KY964149	KY964077	KY964118
D. rudis	MFLUCC 17-1073	*Dioscorea communis (Dioscoreaceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	28.08.2016	KY964222	KY964178	KY964106	KY964135
D. schoeni	**MFLU 15-1279**	***Schoenus nigricans (Cyperaceae)***	**Dead aerial stem**	**Ravenna, Italy**	**E. Camporesi**	**01.05.2015**	**KY964226**	**KY964182**	**KY964109**	**KY964139**
D. schoeni	MFLU 15-2266	*Carduus sp. (Asteraceae)*	Dead aerial stem	Arezzo, Italy	E. Camporesi	26.06.2015	KY964228	KY964184	KY964111	-
D. schoeni	MFLU 15-2609	*Plantago sp. (Plantaginaceae)*	Dead aerial stem	Forlì-Cesena, Italy	E. Camporesi	25.08.2015	KY964229	KY964185	KY964112	KY964141
D. sterilis	MFLUCC 17-1011	*Cytisus sp. (Fabaceae)*	Dead aerial branch	Forlì-Cesena, Italy	E. Camporesi	04.04.2016	KY964211	**KY964167**	**KY964095**	**KY964126**
D. torilicola	**MFLUCC 17-1051**	***Torilis arvensis (Apiaceae)***	**Dead aerial stem**	**Forlì-Cesena, Italy**	**E. Camporesi**	**21.04.2016**	**KY964212**	**KY964168**	**KY964096**	**KY964127**

Table 3 Isolates from GenBank used in phylogenetic analyses (Fig. 2). Ex-type isolates are in bold.

Species	Isolate	Host	ITS	BT	TEF	CAL
D. acaciigena	**CBS 129521**	*Acacia retinodes*	KC343005	KC343973	KC343731	KC343247
D. alleghaniensis	**CBS 495.72**	*Betula alleghaniensis*	KC343007	KC343975	KC343733	KC343249
D. alnea	**CBS 146.46**	*Alnus* sp.	KC343008	KC343976	KC343734	KC343250
	CBS 159.47	*Alnus sp.*	KC343009	KC343977	KC343735	KC343251
D. ampelina	**CBS 114016**	*Vitis vinifera*	AF230751	JX275452	AY745056	AY230751
	CBS 267.80	*Vitis vinifera*	KC343018	KC343986	KC343744	KC343260
D. amygdali	**CBS 126679**	*Prunus dulcis*	KC343022	KC343990	AY343748	KC343264
	CBS 111811	*Vitis vinifera*	KC343019	KC343987	KC343745	KC343261
D. arctii	**DP0482**	*Arctium lappa*	KJ590736	KJ610891	KJ590776	KJ612133
D. asheicola	**CBS 136967**	*Vaccinium ashei*	KJ160562	KJ160518	KJ160594	KJ160542
	CBS 136968	*Vaccinium ashei*	KJ160563	KJ160519	KJ160595	KJ160543
D. australafricana	**CBS 111886**	*Vitis vinifera*	KC343038	KC344006	KC343764	KC343280
	CBS 113487	*Vitis vinifera*	KC343039	KC344007	KC343765	KC343281
D. baccae	**CBS 136972**	*Vaccinium corymbosum*	KJ160565	No	KJ160597	No
	CPC 20585	*Vaccinium corymbosum*	KJ160564	No	KJ160596	No
D. betulae	**CFCC 50469**	*Betula platyphylla*	KT732950	KT733020	KT733016	KT732997
	CFCC 50470	*Betula platyphylla*	KT732951	KT733021	KT733017	KT732998
D. bicincta	**CBS 121004**	*Juglans* sp.	KC343134	KC344102	KC343860	KC343376
D. biguttusis	**CGMCC 3.17081**	*Lithocarpus glabra*	KF576282	KF576306	KF576257	No
	CGMCC 3.17082	*Lithocarpus glabra*	KF576283	KF576307	KF576258	No
D. canthii	**CBS 132533**	*Canthium inerme*	JX069864	KC843230	KC843120	KC843174
D. cassines	**CPC 21916**	*Cassine peragua*	KF777155	No	KF777244	No
D. celastrina	**CBS 139.27**	*Celastrus scandens*	KC343047	KC344015	KC343773	KC343289
D. chamaeropis	**CBS 454.81**	*Chamaerops humilis*	KC343048	KC344016	KC343774	KC343290
	CBS 753.70	*Spartium junceum*	KC343049	KC344017	KC343775	KC343291
D. cucurbitae	**DAOM42078**	*Cucumis sativus*	KM453210	KP118848	KM45321 1	No
	CBS 136.25	*Arctium sp.*	KC343031	KC343999	KC343757	KC343273
D. cynaroidis	**CBS 122676**	*Protea cynaroides*	KC343058	KC344026	KC343784	KC343300
D. cytosporella	**FAU461**	*Citrus limon*	KC843307	KC843221	KC843116	KC843141
	AR5149	*Citrus sinensis*	KC843309	KC843222	KC843118	KC843287
D. diospyricola	**CPC 21169**	*Diospyros whyteana*	KF777156	No	No	No
D. ellipicola	**CGMCC 3.17084**	*Lithocarpus glabra*	KF576270	KF576291	KF576245	No
	CGMCC 3.17085	*Lithocarpus glabra*	KF576271	KF576292	KF576246	No
D. eres	**AR519**	*Ulmus* sp.	KJ210529	KJ420799	KJ210550	KJ434999
	CBS 138598	*Ulmus sp.*	KJ210521	KJ420787	KJ210545	KJ435027
	CBS 439.82	*Cotoneaster sp.*	FJ889450	JX275437	GQ250341	JX197429
	DLR12A	*Vitis vinifera*	KJ210518	KJ420783	KJ210542	KJ434996
	CBS 587.79	*Pinus pantepella*	KC343153	KC344121	KC343879	KC343395

D. foeniculina	**CBS 111553**	*Foeniculum vulgare*	KC343101	KC344069	KC343827	KC343343
	FAU460	*Citrus limon*	KC843304	KC843218	KC843113	KC843138
	ICMP 12285	*Juglans regia*	KC145853	No	KC145937	No
	AR5151	*Citrus latifolia*	KC843303	KC843217	KC843112	KC843137
	CBS 187.27	*Camellia sinesis*	DQ286287	JX275463	DQ286261	KC843122
	CBS 123208	*Foeniculum valgare*	EU814480	JX275464	GQ250315	KC843125
D. fusicola	**CGMCC 3.17087**	*Lithocarpus glabra*	KF576281	KF576305	KF576256	KF576233
	CGMCC 3.17088	*Lithocarpus glabra*	KF576263	KF576287	KF576238	No
D. garethjonesii	**MFLUCC 12-0542a**	Unknown dead leaf	KT459423	KT459441	KT459457	KT459470
D. gulyae	**BRIP 54025**	*Helianthus annuus*	JF431299	No	JN645803	No
	BRIP 53158	*Helianthus annuus*	JF431284	No	JN645799	No
D. helicis	**AR5211**	*Hedera helix*	KJ210538	KJ420828	KJ210559	KJ435043
D. hickoriae	**CBS 145.26**	*Carya glabra*	KC343118	KC344086	KC343844	KC343360
D. longicicola	**CGMCC 3.17089**	*Lithocarpus glabra*	KF576267	KF576291	KF576242	No
	CGMCC 3.17090	*Lithocarpus glabra*	KF576268	KF576292	KF576243	No
D. mahothocarpus	**CGMCC 3.15181**	*Lithocarpus glabra*	KC153096	KF576312	KC153087	No
	CGMCC 3.15182	*Lithocarpus glabra*	KC153097	No	KC153088	No
D. maritima	**DAOMC 250563**	*Picea rubens*	No	KU574616	No	No
D. neilliae	**CBS 144. 27**	*Spiraea* sp.	KC343144	KC344112	KC343870	KC343386
D. neoarctii	**CBS 109490**	*Ambrosia trifida*	KC343145	KC344113	KC343871	KC343387
D. nothofagi	**BRIP 54801**	*Nothofagus cunninghamii*	JX862530	KF170922	JX862536	No
D. novem	**CBS 127270**	*Glycine max*	KC343155	KC344123	KC343881	KC343397
	CBS 127271	*Glycine max*	KC343157	KC344125	KC343883	KC343399
D. ovoicicola	**CGMCC 3.17093**	*Citrus* sp.	KF576265	KF576289	KF576240	KF576223
	CGMCC 3.17092	*Citrus* sp.	KF576264	KF576288	KF576239	KF576222
D. penetriteum	**CGMCC 3.17532**	*Camellia sinensis*	KP267879	KP293459	KP267953	No
D. phaseolorum	**AR4203**	*Phaseolus vulgaris*	KJ590738	KJ610893	KJ590739	KJ612135
D. phragmitis	**CBS 138897**	*Phragmites australis*	KP004445	KP004507	No	No
D. pulla	**CBS 338.89**	*Hedera helix*	KC343152	KC344120	KC343878	KC343394
D. ravennica	**MFLUCC 15–0479**	*Tamarix* sp.	KU900335	KX432254	KX365197	No
	MFLUCC 15–0480	*Tamarix* sp.	KU900336	KX377688	KX426703	No
D. rhusicola	**CBS 129528**	*Rhus pendulina*	JF951146	No	No	No
D. rudis	**AR3422**	*Laburnum anagyroides*	KC843331	KC843177	KC843090	KC843146
	AR3654	*Rosa canina*	KC843338	KC843184	KC843097	KC843153
	ICMP 16419	*Castanea sativa*	KC145904	No	KC145976	No
	DA244	*Brugmansia sp.*	KC843334	KC843180	KC843093	KC843149
	CBS 113201	*Vitis vinifera*	AY485750	JX275454	GQ250327	JX197445

D. saccarata	**CBS 116311**	*Protearepens*	KC343190	KC344158	KC343916	KC343432
D. salicicola	**BRIP 54825**	*Salix purpurea*	JX862531	JX862531	JX862537	No
D. sojae	**FAU635**	*Glycine max*	KJ590719	KJ610875	KJ590762	KJ612116
	CBS 116019	*Caperonia palustris*	KC343175	KC344143	KC343901	KC343417
	FAU455	*Stokesia laevis*	KJ590712	KJ610868	KJ590755	KJ612109
	DP0601	*Glycine max*	KJ590706	KJ610862	KJ590749	KJ612103
	MAFF 410444	*Cucumis melo*	KJ590714	KJ610870	KJ590757	KJ612111
	BRIP 54033	*Helianthus annuus*	JF431295	No	JN645809	No
D. spartinicola	**CBS 140003**	*Spartium junceum*	KR611879	No	No	No
D. sterilis	**CBS 136969**	*Vaccinium corymbosum*	KJ160579	KJ160528	KJ160611	KJ160548
	CPC 20580	*Vaccinium corymbosum*	KJ160582	KJ160531	KJ160614	KJ160551
D. subclavata	**ZJUD95**	*Citrus* sp.	KJ490630	KJ490451	KJ490509	No
	CGMCC 3.17253	*Citrus grandis*	KJ490618	KJ490439	KJ490497	No
D. ternstroemia	**CGMCC 3.15183**	*Ternstroemia gymnanthera*	KC153098	No	KC153089	No
	CGMCC 3.15184	*Ternstroemia gymnanthera*	KC153099	No	KC153090	No
D. toxica	**CBS 534.93**	*Lupinus angustifolius*	KC343220	KC344188	KC343946	KC343462
	CBS 546.93	*Lupinus sp.*	KC343222	KC344190	KC343948	KC343464
D. vaccinii	**CBS 160.32**	*Vaccinium macrocarpon*	AF317578	JX270436	GQ250326	KC343470
	CBS 122116	*Vaccinium corymbosum*	KC343227	KC344195	KC343953	KC343469
	CBS 135436	*Vaccinium corymbosum*	AF317570	KC843225	JQ807380	KC849456
D. virgiliae	**CMW40748**	*Virgilia oroboides*	KP247566	KP247575	No	No
Diaporthella corylina	**CBS 121124**	*Corylus* sp.	KC343004	KC343972	KC343730	KC343246

