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Cryptosphaeria multicontinentalis, a new record for the funga of Iran

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Abstract

During collecting and identifying of *Xylariales* fungi (Ascomycota) of Iran, a specimen was collected from Siahkal forest (Guilan Province, northern Iran) in autumn 2021. Based on morphological and molecular (ITS sequence) data, this fungus was identified as *Cryptosphaeria multicontinentalis*, which is the first report for the funga of Iran. Detailed taxonomic and phylogenetic information related to this species is described and illustrated. In addition, an updated list of species of *Diatrypaceae* is provided from Iran.

Keywords: Biodiversity, mycobiota, *Sordariomycetes*, taxonomy, *Xylariales*

نخستین گزارش از *Cryptosphaeria multicontinentalis* برای قارچ‌های ایران*

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خلاصه

طی نمونه‌برداری و شناسایی قارچ‌های *Xylariales* (Ascomycota) در ایران، نمونه‌ای از جنگل‌های سیاهکل واقع در استان گیلان، در پاییز ۱۴۰۰ جمع‌آوری شد. براساس شواهد ریخت‌شناسی و مولکولی (توالی ناحیه ITS)، این قارچ به عنوان *Cryptosphaeria multicontinentalis* شناسایی شد که نخستین گزارش برای قارچ‌های ایران است. در مقاله حاضر، اطلاعات مرتبط با آرایه‌شناسی و مولکولی این گونه مورد بحث و بررسی قرار گرفته است. همچنین، در بررسی حاضر، فهرست جدیدی از گزارش‌های موجود در ایران از تیره *Diatrypaceae* ارایه شده است.

واژه‌های کلیدی: آرایه‌بندی، تنوع زیستی، میکوبیوتا، *Sordariomycetes*، *Xylariales*

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Introduction

Xylariales (Ascomycota) is a fungal order comprising, amongst others, the large family *Diatrypaceae*, known as saprobes, pathogens and endophytes on economic crops and forest trees worldwide, also occurring in aquatic habitats. There are 27 genera and approximately 1100 species accommodated in this family (Samarakoon *et al.* 2022, Li *et al.* 2023, http://www.indexfungorum.org, accessed Mar. 2023). Like commonly seen in ascomycetes, the life cycle of *Diatrypaceae* usually involves a sexual (teleomorph) as well as an asexual morph (anamorph). The teleomorphs of the family are characterized by perithecial ascomata immersed in or erumpent from the substrate with a poorly to well-developed stroma and usually with more or less pronounced ostiolar necks. The hymenium consists of paraphyses and octo- or polysporous unitunicate asci with an inamyloid or amyloid (I-/I+) apical ring. Ascospores are usually allantoid, subhyaline, and aseptate. Known anamorphs of *Diatrypaceae* are pycnidial, acervular, or comprise free conidiophores; conidia are more or less allantoid, (sub-) hyaline and aseptate as well (Trouillas *et al.* 2010, de Almeida *et al.* 2016).

In recent years, several molecular phylogenetic investigations clarified some phylogenetic relationships within *Diatrypaceae*. Based on rDNA-ITS sequences, Acero *et al.* (2004) published the first more detailed molecular phylogenetic investigation of the family but the did not find any correlation between the resulting clades and morphological concepts used for delimiting genera. Although this publication provided a preliminary assessment of the phylogenetic relationships, it also had two main limitations. The first phylogenetic analyses were based on ITS region (ITS1, 5.8S rRNA gene and ITS2) and the second, on a small percentage of the overall biodiversity of the family. However, an important insight of that study i.e., the commonly applied generic classification was not congruent with the molecular data. Trouillas *et al.* (2010, 2011) performed phylogenetic analyses based on ITS and β-tubulin genes of several species isolated from various grapevine plants in California (USA) and Australia, respectively. More recently (Shang *et al.* 2017, Phookamsak *et al.* 2019, Mehrabi *et al.* 2019), molecular techniques have provided a way of separating species within this family. However, sequence availability of well-identified accessions for *Diatrypaceae* in GenBank is still limited and re-examination of older taxa, epitypification and multi-gene phylogenetic analyses will be required (Zhu *et al.* 2021, Long *et al.* 2021).

During the present survey, plant specimens colonized by members of *Xylariales* were collected from north of Iran (Siahkal, Guilan Province). Among them, one diatrypaceous fungus was identified as *Cryptosphaeria multicontinentalis*, based on their morphological and molecular analyses.

