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Article

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Diatrypella macrospora, a new host and geographical record from Forlì-Cesena, Italy

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Abstract

During a microfungi survey in the Province of Forlì-Cesena, Italy, a diatrypaceous taxon was collected on a dead branch of *Quercus cerris* (Fagaceae, Fagales). Phylogenetic analyses of combined ITS and β-tubulin sequence data identified the taxon as *Diatrypella macrospora*. This represents a new host and geographical record for *D. macrospora*. This new collection is similar to the holotype, but differs in having shorter perithecial necks and smaller ascospores with marked curvature. In this account, a detailed description, colour photographs and phylogenetic analyses are provided to represent the new record of *D. macrospora*.

Keywords – Diatrypaceae – Italy – ITS – morphology – phylogeny

Introduction

Diatrypaceae consists of 22 genera and approximately 1035 species (Wijayawardene et al. 2020, Hyde et al. 2020b, Konta et al. 2020; Dissanayake et al. 2021). The taxa are generally characterized by perithecial ascomata immersed in a stroma, asci with long pedicels, allantoid ascospores and coelomycetous or hyphomycetous asexual morph (Shang et al. 2017, Hyde et al. 2020b). The asexual morphs of Diatrypaceae are morphologically similar and thus cannot be used for taxonomic differentiation (Acero et al. 2004). Genera in Diatrypaceae are delineated mainly based on stromatal morphology e.g., pustulate, valsoid, eutypoid, well-developed or poorly-developed (Vasilyeva & Stephenson 2005, Senwanna et al. 2017). Early treatments of Diatrypaceae were based on morphology, while recent studies have included phylogenetic data (Mehrabi et al. 2016, Dayarathne et al. 2020, Konta et al. 2020).

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Diatrypella was introduced as a segregate of *Diatrype* with polysporous asci (Croxall 1950). Characteristics of *Diatrypella* include conical or truncate, discoid or cushion-like stromata delimited by a black zone on the host tissues, perithecial ascomata, umbilicate or sulcate ostioles, and numerous ovoid to allantoid ascospores. The asexual morph is described as libertella-like (Senwanna et al. 2017, Shang et al. 2017, Hyde et al. 2020a). *Diatrypella* includes approximately 115 species (Wijayawardene et al. 2020). In the last decade, several new species have been introduced using evidence from morphology coupled with ITS and β-tubulin sequence analyses. These include *D. iranensis*, *D. macrospora*, *D. tectonae* and *D. yunnanensis* (Mehrabi et al. 2015, 2016, Shang et al. 2017, Hyde et al. 2020a). Molecular studies, have however, revealed that *Diatrypella* is polyphyletic (Senwanna et al. 2017). Acero et al. (2004) suggested that polysporous asci is a characteristic that evolved independently multiple times during evolution of Diatrypaceae (Acero et al. 2004, Senwanna et al. 2017). This suggests that current features used in the taxonomy of Diatrypaceae may not reflect the evolutionary history (Mehrabi et al. 2016), hence highlighting the importance of using molecular data for delimitation of genera within the family.

Diatrypella taxa have broad geographic distribution as saprobes (e.g. *D. heveae*, *D. quercina*, *D. yunnanensis*), endophytes (e.g. *D. favacea*, *D. frostii*) or occasionally as suspected pathogens (e.g. *D. japonica*, *D. vulgaris*) mainly on woody angiosperms (Pitt et al. 2013, de Almeida et al. 2016, Senwanna et al. 2017, Rudolph et al. 2018, Rashmi et al. 2019, Hyde et al. 2020a, Li et al. 2020). Some species have a broad host range, others such as *D. vitis* (on grapevines) have been reported only on one host genus (Acero et al. 2004, Farr & Rossman 2020).

In the present study we report a new host and geographical record of *Diatrypella macrospora* on *Quercus cerris* from Italy based on combined ITS and β-tubulin sequence analysis. Our comprehensive analysis also highlights the need for extensive revisions within Diatrypaceae.

Materials & methods

Sample collection, morphological studies and isolation

A dead land branch of *Quercus cerris* was collected in the Province of Forlì-Cesena, Italy in July 2019. Samples were brought to the laboratory and kept in paper envelopes. Macroscopic characters (enlarged host surface and ascomata) were examined using a Motic SMZ 168 series stereomicroscope. Microscopic features were observed using a Nikon DS-Ri2 digital camera fitted to a Nikon Eclipse 80i compound microscope. Thin cross sections of stromata were prepared manually and mounted in water on glass microscopic slides. Photomicrographs were prepared with Adobe Photoshop CC v. 20.0.5 (Adobe Systems, USA) and all character measurements were made with Tarosoft Image FrameWork v. 0.9.0.7.

Single ascospore isolation was carried out as described in Senanayake et al. (2020). After germination, ascospores were transferred aseptically to malt extract agar (MEA) medium and incubated at room temperature. A pure culture was obtained and its characteristics were also observed. Dried specimens and living cultures were deposited in the Mae Fah Luang University herbarium, Thailand (MFLU) and the Mae Fah Luang University Culture Collection (MFLUCC) respectively.