Molecular based amplification

Total DNA was extracted from aerial mycelium of 7 day old cultures grown on PDA at 25 C following the modified cetyltrimethyl ammonium bromide (CTAB) method described by Udayanga et al. (2012). Under circumstances where fungi failed to grow in culture, DNA was extracted directly from fruiting bodies using aseptic techniques. For the identification of *Diaporthe*, rDNA internal transcribed spacer (ITS) region was amplified and sequenced for all 44 isolates. The translation elongation factor 1-α (TEF), a portion of the β-tubulin (BT) gene and the calmodulin (CAL) gene were employed to support species identification based on ITS gene sequence data. The rDNA ITS region was amplified using universal primers ITS1 and ITS4 (White et al. 1990). The target region of the TEF gene was amplified using primer pairs EF-728F and EF-986R (Carbone & Kohn 1999). A portion of the BT gene was amplified using the primers BT2a and BT2b (Glass & Donaldson 1995), while the primer pair CAL228F and CAL737R (Carbone & Kohn 1999) was used to amplify the CAL. The PCR reactions were performed in a BIORAD 1000^{TM} thermal cycler in a total volume of 25 μl. The PCR mixture contained TaKaRa Ex-Taq DNA polymerase 0.3 μl, 12.5 μl of 2 × PCR buffer with 2.5 μl of dNTPs, 1 μl of each primer, 9.2 μl of double-distilled water and 100–500 ng of DNA template. DNA samples were detected by electrophoresis and ethidium bromide (EB) staining and were used as templates for PCR amplification. DNA sequencing was performed by Sunbiotech Company, Beijing, China.

Sequence alignment and phylogenetic analyses

All new sequences generated in this study were checked manually and nucleotides at ambiguous positions were clarified with sequences from both strands and aligned with sequences retrieved from GenBank based on recent publications (Liu et al. 2015, Hyde et al. 2016). Combined datasets were aligned using MAFFT (Katoh & Toh 2010, http://mafft.cbrc.jp/alignment/server/) and were manually optimized with BioEdit (Hall 2006) to allow maximum alignment. Maximum Parsimony analysis (MP) was performed with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003). Gaps were treated as missing data, and the ambiguously aligned regions were excluded. Trees were inferred using the heuristic search option with Tree Bisection Reconnection branch swapping and 1000 random sequence additions. Maxtrees was set at 1000, branches of zero length were collapsed, and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony (tree length, consistency index, retention index, rescaled consistency index, and homoplasy index) were calculated for trees generated under different optimality criteria.

The best model of evolution for each gene region was determined with MRMODELTEST v. 2.2 (Nylander 2004), and maximum likelihood analyses were performed in RAXML GUI v. 0.9b2 (Silvestro & Michalak 2010). The RAxML analyses were run with a rapid bootstrap analysis of a random starting tree and 1000 ML bootstrap replicates. The search strategy was set to rapid bootstrapping with one thousand non-parametric bootstrapping iterations using the general time reversible model (GTR) with a discrete gamma distribution. The best scoring trees were selected with final likelihood values. Posterior probabilities (PP) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2003). MrModeltest v. 2.3 (Nylander 2004) was used to perform statistical selection of the best-fit model of nucleotide substitution and was incorporated into the analysis. Six simultaneous Markov chains were run for 1,000,000 generations, and the trees were sampled every 100th generation. The 2000 trees representing the burn-in phase of the analyses were discarded, and the remaining 8000 trees were used for calculating PP in the majority rule consensus tree. The fungal strains isolated in this study are listed in Table 2 with details of the type cultures and sequence data. Novel sequence data were deposited in GenBank (Table 2), alignments in TreeBASE (www.treebase.org, submission no. S20936), and taxonomic novelties in the Faces of Fungi database (Jayasiri et al. 2015) and Index Fungorum (Index Fungorum 2016).

Results

Phylogenetic analyses

The collection of saprobic specimens from numerous woody hosts in Italy (Fig. 1) resulted in the isolation of 44 isolates of *Diaporthe* (Fig. 2). The ITS, TEF, BT and CAL sequences were determined to be approximately 530, 350, 510 and 410 bp, respectively.

The combined ITS, TEF, BT and CAL sequences of *Diaporthe* contained data for 144 isolates, including one outgroup taxon, and consisted of 44 isolates from this study and other sequences originating from GenBank (Table 3). Out of a total of 1998 characters, 882 were constant, and 295 were variable and parsimony uninformative. The remaining 821 parsimony-informative characters resulted in 10 most parsimonious trees (TL = 4190, CI = 0.464, RI = 0.883, RC = 0.410, HI = 0.536) and the best tree is shown in Fig. 2. The maximum parsimony (MP) and Bayesian (BM) analyses produced trees with nearly identical topologies (Bayesian tree not shown). The isolates obtained in this study grouped into 15 distinct clades. The majority (12 isolates) grouped with the ex-epitype isolate of *Diaporthe eres* (AR5193); nine isolates clustered with the ex-epitype of *D. foeniculina* (CBS 111553); four isolates clustered with *D. rhusicola* (CBS 129528) and another four isolates clustered with *D. rudis* (AR3422). Moreover, four isolates grouped each with *D. gulyae* (BRIP 54025), *D. novem* (CBS 127270), *D. ravennica* (MFLUCC 15-0479) and *D. sterilis* (CBS 136969). Eleven isolates did not cluster with any known *Diaporthe* species and thus seven novel species, *D. acericola*, *D. cichorii*, *D. dorycnii*, *D. lonicerae*, *D. pseudotsugae*, *D. schoeni* and *D. torilicola* are introduced based on morphology and phylogenetic placement (Fig. 2).

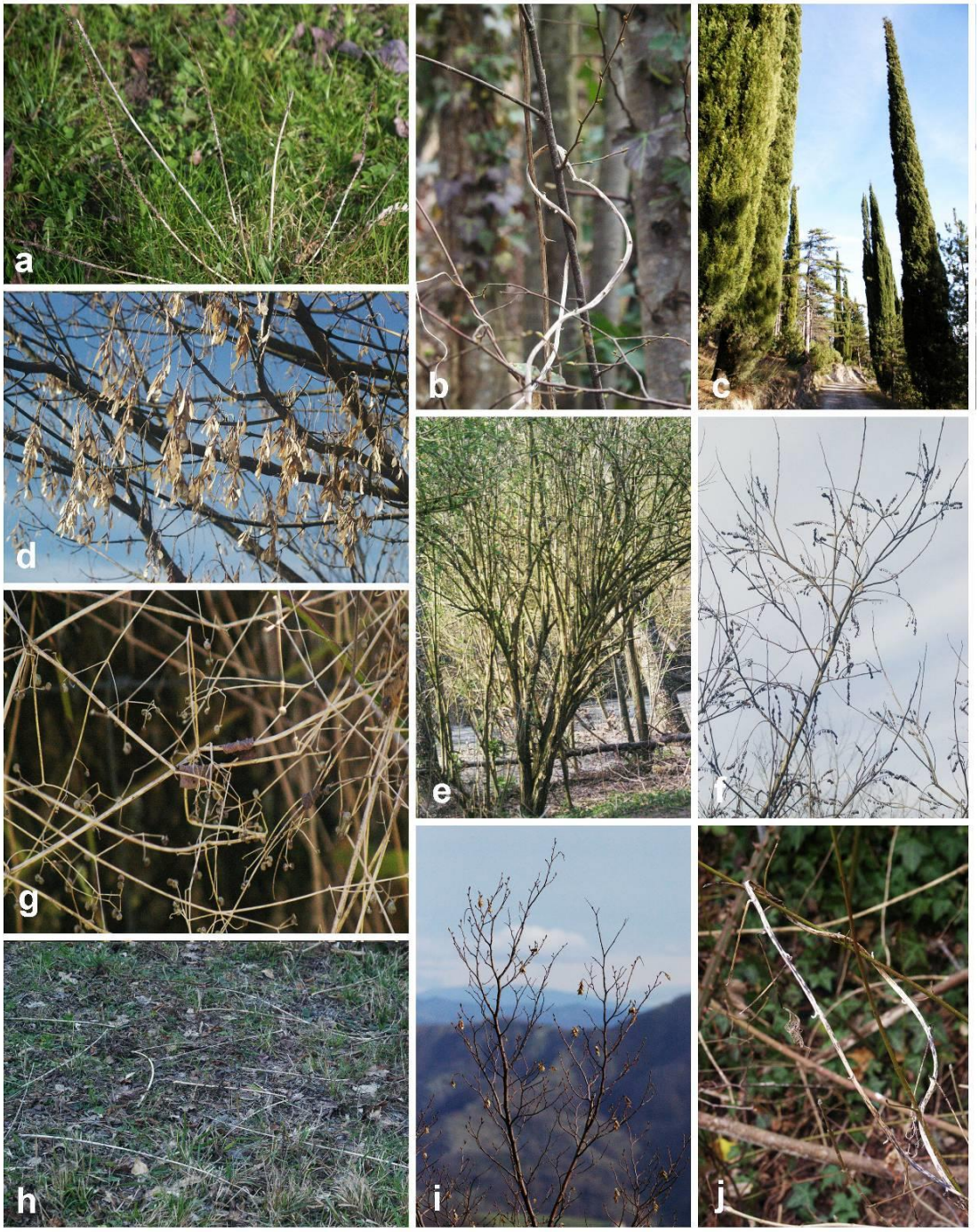
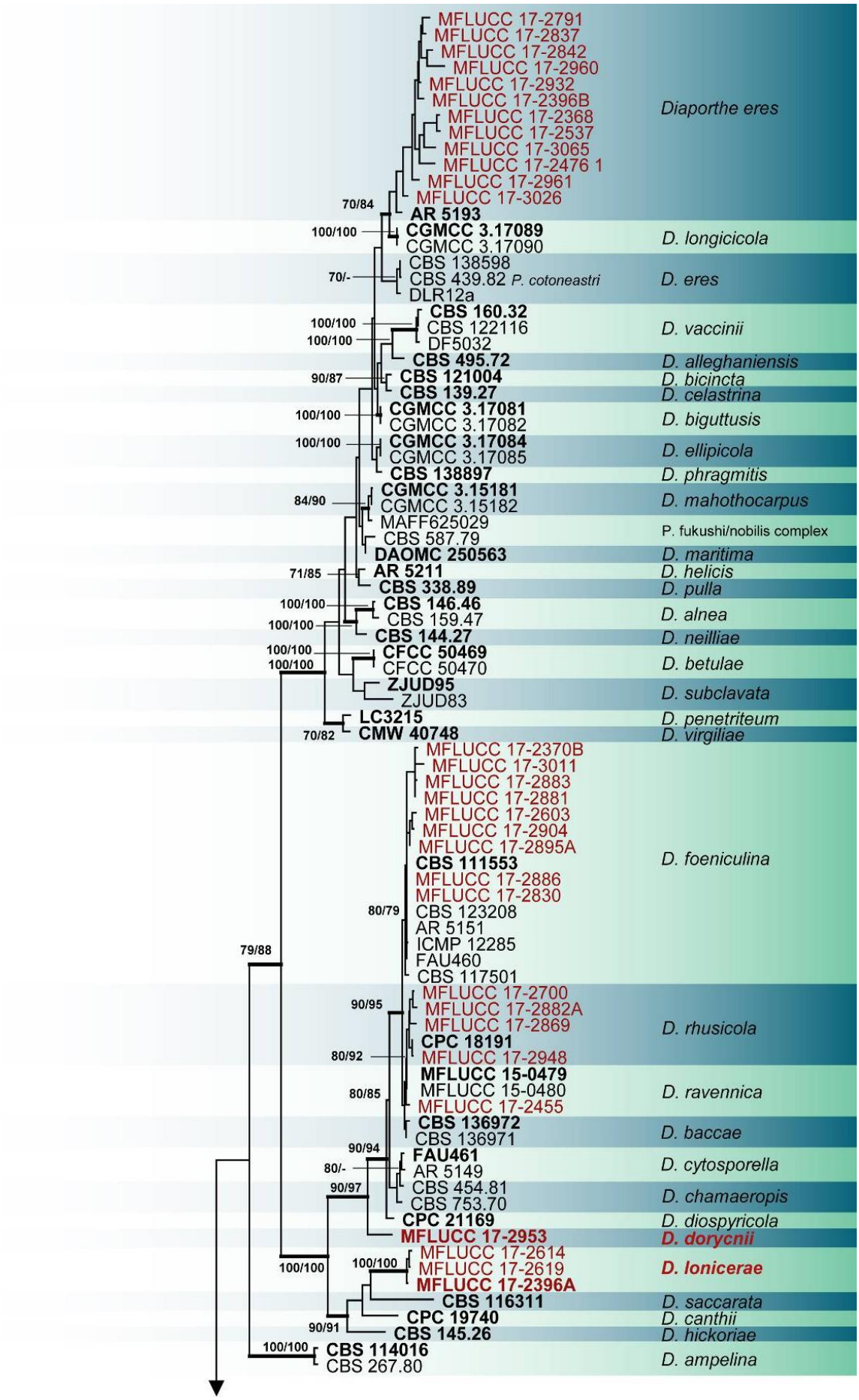