Materials and Methods

- Morphological observation and isolation

The specimen was collected from Siahkal (Guilan Province, northern Iran). Single ascospore isolate was obtained directly from fungal perithecia on wood specimen using 2% malt-extract-agar (MEA). For examination of culture macro-morphology, the strain was grown on the same culture. Living culture was deposited in the culture collection of the Iranian Research Institute of Plant Protection (IRANC), Tehran, Iran. Dried specimen was deposited in the University of Guilan Mycological Herbarium (GUM), Rasht, Iran. For light microscopy, specimen was examined according to Pourmoghaddam *et al.* (2014).

- DNA extraction, PCR and sequencing

Total DNA was extracted using Thermolysis method (Zhang *et al.* 2010). The complete rDNA ITS region was amplified using general primer pairs (ITS5/ITS4, White *et al.* 1990). The amplicon was then sent to Codon Genetic Group (Tehran, Iran) to be sequenced. The sequence generated in this study was deposited in the GenBank under accession No. OQ613356.

Table 1. Vouchers and accession numbers of sequences used in the phylogenetic analyses [Type specimens labeled with HT (holotype) in bold was isolated/sequenced in the present study.]

Taxon	Specimen No.	Origin	GenBank No.	Reference
Cryptosphaeria avicenniae(HT)	NFCCI-4248	India	MH304406	Dayarathne *at al.* (2020)
C. ligniota	CBS 273.87	Switzerland	KT425233	Trouillas *et al.* (2015)
C. multicontinentalis(HT)	DSIERRA600	USA	KT425184	Trouillas *et al.* (2015)
C. multicontinentalis	NSW01PO	Australia	KT425236	Trouillas *et al.* (2015)
C. multicontinentalis	ARG02	Argentina	KT425239	Trouillas *et al.* (2015)
C. multicontinentalis	**IRAN 4835C**	**Iran**	**OQ613356**	**This study**
C. pullmanensis	ATCC 52655	USA	KT425235	Trouillas *et al.* (2015)
C. subcutanea	CBS 240.87	Norway	KT425232	Acero *et al.* (2004)
Diatrype bullata	UCDDCh400	USA	DQ006946	Rolshausen *et al.* (2006)
D. disciformis	CBS 205.87	Switzerland	AJ302437	Acero *et al.* (2004)
D. spilomea	CBS 212.87	Switzerland	AJ302433	Acero *et al.* (2004)
D. stigma	DCASH200	USA	GQ293947	Trouillas *et al.* (2010)
D. undulata	CBS 271.87	Switzerland	AJ302436	Acero *et al.* (2004)
D. virescens	ANM 1075	USA	KU320619	de Almeida *et al.* (2016)
Diatrypella atlantica(HT)	HUEFS 194228	Brazil	KM396615	de Almeida *et al.* (2016)
D. frostii	ATCC 52484	Unknown	AJ302441	Acero *et al.* (2004)
D. iranensis(HT)	IRAN 2280C	Iran	KM245033	Mehrabi *et al.* (2015)
D. macrospora(HT)	IRAN 2344C	Iran	KR605648	Mehrabi *et al.* (2016)
D. major	ANM 1947	USA	KU320613	de Almeida *et al.* (2016)
D. quercina	F-091, 966	Spain	AJ302444	Acero *et al.* (2004)
D. verruciformis	DHB500	USA	GQ293925	Trouillas *et al.* (2010)
D. vulgaris(HT)	CBS 128327	Australia	HQ692590	Trouillas *et al.* (2011)
Eutypella cerviculata	CBS 221.87	Switzerland	AJ302468	Acero *et al.* (2004)
E. citricola	CBS128330	Australia	HQ692579	Trouillas *et al.* (2011)
E. leprosa	CBS 276.87	Switzerland	AJ302463	Acero *et al.* (2004)
E. microtheca(HT)	CBS1 28336	Australia	HQ692569	Trouillas *et al.* (2011)
E. persica	IRAN 2540C	Iran	KX828144	Mehrabi *et al.* (2019)
E. quercina	IRAN 2543C	Iran	KX828139	Mehrabi *et al.* (2019)
E. semicircularis(HT)	MP 4996	Panama	JQ517314	Chacón *et al.* (2013)
E. vitis	ATCC 64171	USA	AJ302466	Acero *et al.* (2004)
Kretzschmaria deusta	MUCL 57705	Iran	MH084755	Pourmoghaddam *et al.* (2018)
K. hedjaroudei(HT)	MUCL 57706	Iran	MH084757	Pourmoghaddam *et al.* (2018)
Peroneutypa alsophila	CBS 250.87	France	AJ302467	Acero *et al.* (2004)
P. curvispora	HUEFS 136877	Brazil	KM396641	de Almeida *et al.* (2016)
P. diminutispora(HT)	HUEFS 192196	Brazil	KM396647	de Almeida *et al.* (2016)
P. scoparia	DFMAL100	France	GQ293962	Trouillas *et al.* (2010)