DNA extraction, PCR amplification and phylogenetic analyses

The methods used in DNA extraction, PCR amplification, sequence analyses and genetic analyses are as outlined in Dissanayake et al. (2020) with the following or modifications. The internal transcribed spacer (ITS) and β-tubulin loci were amplified by polymerase chain reaction. The primers used for amplification were: ITS5 and ITS4 (White et al. 1990) for ITS and T1 and Bt2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997) for β-tubulin. The PCR conditions for both ITS and β-tubulin were set as follows: initial denaturation of 94°C for 3 mins, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 55°C for 50s, elongation at 72°C for 1 min and final extension at 72°C for 10 mins.

DNA sequencing was performed at BGI Shenzhen, China. The closest related strains and sequences spanning the diversity of Diatrypaceae were compiled following recent publications (Dayarathne et al. 2020, Konta et al. 2020) (Table 1). For the Maximum likelihood (ML) analysis, the optimal ML tree was obtained using 1000 separate runs and GTR+GAMMA was used as the model for nucleotide substitution. For Bayesian inference analyses (BI), two parallel runs, each consisting of four simultaneous Markov chains were executed for 4,000,000 generations, sampling one tree every 1000th generation. 25% of the trees were discarded as the burn-in phase in the analysis. The remaining trees were used to calculate posterior probabilities in the majority rule consensus tree. Convergence was determined when the average standard deviation of split frequencies reached 0.01.

Table 1 GenBank accession numbers for the strains used in this study. The newly isolated strain of *Diatrypella macrospora*, is shaded. The type species of each genus is indicated as ^{T} and ex-type strains are in **bold**

Species	Strains	GenBank accession numbers	
		ITS	β-tubulin
Allocryptovalsa cryptovalsoidea	**HVFIG02**	**HQ692573**	**HQ692524**
Allocryptovalsa cryptovalsoidea	HVFIG05	HQ692574	HQ692525
Allocryptovalsa elaeidis	**MFLUCC 15-0707**	**MN308410**	**MN340296**
***Allocryptovalsa polyspora*^{T}**	**MFLUCC 17-0364**	**MF959500**	**MG334556**
Allocryptovalsa rabenhorstii	WA08CB	HQ692619	HQ692523
Allocryptovalsa rabenhorstii	WA07CO	HQ692620	HQ692522
***Allodiatrype arengae*^{T}**	**MFLUCC 15-0713**	**MN308411**	**MN340297**
Allodiatrype elaeidicola	**MFLUCC 15-0737a**	**MN308415**	**MN340299**
Allodiatrype elaeidicola	**MFLUCC 15-0737b**	**MN308416**	**-**
Allodiatrype elaeidis	**MFLUCC 15-0708a**	**MN308412**	**MN340298**
Allodiatrype elaeidis	**MFLUCC 15-0708b**	**MN308413**	**-**
Allodiatrype thailandica	MFLUCC 14-1210	KU315392	-
Allodiatrype thailandica	**MFLUCC 15-0711**	**MN308414**	**-**
Anthostoma decipiens^{T}	IPV-FW349	AM399021	-
Anthostoma decipiens^{T}	JL567	JN975370	JN975407
Cryptosphaeria eunomia^{T}	C1C, CBS 216.87	AJ302417	-
Cryptosphaeria eunomia^{T}	C5C, CBS 223.87	AJ302421	-
Cryptosphaeria ligniota	**CBS 273.87**	**KT425233**	**KT425168**
Cryptosphaeria moravica/ Eutypa petrakii	CBS 244.87	HM164735	HM164769
Cryptosphaeria pullmanensis	ATCC 52655	KT425235	KT425170
Cryptosphaeria pullmanensis	HBPF24	KT425202	GQ294014
Cryptosphaeria subcutanea	**CBS 240.87**	**KT425232**	**KT425167**
Cryptosphaeria subcutanea	DSUB100A	KT425189	KT425124
Cryptovalsa ampelina	A001	GQ293901	GQ293972
Cryptovalsa ampelina	DRO101	GQ293902	GQ293982
Diatrype brunneospora	CNP01	HM581946	HQ692478
Diatrype bullata	UCDDCh400	DQ006946	DQ007002
Diatrype bullata	D6C, CBS 215.87	AJ302422	-
Diatrype decorticata	1056	KU320621	-
Diatrype disciformis^{T}	D21C, CBS 205.87	AJ302437	-
Diatrype disciformis^{T}	IRAN 2347C	KR605644	KY352434
Diatrype enteroxantha	HUEFS155114	KM396617	KT003700
Diatrype enteroxantha	HUEFS155116	KM396618	KT022236
Diatrype macowaniana	D15C, CBS 214.87	AJ302431	-

Table 1 Continued.