Figure 1 – Habitats of *Diaporthe* species in Italy. **a** *Plantago* sp., **b** *Tamus communis* on *Rubus* sp. and *Ostrya carpinifolia*. **c** *Cupressus sempervirens*. **d** *Acer negundo*. **e** *Sambucus nigra*. **f** *Amorpha fruticosa*. **g** *Galium aparine*. **h** land stem-leaf under *Ailanthus altissima*. **i** *Ostrya carpinifolia*. **j** *Tamus communis* on *Cornus sanguinea*. Photos by Erio Camporesi.

Morphology and culture characteristics

All 44 isolates identified based on the phylogenetic analyses using the combined data comprised 15 *Diaporthe* species (*Diaporthe acericola, D. cichorii, D. dorycnii, D. eres, D. foeniculina, D. gulyae, D. lonicerae, D. novem, D. pseudotsugae, D. ravennica, D. rhusicola, D. rudis, D. schoeni, D. sterilis* and *D. torilicola*) and were further characterized on the basis of colony morphology and conidial characteristics. Growth of all isolates was rapid on PDA, with mycelia covering the entire surface of the Petri dishes. Aerial mycelium was initially white and turned dirty white or greyish after 4–5 days of incubation at 25 C in the dark. For all isolates, structures of the asexual morph appeared within 2–4 weeks of incubation. Sexual structures did not form on PDA throughout the growth period. All species showed morphological features typical of the genus.


MFLUCC 17-2791
MFLUCC 17-2837
MFLUCC 17-2842
MFLUCC 17-2960
MFLUCC 17-2932
MFLUCC 17-2396B
MFLUCC 17-2368
MFLUCC 17-2537
MFLUCC 17-3065
MFLUCC 17-2476 1
MFLUCC 17-2961
MFLUCC 17-3026
Diaporthe eres
70/84
AR 5193
100/100
CGMCC 3.17089
CGMCC 3.17090
D. longicicola
CBS 138598
70/-
CBS 439.82 P. cotoneastri
DLR12a
D. eres
CBS 160.32
100/100
CBS 122116
100/100
DF5032
D. vaccinii
CBS 495.72
D. alleghaniensis
90/87
CBS 121004
D. bicincta
CBS 139.27
D. celastrina
100/100
CGMCC 3.17081
CGMCC 3.17082
D. biguttusis
100/100
CGMCC 3.17084
CGMCC 3.17085
D. ellipicola
CBS 138897
D. phragmitis
84/90
CGMCC 3.15181
CGMCC 3.15182
D. mahothocarpus
MAFF625029
CBS 587.79
P. fukushi/nobilis complex
DAOMC 250563
D. maritima
71/85
AR 5211
D. helicis
CBS 338.89
D. pulla
100/100
CBS 146.46
CBS 159.47
D. alnea
100/100
CBS 144.27
D. neilliae
100/100
CFCC 50469
100/100
CFCC 50470
D. betulae
ZJUD95
ZJUD83
D. subclavata
LC3215
D. penetriteum
70/82
CMW 40748
D. virgiliae
MFLUCC 17-2370B
MFLUCC 17-3011
MFLUCC 17-2883
MFLUCC 17-2881
MFLUCC 17-2603
MFLUCC 17-2904
MFLUCC 17-2895A
CBS 111553
MFLUCC 17-2886
MFLUCC 17-2830
80/79
CBS 123208
AR 5151
ICMP 12285
FAU460
CBS 117501
D. foeniculina
79/88
MFLUCC 17-2700
90/95
MFLUCC 17-2882A
MFLUCC 17-2869
CPC 18191
80/92
MFLUCC 17-2948
D. rhusicola
MFLUCC 15-0479
80/85
MFLUCC 15-0480
MFLUCC 17-2455
D. ravennica
CBS 136972
CBS 136971
D. baccae
90/94
FAU461
80/-
AR 5149
D. cytosporella
90/97
CBS 454.81
CBS 753.70
D. chamaeropis
CPC 21169
D. diospyricola
MFLUCC 17-2953
D. dorycnii
MFLUCC 17-2614
100/100
100/100
MFLUCC 17-2619
MFLUCC 17-2396A
D. lonicerae
CBS 116311
D. saccarata
CPC 19740
D. canthii
90/91
CBS 145.26
D. hickoriae
100/100
CBS 114016
CBS 267.80
D. ampelina

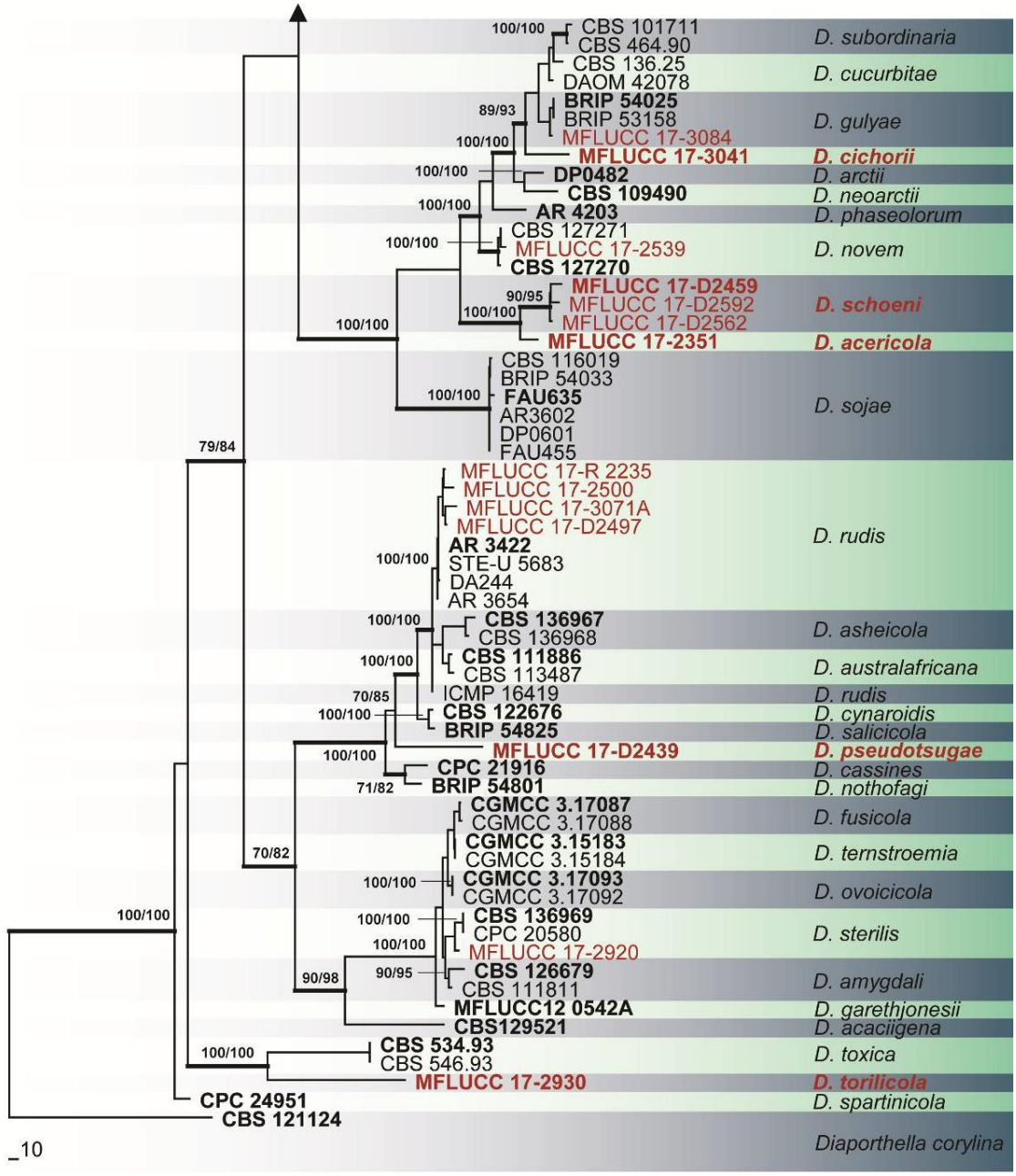


Figure 2 – Phylogram generated from maximum likelihood analysis of *Diaporthe* species isolated in this study and their phylogenetically closely related species based on combined ITS, TEF, BT and CAL sequence data. Parsimony bootstrap support values for ML≥70 %, MP≥70 %, are indicated above the nodes and the branches are in bold indicate Bayesian posterior probabilities ≥0.9. The tree is rooted with *Diaporthella corylina* (CBS 121124). Isolate numbers of ex-types and reference strains are in bold. Taxa isolated in this study are in red and the ex-type isolate numbers of novel species are in bold.