- Phylogenetic analyses

For the phylogenetic placement of the *Diatrypaceae* taxa included in our analyses, a representative ITS matrix including 36 members of the families was produced with two outgroups from *Xylariaceae* (*Kretzschmaria deusta* (Hoffm.) P.M.D. Martin and *K. hedjaroudei* Pourmogh. and Khodap.). All alignments were generated with the server version of MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft) (Katoh *et al.* 2019), checked and refined manually using MEGA Ver. 7 (Kumar *et al.* 2016). After exclusion of ambiguously aligned regions and long gaps, the final matrix contained 635 nucleotide characters. Details on the sequences used in the phylogenetic analyses are provided in table 1.

Maximum Likelihood (ML) analyses were performed by RAxML (Stamatakis 2006) as implemented in raxmlGUI Ver. 1.3 (Silvestro and Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates. Bootstrap values ≤70% are considered as low, between 70 and 90% as intermediate, and ≥90% as high.

Results

- Molecular phylogeny

The matrix used for phylogenetic analyses comprised 635 characters, of which 206 characters were parsimony informative, 69 characters were parsimony uninformative, and 361 characters were constant. Figure 1 shows a simplified phylogram of the best ML tree (lnL = − 4,258.5007) obtained by RAxML. The Iranian sequence of *Cryptosphaeria multicontinentalis* is almost identical to the type sequence (KT425184, specimen: DSIERRA600, from USA) and they clustered together with intermediate ML (81%) support.

- Taxonomy

Cryptosphaeria multicontinentalis Trouillas, F. Peduto, Inderb. and Gubler, Stud. Mycologia. 107: 1308 (2015) (Fig. 2)

Teleomorph: Stromata poorly developed, immersed in the bark, raising the epidermis and forming blister-like areas. Perithecia were produced in a single layer, black, spherical, 0.5–7.7 mm high × 0.5–7.7 mm wide. Ostioles with short necks and emerging separately. Asci cylindrical to fusiform, long-stipitate, eight-spored, with an inamyloid apical ring, spore-bearing part 45–65 × 5–10 µm. Ascospores allantoid, pale yellow, 8–10(–12.5) × (1.5–)2–2.5 µm.

Anamorph: Colonies on MEA covering a nine cm Petri dish in 1 wk, at first white, becoming grey from center, azonate; finally, attaining grey to dark grey after 30 days. Anamorph libertella-like. Conidiophores variables in length, hyaline to light olive. Conidia filiform, falcate, hyaline, 15–22 × 1.5–2 µm (Fig. 3).

Specimen examined: IRAN: Guilan Province, Siahkal, 36°57'17"N, 49°52'15"E, on dead branch of *Populus* sp., 23.10.2021, leg. M.J. Pourmoghaddam (GUM 1152; living culture IRAN 4835C).

Notes: Most of the characters of the Iranian specimen is in accordance with the type species description provided by Trouillas *et al.* (2015), aside from variations in the size of ascospores [8–10(–12.5) × (1.5–)2–2.5 vs. (8–)9–11(–14) × 2–3(–4) µm]. This species is morphologically similar to *C. ligniota* but can be distinguished by its different ascospores size (Rappaz 1987, https://fungi.myspecies.info/all-fungi/cryptosphaeria-ligniota). The ITS sequence of the Iranian collection (OQ613356) is almost identical (one substitution) to the type sequence of *C. multicontinentalis* (KT425184, specimen: DSIERRA600), which also supports the erection of the novelties for the Iranian mycobiota (Fig. 1).