The new species of *Diaporthe* described here are phylogenetically distinct from all previously described species for which sequence data are available.

Taxonomy

Seven undescribed species of *Diaporthe* were recognized by DNA sequence analysis, together with culture morphology, and with description of anamorphic structures. Two of the novel species, *D. pseudotsugae* and *D. schoeni* did not grow under the conditions used in this study and we could not obtain single conidial cultures. Therefore, DNA was extracted directly from the conidiomata/ascomata.

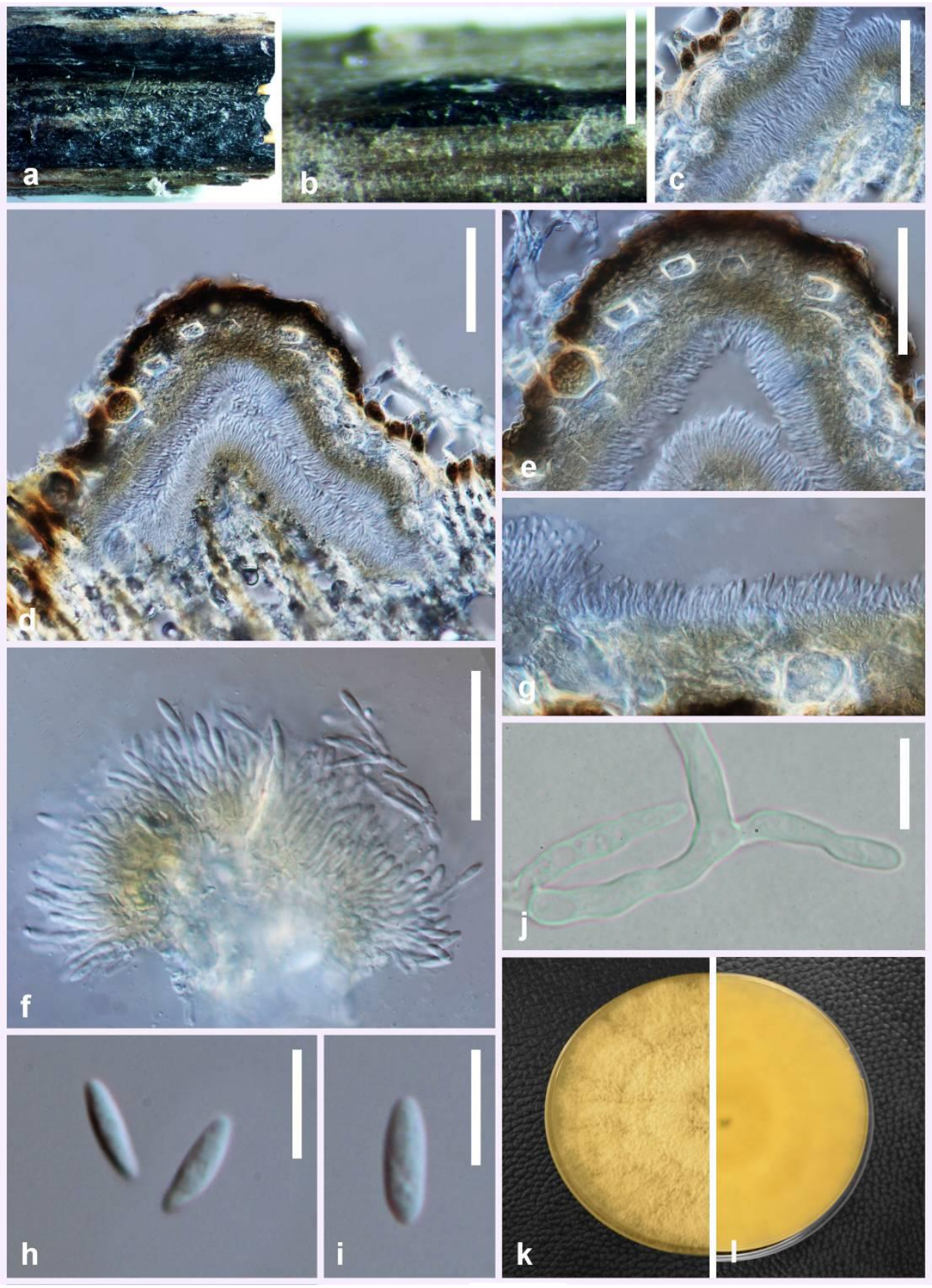


Figure 3 – *Diaporthe acericola* (MFLU 15-3254, holotype). **a, b** Conidiomata on host surface. **d** Cross section of conidioma. **c,e** Peridium. **f, g** Conidia attached to conidiogenous cells. **h, i** Alpha conidia. **j** Germinating spore. **k, l** Culture on PDA after one week. Scale bars: b = 0.5 mm, c–f = 100 µm, g–i = 10 µm.

Diaporthe acericola Dissanayake, Camporesi & K.D. Hyde, ***sp. nov.*** Fig. 3

Index fungorum number: IF553186; Facesoffungi number: FoF 03270

Etymology – The specific epithet *acericola* is based on the host genus (*Acer*).

Holotype – MFLU 15-3254

Saprobic on aerial branch and samaras of *Acer negundo* L. Sexual morph: Not observed. Asexual morph: Conidiomata up to 460 μm in diameter, 285 μm high, superficial, solitary, scattered on host, oval, black. Peridium 65–77 μm thick, inner layer composed of light brown *textura angularis*, outer layer composed of dark brown *textura angularis*. Conidiophores 21–35 ×1.5–2.5 μm ($\bar{x}$ = 27 × 2 μm), cylindrical, aseptate, densely aggregated, straight or sinuous, terminal, slightly tapered towards the apex. Conidiogenous cells 10–15 × 2–3 μm, phialidic, cylindrical, terminal and lateral. Alpha conidia 9.7–13.5 ×3–4.5 μm ($\bar{x}$ = 11 × 4 μm), hyaline, fusiform or oval, both ends obtuse. Beta conidia not observed.

Culture characteristics – Colonies on PDA covering entire Petri dishes after seven days at 25 °C, grey, with scant aerial mycelium; reverse fuscous black. Surface dirty white with profuse aerial mycelium, reverse umber.

Material examined – ITALY, Forlì-Cesena Province, San Colombano – Meldola, on dead aerial branches and samaras of *Acer negundo* (*Sapindaceae*), 22 January 2015, Erio Camporesi; (MFLU 15-3254, holotype); ex-type living culture MFLUCC 17-0956.

Notes – *Diaporthe acericola* forms a sister clade to *D. schoeni* which is also a new species introduced in this study (Fig. 2). However, the two species differed by 62 nucleotides in the concatenated alignment, of which 13 were distinct in the ITS region, 26 in the TEF region, 2 in the BT region and 21 in the CAL region. Morphologically, *D. acericola* differs from *D. schoeni* in having larger conidiomata and smaller conidia (Figs 3, 8). Conidia of *D. acericola* are obtuse at both ends, while the conidia of *D. schoeni* are slightly acute and tapered at both ends.

Diaporthe cichorii Dissanayake, Camporesi & K.D. Hyde, ***sp. nov.*** Fig. 4

Index fungorum number: IF553187; Facesoffungi number: FoF 03271

Etymology – The specific epithet *cichorii* is based on the host genus (*Cichorium*).

Holotype – MFLU 16-2168

Saprobic on dead aerial stem of *Cichorium intybus* L. Sexual morph: Not observed. Asexual morph: Conidiomata up to 540 μm in diameter, 390 μm high, superficial, solitary or aggregated, globose to oval, dark brown to black, clustered in groups of 2-5 conidiomata. Peridium 47–58 μm thick, inner layer composed of light brown *textura angularis*, outer layer composed of dark brown *textura angularis*. Conidiophores 24–37 × 1.5–3 μm ($\bar{x}$ = 29 × 3 μm), cylindrical, aseptate, densely aggregated, straight or sinuous, terminal, slightly tapered towards the apex. Conidiogenous cells 7–10 × 2–3 μm, hyaline, subcylindrical, filiform, straight to curved, tapering towards the apex. Alpha conidia 10–14 × 3–4 μm ($\bar{x}$ = 12 × 3 μm) hyaline, fusiform or oval, both ends obtuse. Beta conidia not observed.

Culture characteristics – Colonies on PDA flat, with an entire edge, mycelium growing in concentric rings, cottony texture, white to smoke-grey; colonies reaching up to 64 mm diameter after one week at 25 °C; reverse buff and isabelline.

Material examined – ITALY, Forlì-Cesena Province, Santa Sofia, on dead aerial stem of *Cichorium intybus* (*Asteraceae*), 17 July 2016, Erio Camporesi; (MFLU 16-2168, holotype); ex-type living culture MFLUCC 17-1023.

Notes – *Diaporthe cichorii* occurs in a clade separate from *D. gulyae*, *D. cucurbitae* and *D. subordinaria* and differs from *D. gulyae* by 19 nucleotides in the concatenated alignment, in which 7 were distinct in the ITS region and 12 in the TEF region. Though the sequences of BT region and CAL region are available for *D. cichorii*, the sequences of those regions are unavailable for *D. gulyae*. Morphologically, the conidiomata of *D. gulyae* are up to 3 mm in diam, whereas in *D. cichorii* they are up to 540 μm in diameter Alpha conidia of *D. gulyae* are smaller (6.5–9 μm) compared to those of *D. cichorii* (10–14 μm).

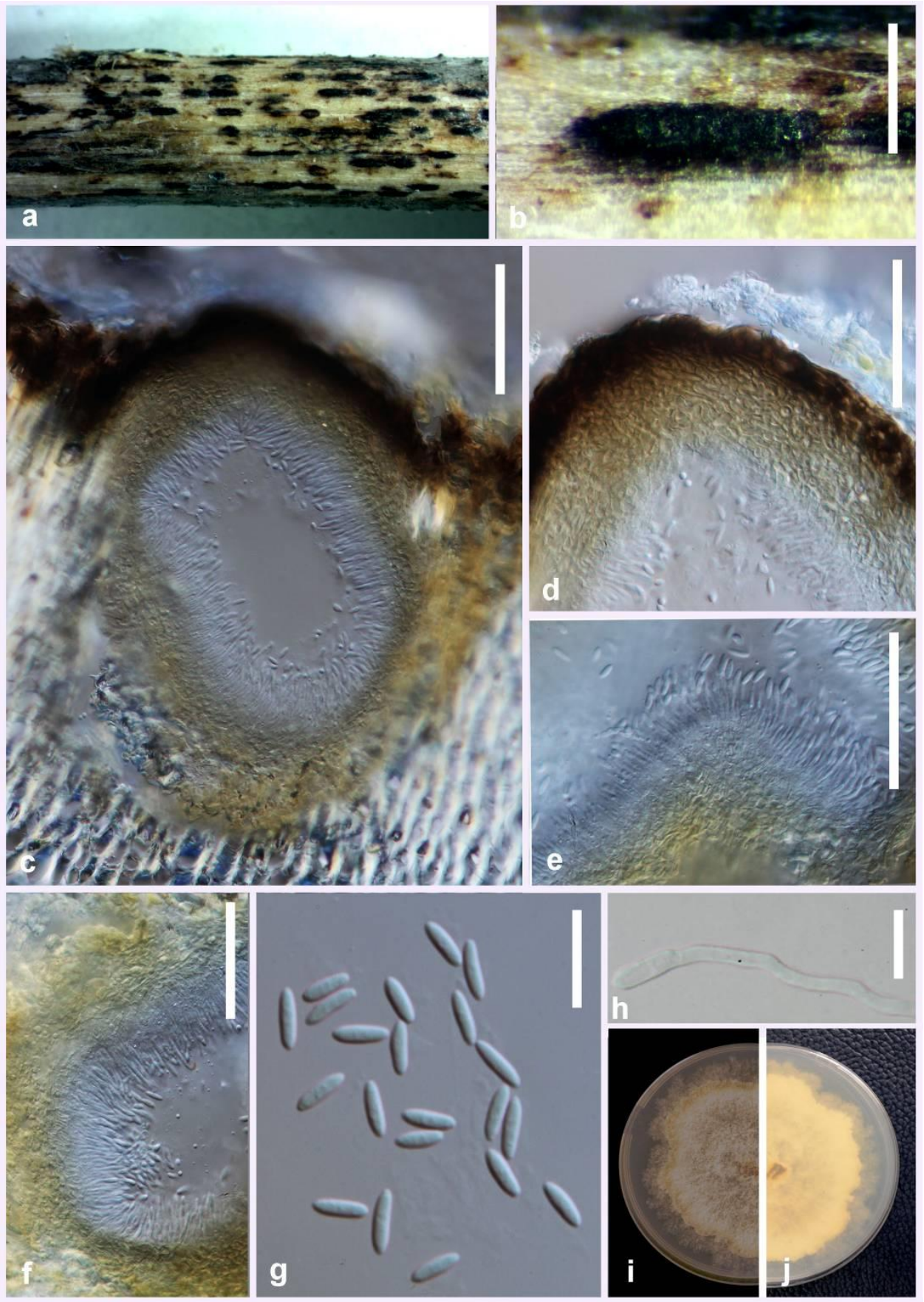


Figure 4 – *Diaporthe cichorii* (MFLU 16-2168, holotype). **a, b** Conidiomata on host surface. **c** Cross section of conidioma. **d** Peridium. **e, f** Conidia attached to conidiogenous cells. **g** Alpha conidia. **h** Germinating spore. **i, j** Culture on PDA after one week. Scale bars: b = 1 mm, c–f = 100 µm, g = 20 µm, h = 10 µm.

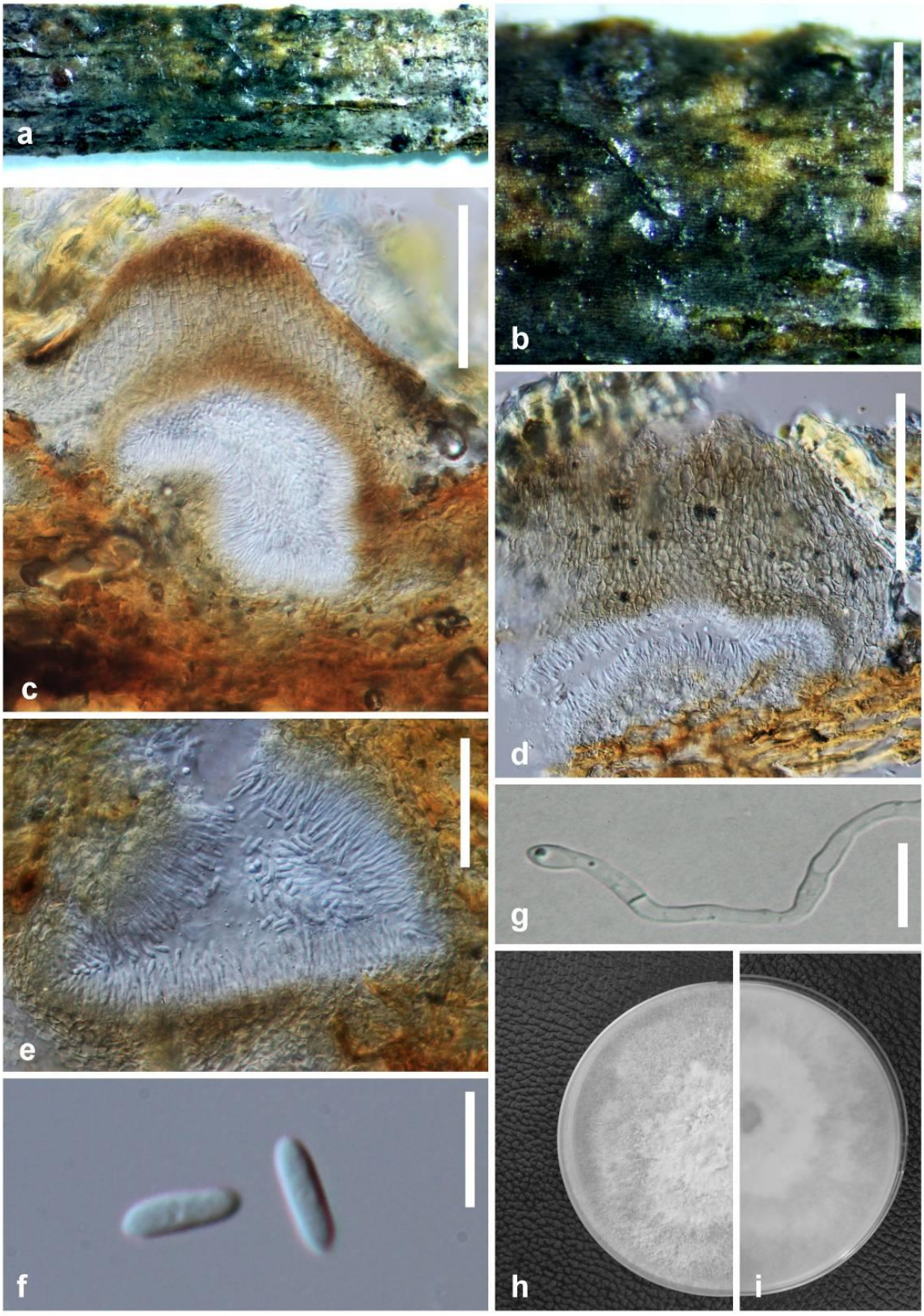


Figure 5 – *Diaporthe dorycnii* (MFLU 16-1322, holotype). **a, b** Conidiomata on host surface. **c** Cross section of conidioma. **d** Peridium. **e,** Conidia attached to conidiogenous cells. **f** Alpha conidia. **g** Germinating conidium. **h, i** Culture on PDA after one week. Scale bars: b = 1 mm, c–e = 100 µm, f = 10 µm, g = 20 µm.

Diaporthe dorycnii Dissanayake, Camporesi & K.D. Hyde, ***sp. nov.*** Fig. 5

Index fungorum number: IF553188; Facesoffungi number: FoF 03272

Etymology – The specific epithet *dorycnii* is based on the host genus (*Dorycnium*).

Holotype – MFLU 16-1322

Saprobic on dead aerial stem of *Dorycnium hirsutum* L. Sexual morph: Not observed. Asexual morph: Conidiomata up to 420 μm in diameter, 380 μm high, superficial or immersed, solitary or gregarious, scattered on host surface, globose, dark brown to black, clustered in groups of 2-5 conidiomata. Peridium 35–50 μm thick, inner layer composed of light brown *textura angularis*, outer layer composed of dark brown *textura angularis*. Conidiophores 21–35 × 1.5–2.5 μm ($\bar{x}$ = 27 × 2 μm), cylindrical, aseptate, densely aggregated, straight or sinuous, terminal, slightly tapered towards the apex. Conidiogenous cells 13–19 × 2–3 μm hyaline, subcylindrical and filiform, straight, tapering towards the apex. Alpha conidia 9–13.5 × 3–4 μm ($\bar{x}$ = 11 × 4 μm) hyaline, biguttulate, fusiform or oval, both ends obtuse. Beta conidia not observed.

Culture characteristics – Colonies on PDA covering entire Petri dishes after 10 days, flat, with an entire edge, aerial mycelium forming concentric rings with cottony texture, white, olivaceous on surface.

Material examined – ITALY, Forlì-Cesena Province, Fiumicello di Premilcuore, on dead aerial stem of *Dorycnium hirsutum* (*Fabaceae*), 2 May 2016, Erio Camporesi; (MFLU 16-1322, holotype); ex-type living culture MFLUCC 17-1015.

Notes – *Diaporthe dorycnii* occurs in a clade separate from *D. diospyricola*, *D. chamaeropsis* and *D. cytosporella* with high bootstrap support (Fig. 2). *Diaporthe diospyricola* differs from *D. dorycnii*, in the presence of beta conidia. Phylogenetically, *D. diospyricola* is the closest species to *D. dorycnii* (Fig. 2), differing by 24 nucleotides in the ITS region. Though the sequences of EF region, BT region and CAL region are available for *D. dorycnii*, the sequences of those regions are unavailable for *D. diospyricola* and thus the nucleotide comparison is incomplete.

Diaporthe lonicerae Dissanayake, Camporesi & K.D. Hyde, ***sp. nov.*** Fig. 6

Index fungorum number: IF553189; Facesoffungi number: FoF 03273

Etymology – The specific epithet *lonicerae* is based on the host genus (*Lonicera*).

Holotype – MFLU 15-3511

Saprobic on dead aerial branch of *Lonicera* sp. Sexual morph: Not observed. Asexual morph: Conidiomata up to 680 μm in diameter, superficial, solitary, scattered on PDA, globose, dark brown to black, clustered in groups of 2–5 pycnidia. Peridium 15–60 μm thick, inner layer composed of light brown *textura angularis*, outer layer composed of dark brown *textura angularis*. Conidiophores 21–35 × 1.5–2.5 μm ($\bar{x}$ = 27 × 2 μm), cylindrical, aseptate, densely aggregated, straight or sinuous, terminal, slightly tapered towards the apex.