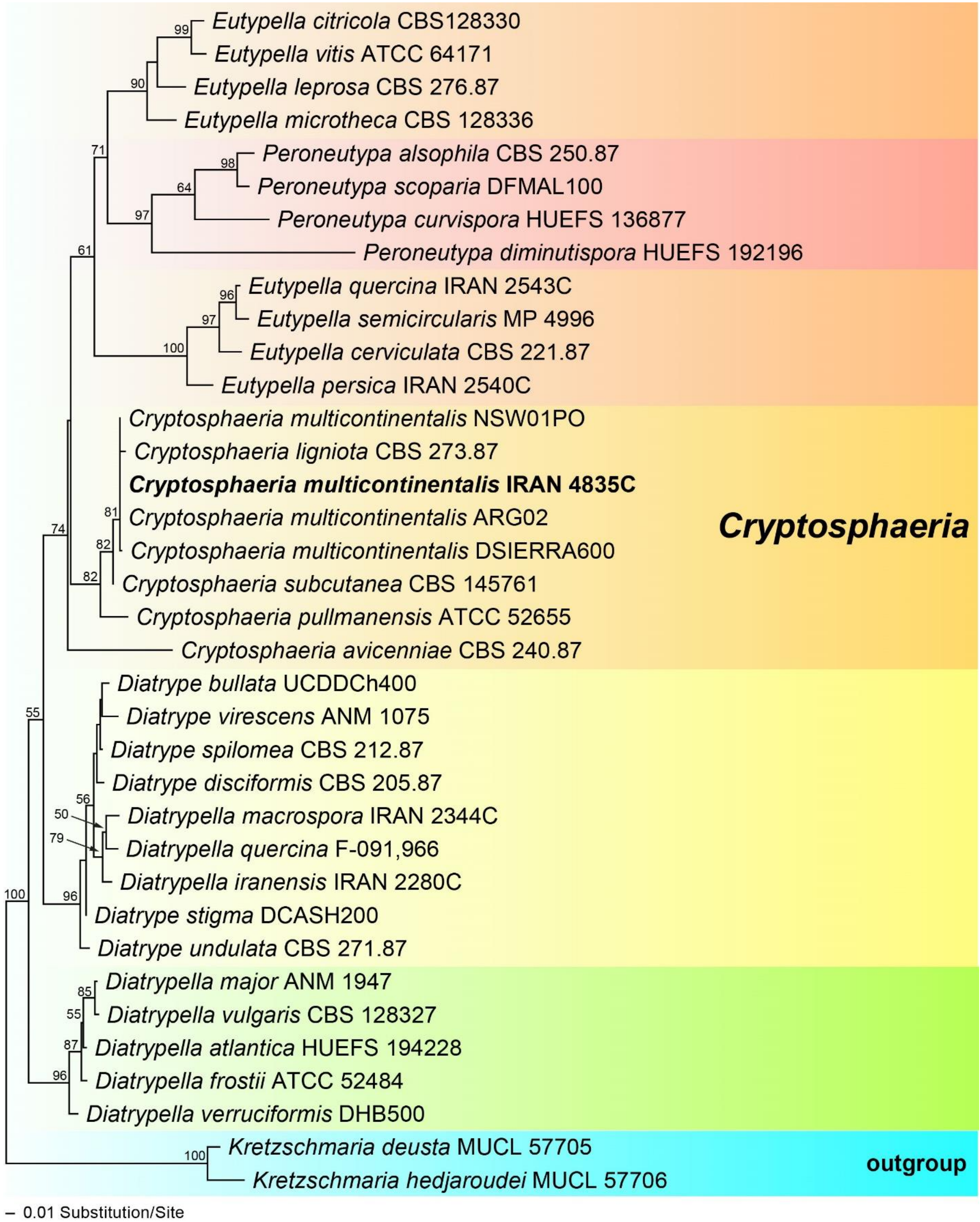

Fig. 1. Phylogram of the best ML trees (lnL = − 4,258.5007) revealed by RAxML from an analysis of the ITS matrix of selected *Diatrypaceae*. Strain in bold was sequenced in the current study. ML bootstrap supports above 50% are given above or below the branches.