Conidiogenous cells 8–11 × 2–3 μm hyaline, subcylindrical, straight to curved, tapering towards the apex. Alpha conidia 12.5–16 × 3.5–4 μm ($\bar{x}$ = 14.5 × 4 μm) hyaline, biguttulate, fusiform or oval, both ends obtuse. Beta conidia 32–39 × 1–1.5 μm ($\bar{x}$ = 36 × 1.5 μm) hyaline, aseptate, filiform, hamate, tapering towards both ends.

Culture characteristics – Colonies on PDA covering entire Petri dishes after 10 days, flat, with an entire edge, aerial mycelium forming irregular concentric rings with cottony texture, olivaceous-buff, isabelline to honey on surface.

Material examined – ITALY, Forlì-Cesena Province, Predappio Alta, on dead aerial branch of *Lonicera* sp. (*Caprifoliaceae*), 28 Febrary 2015, Erio Camporesi; (MFLU 15-3511, holotype); ex-type living culture MFLUCC 17-0963.

Notes – *Diaporthe lonicerae* clusters closer to *D. saccarata*, *D. canthi* and *D. hickoriae*. Phylogenetically, *D. saccarata* is the closest species to *D. lonicerae* (Fig. 2), differing by 107 nucleotides in the concatenated alignment, in which 19 were distinct in the ITS region, 34 in the TEF region, 16 in the BT region and 38 in the CAL region. Both species possess beta conidia and morphologically, *D. saccarata* differs from *D. lonicerae*, in having 1-septate alpha conidia (Mostert et al. 2001).

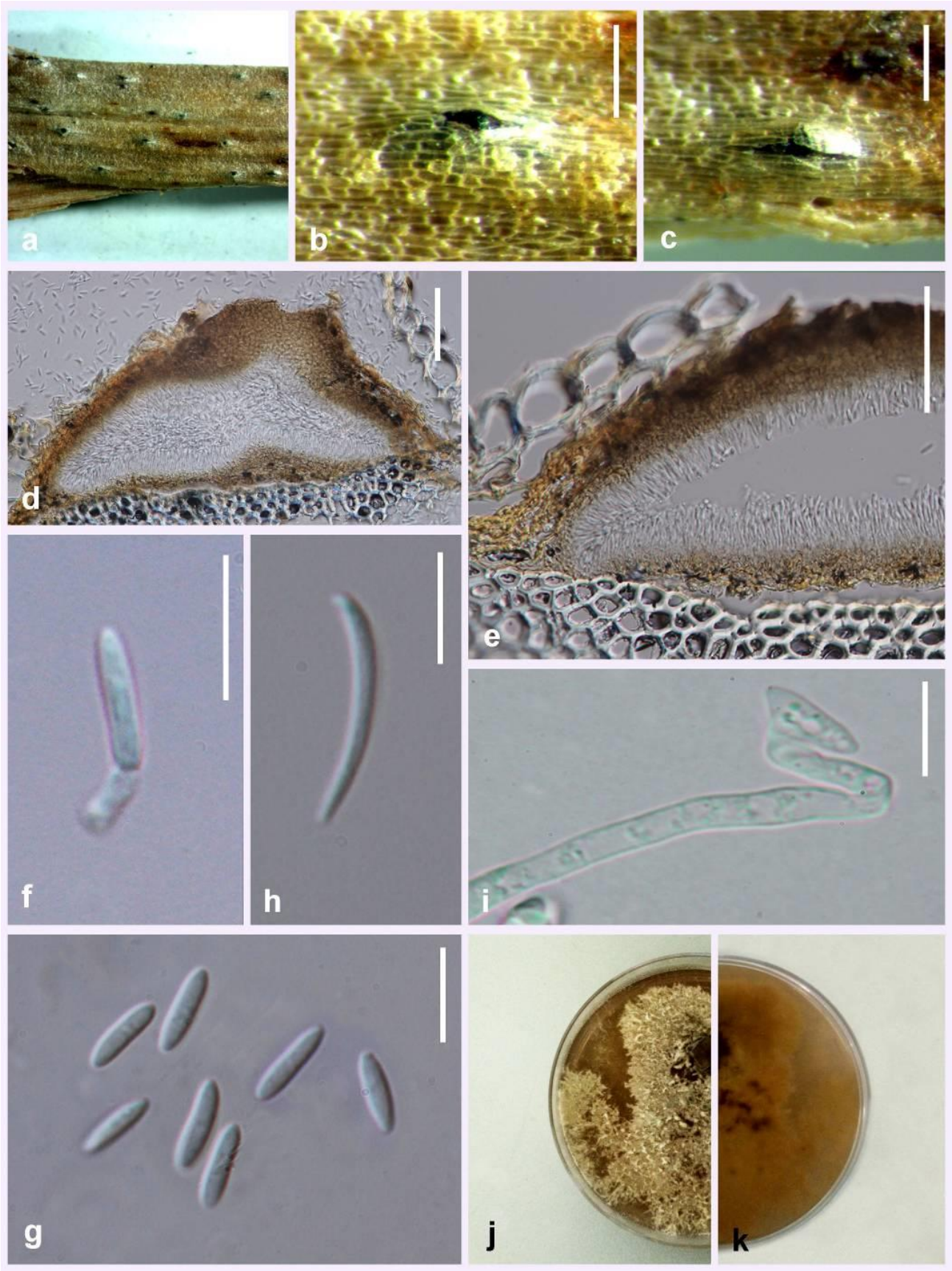


Figure 6 – *Diaporthe lonicerae* (MFLU 15-3511, holotype). **a**–**c** Conidiomata on host surface. **d** Cross section of conidioma. **e** Peridium. **f** Alpha conidium attached to conidiogenous cells. **g** Alpha conidia. **h** Beta conidium. **i** Germinating conidium. **j, k** Culture on PDA after two weeks. Scale bars: b, c = 1 mm, d, e = 100 µm, f, g = 15 µm.

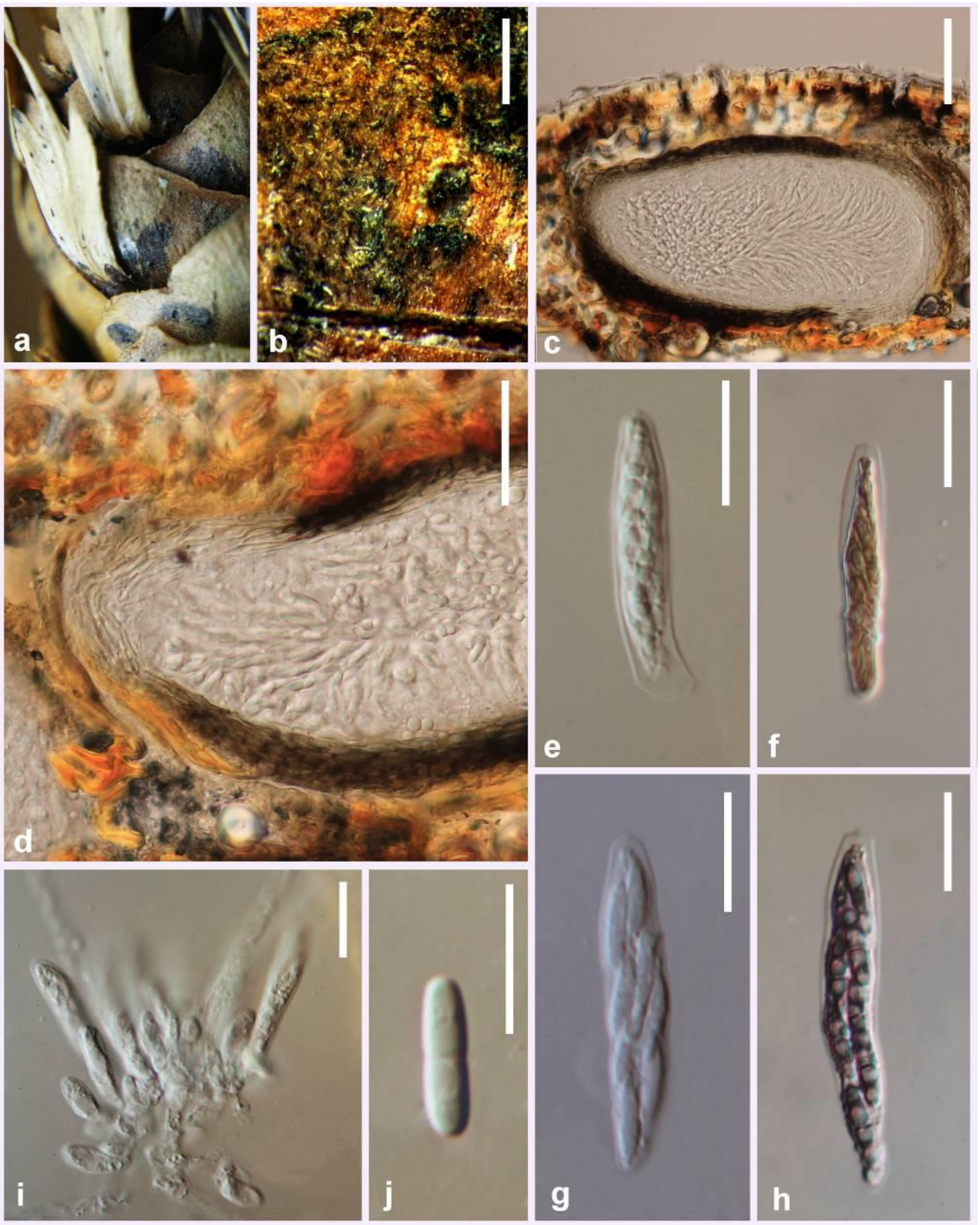


Figure 7 – *Diaporthe pseudotsugae* (MFLU 15-3228, holotype). **a, b** Ascomata on host surface. **c** Cross section of ascoma. **d** Peridium. **e** Immature ascus. **f** Immature ascus immersed in Indian ink. **g** Mature ascus. **h** Mature ascus mounted in methylene blue. **i** Cluster of immature asci. **j** ascospore. Scale bars: b = 1 mm, c, d = 100 µm, e–i = 30 µm, j = 20 µm.

Diaporthe pseudotsugae Dissanayake, Camporesi & K.D. Hyde, ***sp. nov.*** Fig. 7

Index fungorum number: IF553190; Facesoffungi number: FoF 03274

Etymology – The specific epithet *pseudotsugae* is based on the host genus (*Pseudotsuga*).

Holotype – MFLU 15-1274

Saprobic on dead land cones of *Pseudotsuga menziesii* (Mirb.). Sexual morph: Ascomata up to 465 μm in diameter, 255 μm high, black, globose to oval, clustered in groups, deeply immersed in host tissue protruding through substrata. Peridium 28–42 μm thick, inner layer composed of light brown *textura angularis*, outer layer composed of dark brown *textura angularis*. Asci 60–85 × 21–37 μm ($\bar{x}$ = 75 × 29 μm), unitunicate, 8-spored, sessile, elongate to clavate. Ascospores 19–21 × 6–8 μm ($\bar{x}$ = 20 × 7 μm), hyaline, two-celled, often 4-guttulate, with larger guttules at centre and smaller ones at the ends, elongated to elliptical. Asexual morph: Not observed.

Material examined – ITALY, Forlì-Cesena Province, Premilcuore, on dead land cones of *Pseudotsuga menziesii* (*Pinaceae*), 10 April 2015, Erio Camporesi; (MFLU 15-1274, holotype).

Notes – We could not obtain a culture from single ascospore. Therefore, DNA was extracted directly from the ascomata. *Diaporthe pseudotsugae* occurs in a clade separate from *D. salicicola*, *D. cynaroidis*, *D. cassines* and *D. nothofagi*. Although *D. pseudotsugae* is a sexual morph, none of the above mentioned species possess any sexual morph. Phylogenetically, *D. cassines* is the closest species to *D. pseudotsugae* (Fig. 2), differing by 64 nucleotides in the concatenated alignment, in which 34 were distinct in the ITS region, 30 in the TEF region. Though the sequences of BT region and CAL region are available for *D. pseudotsugae,* the sequences of those regions are unavailable for *D. cassines*.

Diaporthe schoeni Dissanayake, Camporesi & K.D. Hyde, ***sp. nov.*** Fig. 8

Index fungorum number: IF553191; Facesoffungi number: FoF 03275

Etymology – The specific epithet *schoeni* is based on the host genus (*Schoenus*).

Holotype – MFLU 15-1279

Saprobic on dead aerial stem of *Schoenus nigricans* L. Sexual morph: Not observed. Asexual morph: Conidiomata up to 210 μm in diameter, 110 μm high, immersed, solitary or gregarious, scattered on host surface, globose to oval, dark brown to black. Peridium 9–32 μm thick, inner layer composed of light brown *textura angularis*, outer layer composed of dark brown *textura angularis*. Conidiophores absent, Conidiogenous cells 21–35 × 1.5–2.5 μm ($\bar{x}$ = 27 × 2 μm), cylindrical, aseptate, densely aggregated, straight or sinuous, terminal, slightly tapered towards the apex. Alpha conidia 11–14.5 × 2–3 μm ($\bar{x}$ = 13.5 × 3 μm), hyaline, fusiform or oval, both ends slightly acute and tapered. Beta conidia 21–33 × 1–1.5 μm ($\bar{x}$ = 27 × 1.5 μm), rarely found among alpha conidia, hyaline, aseptate, filiform, hamate, tapering towards both ends.

Material examined – ITALY, Ravenna Province, Lido di Dante, on dead aerial stem of *Schoenus nigricans* (*Cyperaceae*), 1 May 2015, Erio Camporesi; (MFLU 15-1279, holotype).

Notes – We could not obtain a culture from single conidia. Therefore, fungal DNA was extracted directly from the conidiomata. Three isolates of *D. schoeni* were isolated from three different hosts, *Carduus* sp. (*Asteraceae*), *Plantago* sp. (*Plantaginaceae*) and *Schoenus nigricans* (*Cyperaceae*). However, any of those isolates were failed to germinate. *Diaporthe schoeni* occurs in a clade closer to *D. acericola* (Fig. 2). Both species can be differentiated by smaller conidiomata and larger conidia of *D. schoeni*. Conidia of *D. acericola* are obtuse at both ends, while the conidia of *D. schoeni* are slightly acute and tapered at both ends (Figs 3, 8). Phylogenetically, *D. schoeni* differs from *D. acericola* by 62 nucleotides in the concatenated alignment, of which 13 were distinct in the ITS region, 26 in the TEF region, 2 in the BT region and 21 in the CAL region.

Diaporthe torilicola Dissanayake, Camporesi & K.D. Hyde, ***sp. nov.*** Fig. 9

Index fungorum number: IF553192; Facesoffungi number: FoF 03276

Etymology – The specific epithet *torilicola* is based on the host genus (*Torilis*).

Holotype – MFLU 16-1166

Pathogenic on *Torilis arvensis* (Huds.). Sexual morph: Not observed. Asexual morph: Conidiomata up to 300 μm in diameter, superficial, solitary, scattered on PDA, globose, dark brown to black, clustered in groups of 2–5 pycnidia. Peridium 16–20 μm thick, inner layer composed of light brown *textura angularis*, outer layer composed of dark brown *textura angularis*. Conidiophores 21–35 × 1.5–2.5 μm ($\bar{x}$ = 27 × 2 μm), cylindrical, aseptate, densely aggregated,

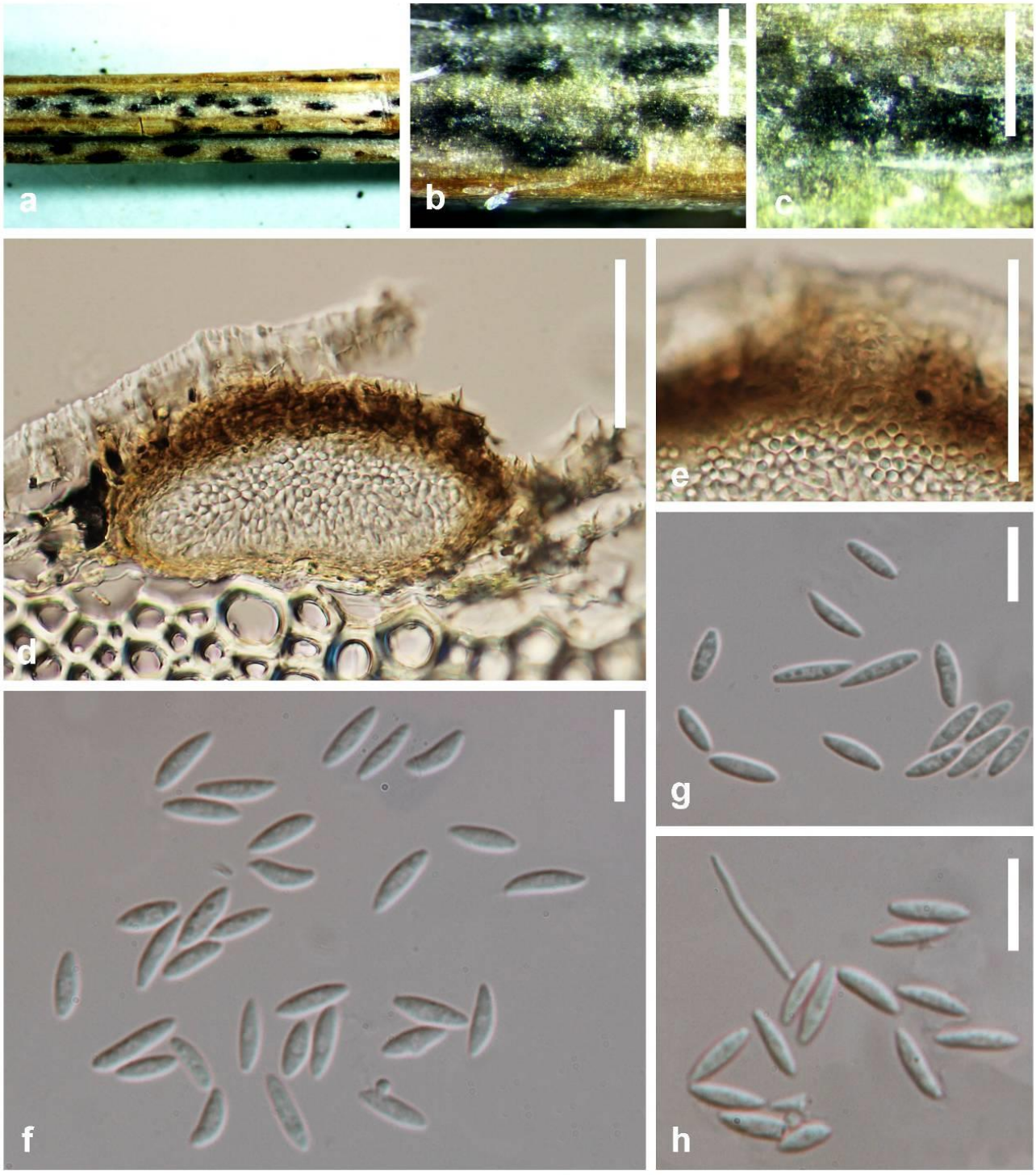


Figure 8 – *Diaporthe schoeni* (MFLU 15-1279, holotype). **a**–**c** Conidiomata on host surface. **d** Cross section of conidiomata. **e** Peridium. **f, g** Alpha conidia. **h** Alpha conidia with a beta conidium. Scale bars: b, c = 1 mm, d,e = 100 µm, f–h = 15 µm.

straight or sinuous, terminal, slightly tapered towards the apex. Alpha conidia 6–8.5 × 2–3 µm ($\bar{x}$ = 8 × 3 µm) hyaline, biguttulate, fusiform or oval, both ends obtuse. Beta conidia 18–37 ×1–1.5 µm ($\bar{x}$ = 27 × 1.5 µm) hyaline, aseptate, filiform, hamate, guttulate, tapering towards both ends.

Culture characteristics – Colonies on PDA covering entire Petri dishes after 10 days, grey, with scant aerial mycelium; reverse fuscous black. Colonies on PDA flat, with entire edge, cottony, olivaceous buff, with aerial mycelium in concentric rings, with olivaceous patches; colonies reaching entire petri dish after 2 wk at 25 °C; reverse olivaceous buff and greenish olivaceous.