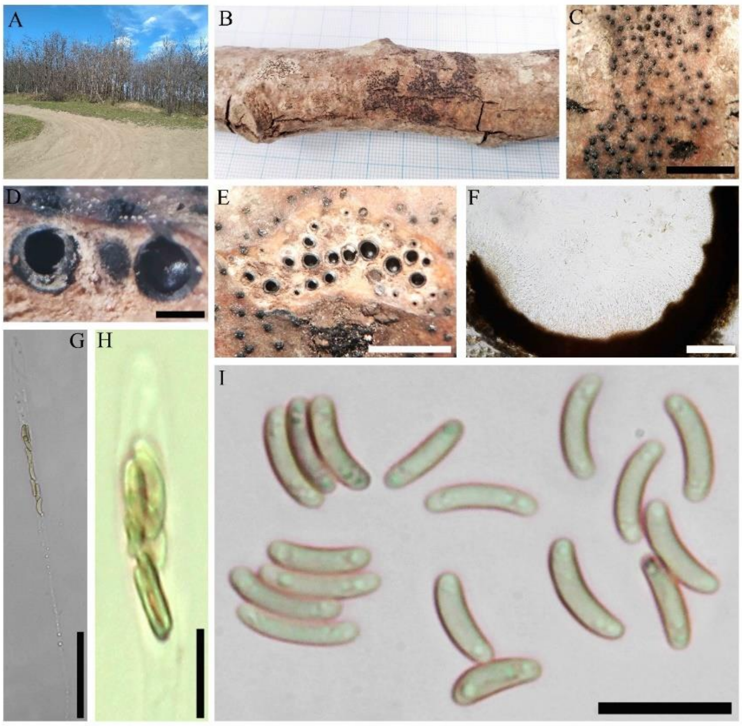

Fig. 2. *Cryptosphaeria multicontinentalis* (GUM 1152): A. Collection site, B. Stromata habit, C. Close-up view of stromatal surface, showing ostioles, D. Stroma in longitudinal section showing perithecia, E. Stroma in horizontal section showing perithecia, F. Longitudinal section showing perithecia, paraphyses, immature and mature asci in water, G. Mature ascus with long-stipitate in water, H. Mature ascus in Melzer's reagent with an inamyloid apical ring, I. Ascospores in water (Bars = C: 0.5 cm, D: 0.5 mm, E: 0.2 cm, F: 100 µm, G: 50 µm, H, I: 10 µm).

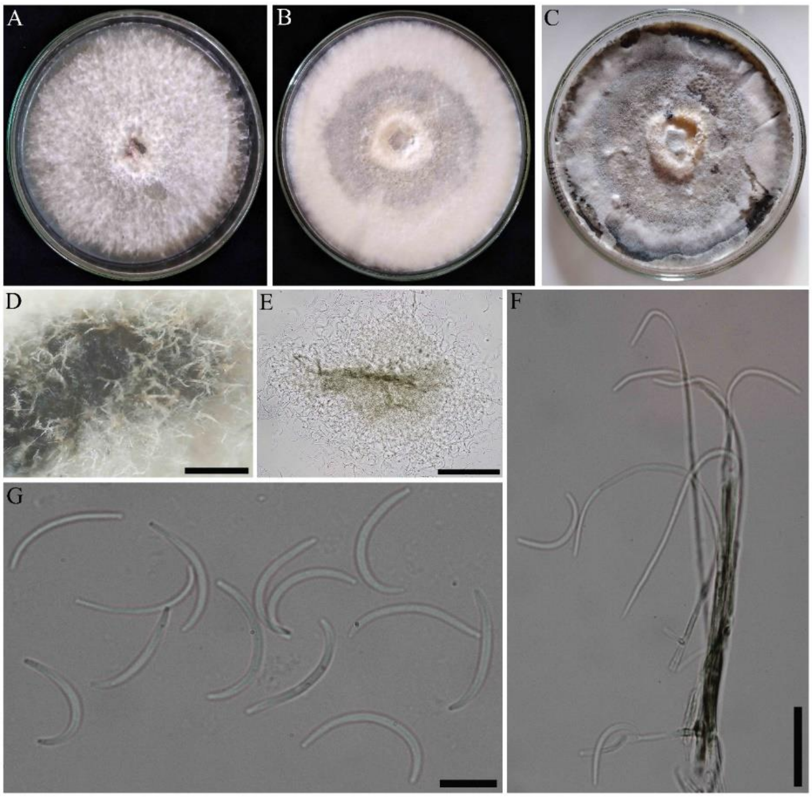

Fig. 3. Culture and anamorphic structures of *Cryptosphaeria multicontinentalis* (IRAN 4835C) on MEA: A–C. Surface of colony after (A) 7 (B) 14, and (C) 30 days of incubation, D. Conidiomata on surface of colony after 40 days of incubation, E. A conidioma in water, F. Conidiophores, conidiogenous cells and conidia in water, G. Conidia in water (Bars = D: 0.1 cm, E: 100 µm, F: 20 µm, G: 10 µm).