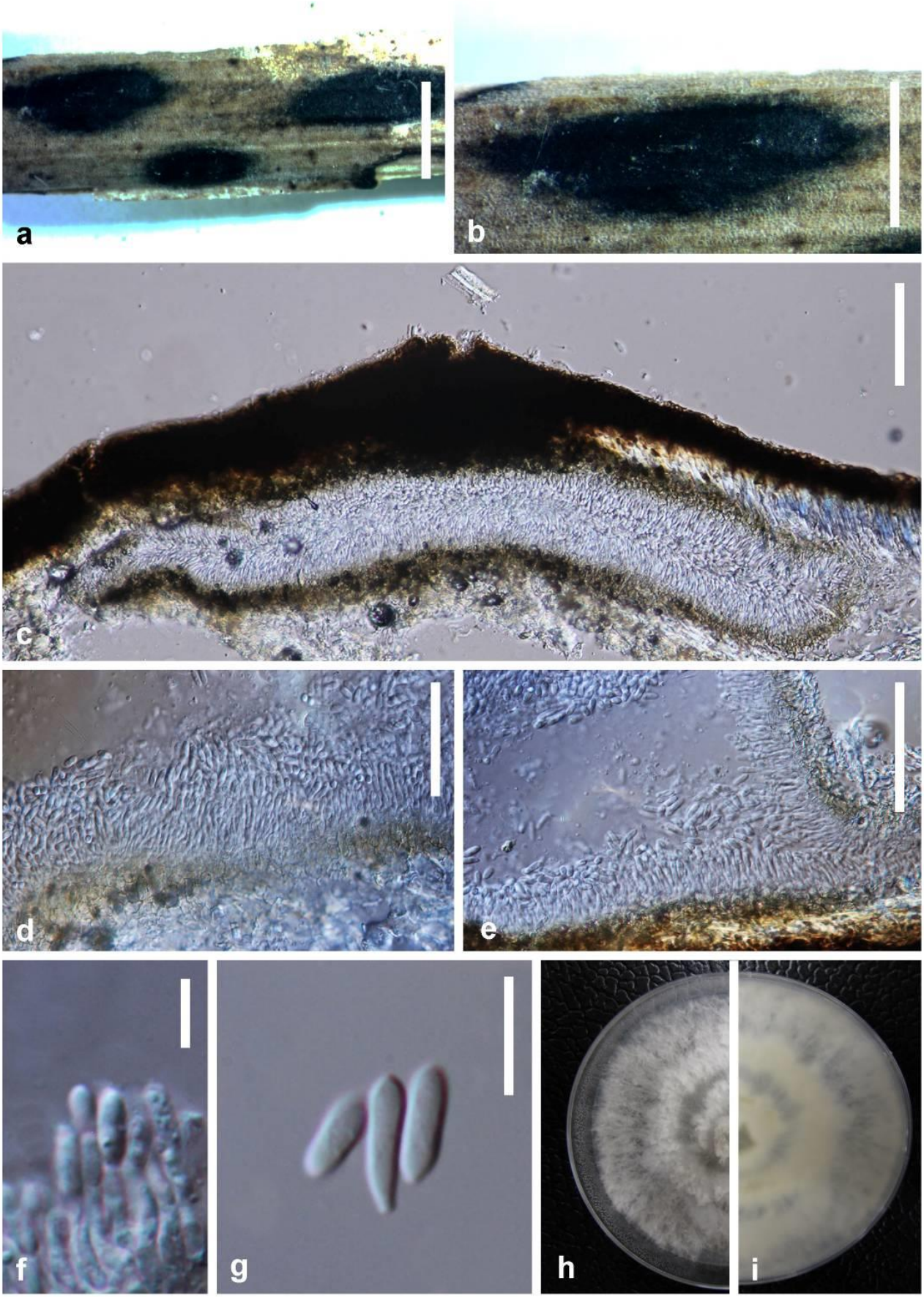


Figure 9 – *Diaporthe torilicola* (MFLU 16-1166, holotype). **a, b** Conidiomata on host surface. **c** Cross section of conidiomata. **d–f** Conidia attached to conidiogenous cells. **g** Alpha conidia. **h, i** Culture on PDA after one week. Scale bars: a, b = 0.5 mm, c–e = 100 µm, f, g = 10 µm.

Material examined – ITALY, Forlì-Cesena Province, Monte Pallareto - Meldola dead aerial stem of *Torilis arvensis* (*Apiaceae*), 12 April 2016, Erio Camporesi; (MFLU 16-1166, holotype); ex-type living culture MFLUCC 17-1051.

Notes – In the phylogenetic analysis, *D. torilicola* forms a sister clade to *D. toxica*. Williamson et al. (1994) designated the name *D. toxica* for the sexual state of the toxicogenic variety, *P. leptostromiformis var. leptostromiformis*. Phylogenetically, *D. toxica* differs from *D. torilicola* by 92 nucleotides in the concatenated alignment, in which 26 were distinct in the ITS region, 27 in the TEF region, 17 in the BT region and 22 in the CAL region.

Discussion

Studies on *Diaporthe*, dealing with the phylogenetic traits and morphology of isolates associated with various hosts, have increased in recent years, enabling the worldwide identification of taxa at the species level (Gomes et al. 2013, Udayanga et al. 2014a, b). In this study, seven new species have been described in *Diaporthe*, on the basis of morphological and molecular characteristics. Two of the novel species, *D. pseudotsugae* and *D. schoeni* did not grow under the conditions of this study and single spore isolates could not be obtained. In addition to the new species, eight known species of *Diaporthe* (*D. eres*, *D. foeniculina*, *D. gulyae*, *D. novem*, *D. ravennica*, *D. rhusicola*, *D. rudis* and *D. sterilis*) were identified. Apart from *D. eres*, *D. foeniculina* and *D. ravennica*; none of the other species were identified in previous studies on Italian hosts, which probably implies an association with geographic origin and/or host species. A phylogenetic tree derived from an alignment of ITS sequences is beneficial as a guide for identification of isolates of *Diaporthe* species (Udayanga et al. 2012, Tan et al. 2013). ITS sequences offer convincing proof for species demarcation where a limited number of taxa are analyzed, such as species associated with the same host (Santos & Phillips 2009, Santos et al. 2011, Thompson et al. 2011). However, confusion arises when a large number of species from an extensive range of host species are examined. Santos et al. (2010) proposed that TEF is a superior phylogenetic marker in *Diaporthe* than ITS, and has been commonly used as a secondary locus for phylogenetic studies (Santos et al. 2011, Udayanga et al. 2012, Dissanayake et al. 2015). Gomes et al. (2013) studied five loci from 95 species. They stated that TEF poorly distinguished species, and recommended that histone and BT were suitable possibilities as subordinate phylogenetic markers to accompany the authorized fungi barcode, ITS. In this study, a combined four gene analyses of ITS, TEF, BT and CAL was used to study eight known *Diaporthe* species and to assist in the introduction of seven new *Diaporthe* species.

Diaporthe eres was the most frequent species in the present study, comprising 27% of the isolates, and was associated with *Galega officinalis* (*Fabaceae*), *Juglans regia* (*Juglandaceae*), *Lonicera* sp. (*Caprifoliaceae*), *Ostrya carpinifolia* (*Betulaceae*), *Picea excels* (*Pinaceae*), *Pinus pinaster* (*Pinaceae*), *Populus nigra* (*Salicaceae*), *Rhamnus alpinus* (*Rhamnaceae*), *Salix caprea* (*Salicaceae*), *Sambucus nigra* (*Adoxaceae*), *Sanguisorba minor* (*Rosaceae*) and *Sonchus oleraceus* (*Asteraceae*) in the provinces of Arezzo and Forlì-Cesena (Table 2). In all of the studies conducted in Italy, involving gene sequencing, this species was detected as the most common, but not the most virulent (Gomes et al. 2013, Cinelli et al. 2016, Udayanga et al. 2015). Phylogenetic studies indicated that, as well as the aforementioned phenomenon, there is low posterior probability support between the internal branches of the *D. eres* clade, indicating a large intraspecific diversity in this species (Gomes et al. 2013, Dissanayake et al. 2017a, b). After several phylogenetic studies of *D. eres*, from 2005 to the present day, including sampled plants and areas previously unexplored, it was shown how this morphological species is complex, harbouring several cryptic species with various hosts in different geographical locations (Crous 2005, Gao et al. 2016, Gomes et al. 2013, Dissanayake et al. 2015, 2017a, Udayanga et al. 2014b).

Diaporthe foeniculina was the second most common species, with 20% of the isolates in this study, and was associated with *Achillea millefolium* (*Asteraceae*), *Ailanthus altissima* (*Simaroubaceae*), *Arctium minus* (*Asteraceae*), *Cupressus sepervirens* (*Cupressaceae*), *Hemerocallis fulva* (*Hemerocallidoiceae*), *Lunaria rediviva* (*Brassicaceae*), *Melilotus officinalis*

(*Fabaceae*), *Vicia* sp. (*Fabaceae*) and *Wisteria sinensis* (*Fabaceae*) in the provinces of Arezzo and Forlì-Cesena (Table 2). Recently, *D. foeniculina* was epitypified by Udayanga et al. (2014a) and the utility of individual genes for accurate circumscription of this species was assessed. *Diaporthe foeniculina*, including the synonym *D. neotheicola*, is recognized as a species with an extensive host range (Udayanga et al. 2014a). Regarding *D. neotheicola*, this species has been reported to cause diseases of temperate and tropical fruits in Australia, Europe and South Africa (Golzar et al. 2012, Thomidis et al. 2013).

Diaporthe rudis was isolated from *Anthoxanthum odoratum* (*Poaceae*), *Carlina vulgaris* (*Asteraceae*), *Cornus* sp. (*Cornaceae*) and *Dioscorea communis* (*Dioscoreaceae*). Since its description, this species has been identified around the world as being associated with numerous hosts (Udayanga et al. 2014a, Chen et al. 2014a, b, Huang et al. 2015, Lombard et al. 2014), which highlights its high degree of dissemination, distribution and wide host range, similar to *D. eres*. Udayanga et al. (2014a) determined *D. viticola* to be a synonym of *D. rudis*, which was previously recognized as a distinct taxon.

Diaporthe rhusicola occurred at the same frequency as *D. rudis*, with 9% of isolates taken from dead aerial stem or branch in *Amorpha fruticosa* (*Fabaceae*), *Angelica sylvestris* (*Apiaceae*), *Platanus hybrida* (*Platanaceae*) and *Rubus* sp. (*Rosaceae*). *Diaporthe rhusicola* was described and first reported in South Africa as causing leaf spots of *Rhus pendulina* (Crous et al. 2011) and was subsequently proved to be pathogenic in English walnut (Chen et al. 2014b) and pistachio in California (Chen et al. 2014a). The other known *Diaporthe* species (*D. gulyae*, *D. novem*, *D. ravennica* and *D. sterilis*) were isolated from Italian hosts *Heracleum sphondylium* (*Apiaceae*), *Galium* sp. (*Rubiaceae*), *Salvia* sp. (*Lamiaceae*) and *Cytisus* sp. (*Fabaceae*) respectively.

Except for *D. lonicerae* and *D. schoeni*, all other novel species identified in this study were associated with only one host (Table 2). *Diaporthe acericola*, *D. cichorii*, *D. dorycnii*, *D. pseudotsugae*, and *D. torilicola* were isolated from *Acer negundo* (*Sapindaceae*), *Cichorium intybus* (*Asteraceae*), *Dorycnium hirsutum* (*Fabaceae*), *Pseudotsuga menziesii* (*Pinaceae*) and *Torilis arvensis* (*Apiaceae*) respectively. *Diaporthe lonicerae* was isolated from *Lonicera* sp. (*Caprifoliaceae*), *Laurus nobilis* (*Lauraceae*) and *Torilis arvensis* (*Apiaceae*) in Forlì-Cesena province, while *D. schoeni* was isolated from *Schoenus nigricans* (*Cyperaceae*), *Carduus* sp. (*Asteraceae*) and *Plantago* sp. (*Plantaginaceae*) in Arezzo, Forlì-Cesena and Ravenna provinces.

The discovery of these species of *Diaporthe* on diverse hosts and in different geographical localities in Italy as well as worldwide shows the polyphagous and cosmopolitan behavior of species in this genus. Certainly, it is obvious that performing complementary studies based on sequencing at least four gene regions of *Diaporthe* species is essential in order to support reliable species identification. Such studies are necessary to investigate this group of fungi in different unexploited biomes, to reveal the degree of diversity and to support more suitable control measures to prevent their dissemination.

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