Discussion

Molecular data support the placement of *Diatrypaceae* as a distinct well-defined family within the *Sordariomycetes* (Hyde *et al.* 2020). Most genera in *Diatrypaceae* are saprobes and some are endophytes such as *Diatrypella frostii* and *Peroneutypa scoparia* (de Almeida *et al.* 2016). Strikingly, several species are serious threats to forest trees causing canker, dieback, and grapevine trunk diseases (e.g. *Cryptosphaeria populina* caused canker in *Populus* species or *C. pullmanensis* caused canker in *Populus alba* and *Salix alba*) (Zhu *et al.* 2021). Some diatrypaceous fungi have been found only on one host, such as *Diatrypella betulina* on *Betula* and *Eutypella quaternata* on *Fagus sylvatica*, while others show a broad host range, such as *Diatrype bullata* and *Eutypa flavovirens* (Acero *et al.* 2004).

The genus *Cryptosphaeria* was introduced by Greville (1822). Species produce eight-spored, usually spindle-shaped asci with long stipitate and subolivaceous to brown ascospores (Trouillas *et al.* 2015). This genus comprises more than 30 species (https://www.indexfungorum.org/Names/Names.asp, accessed 13 Mar. 2023), but the generic concepts of the genus and family have been unresolved, thus some species were transferred from one genus to another, often by applying single or few morphological characters for generic delimitation which, however, have proven unsuitable for a phylogenetic classification system (Long *et al.* 2021).

In recent years, several studies on *Diatrypaceae* have been conducted in Iran (Mehrabi & Hemmati 2013, Mehrabi *et al.* 2015, 2016, 2017, 2019, Jamali & Yalveh 2017, Bakhshi *et al.* 2022, Ershad 2022, Khodaparast *et al.* 2022). Below, a list of the diatrypaceous species is provided which has been reported from Iran until the end of 2022 (Table 2).

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Table 2. Distribution of diatrypaceous fungi in Iran

Taxon	Host	Collection site (province)	Reference
Cryptosphaeria multicontinentalis	*Populus* sp.	Guilan	This study
C. pullmanensis	*Populus nigra*	Zanjan	Mehrabi *et al.* (2017)
Cryptovalsa ampelina	*Juglans regia*	Kermanshah	Mehrabi *et al.* (2015)
C. mori	In dead stems and branches	Not available	Ershad 2022
C. rabenhorstii[*]	*Citrus* sp.	Mazandaran	Mehrabi *et al.* (2016)
Diatrype disciformis	*Fagus orientalis*/ *Alnus* sp.	Guilan	Pourmoghaddam *et al.* (2015), Mehrabi *et al.* (2016)
D. stigma	*Morus alba*	Guilan, Mazandaran	Ershad 2022
D. whitmanensis	*Amygdalus communis*/*Ulmus carpinifolia* var. *umbraculifera*	Isfahan, Fars, Kerman, Kohgiluyeh-va-Boyerahmad	Hashemi & Mohammadi (2016)
Diatrypella iranensis	*Quercus brantii*	Kohgiluyeh-va-Boyerahmad	Mehrabi *et al.* (2015)
D. macrospora	*Q. brantii*	Kohgiluyeh-va-Boyerahmad	Mehrabi *et al.* (2016)
Eutypa lata	*Malus pumila*/ *Vitis sylvestris*	Isfahan, Tehran, E. & W. Azerbaijan	Nourian *et al.* (2021), Ershad 2022
E. ludibunda	In dead stems and branches	Guilan	Ershad 2022
Eutypella citricola[**]	*Salix* sp.	Guilan	Mehrabi *et al.* (2016)

Table 2 (contd)

E. persica	*Alnus* sp.	Guilan	Mehrabi *et al.* (2019)
E. quercina	*Quercus* sp.	E. Azerbaijan	Mehrabi *et al.* (2019)
*E. vitis****	Persimmon trees	Mazandaran	Jabbari Firoozjah *et al.* (2015)
Libertella platani	*Platanus orientalis*	Kermanshah	Jamali & Yalveh (2017)
L. quercina	*Quercus brantii*	Kohgiluyeh-va-Boyerahmad	Mehrabi & Hemmati (2013)
Monosporascus cannonballus	*Cucumis melo*	Several provinces	Ershad 2022
M. monsporus	*Iris* sp.	Not available	Hawksworth & Ciccarone (1979)
Peroneutypa iranica	*Wisteria sinensis*	Guilan	Mehrabi *et al.* (2019)
P. scoparia	*Gledischia* sp.	Guilan	Mehrabi *et al.* (2016)
Quaternaria quaternata	*Fagus* sp.	Guilan	Mehrabi *et al.* (2016)

* Current name: *Allocryptovalsa rabenhorstii*
** Current name: *Paraeutypella citricola*
*** Current name: *Paraeutypella vitis*

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