Species	Strains	GenBank accession numbers	
		ITS	**β-tubulin**
Diatrype mangrovei	MFLUCC 17-0412	MH304407	-
Diatrype mangrovei	MFLUCC 17-0391	MH304408	-
Diatrype mangrovei	MFLUCC 17-0394	MH304409	-
Diatrype oregonensis	CA117	GQ293934	GQ293996
Diatrype oregonensis	DPL200	GQ293940	GQ293999
Diatrype palmicola	MFLUCC 11-0018	KP744439	-
Diatrype palmicola	MFLUCC 11-0020	KP744438	-
Diatrype polycocca	D16C, CBS 213.87	AJ302432	-
Diatrype prominens	ATCC: MYA-4410	FJ430594	-
Diatrype prominens	SBen212	KU721868	-
Diatrype sp.	H2/4b	MG020309	-
Diatrype sp.	H3/2b	MG020294	-
Diatrype sp.	H2/5c	MG020292	-
Diatrype spilomea	D17C	AJ302433	-
Diatrype stigma	DCASH200	GQ293947	GQ294003
Diatrype stigma	UCD23-Oe	JX515704	JX515670
Diatrype undulata	D20C, CBS 271.87	AJ302436	-
Diatrype undulata	Olrim324	AY354239	-
Diatrype whitmanensis	CDB011	GQ293954	GQ294010
Diatrype whitmanensis	DCHES100	GQ293951	GQ294008
Diatrypella atlantica	HUEFS 136873	KM396614	KR259647
Diatrypella atlantica	**HUEFS 194228**	**KM396615**	**KR363998**
Diatrypella banksiae	CPC 29118	KY173402	-
Diatrypella banksiae	CPC 29054	KY173401	-
Diatrypella cephalanthi	CBS 161.32	MH855258	-
Diatrypella delonicis	**MFLUCC 15-1014**	**MH812994**	**MH847790**
Diatrypella delonicis	MFLU 16-1032	MH812995	MH847791
Diatrypella elaeidis	**MFLUCC 15-0279**	**MN308417**	**MN340300**
Diatrypella favacea	Isolate 380	KU320616	-
Diatrypella favacea	CBS 198.49	MH856491	-
Diatrypella frostii	UFMGCB 1917	HQ377280	-
Diatrypella heveae	**MFLUCC 17-0368**	**MF959501**	**MG334557**
Diatrypella heveae	MFLUCC 15-0274	MN308418	MN340301
Diatrypella iranensis	**IRAN 2280C KDQ18**	**KM245033**	**KY352429**
Diatrypella macrospora	**IRAN 2344C KDQ15**	**KR605648**	**KY352430**
Diatrypella macrospora	MFLUCC 21-0010	MW647094	MW677962
Diatrypella major	Isolate 1058	KU320613	-
Diatrypella prominens	DL28A, ATCC 64182	AJ302442	-
Diatrypella pulvinata	H048	FR715523	FR715495
Diatrypella quercina	DL30M	AJ302444	-
Diatrypella quercina	CBS 108.18	MH854666	-
Diatrypella sp.	C6	KX611072	-
Diatrypella sp.	ENQ55	KX828138	KY352431
Diatrypella sp.	MNQ75B	KX828158	KY352432
Diatrypella tectonae	**MFLUCC 12-0172a**	**KY283084**	**-**
Diatrypella tectonae	**MFLUCC 12-0172b**	**KY283085**	**KY421043**
Diatrypella verruciformis^{T}	UCROK1467	JX144793	JX174093
Diatrypella verruciformis^{T}	UCROK754	JX144783	JX174083
Diatrypella vulgaris	HVFRA02	HQ692591	HQ692503
Diatrypella vulgaris	**HVGRF03**	**HQ692590**	**HQ692502**
Diatrypella yunnanensis	VT01	MN653008	-

Table 1 Continued.

Species	Strains	GenBank accession numbers	
		ITS	**β-tubulin**
Eutypa armeniacae	ATCC 28120	DQ006948	DQ006975
Eutypa astroidea	E49C, CBS 292.87	AJ302458	DQ006966
Eutypa flavovirens	E48C, CBS 272.87	AJ302457	DQ006959
Eutypa laevata	E40C CBS 291.87	AJ302449	-
Eutypa lata^{T}	CBS 290.87	HM164736	HM164770
Eutypa lata^{T}	EP18	HQ692611	HQ692501
Eutypa lata^{T}	RGA01	HQ692614	HQ692497
Eutypa lejoplaca	CBS 248.87	DQ006922	DQ006974
Eutypa leptoplaca	CBS 287.87	DQ006924	DQ006961
Eutypa maura	CBS 219.87	DQ006926	DQ006967
Eutypa microasca	**BAFC 51550**	**KF964566**	**KF964572**
Eutypa sparsa	3802 3b	AY684220	AY684201
Eutypella cerviculata^{T}	EL59C	AJ302468	-
Eutypella cerviculata^{T}	M68	JF340269	-
Eutypella leprosa	EL54C, CBS 276.87	AJ302463	-
Eutypella leprosa	Isolate 60	KU320622	-
Eutypella microtheca	ADEL200	HQ692559	HQ692527
Eutypella microtheca	BCMX01	KC405563	KC405560
Eutypella parasitica	CBS 210.39	DQ118966	-
Eutypella semicircularis	MP4669	JQ517314	-
Halodiatrype avicenniae	**MFLUCC 15-0953**	**KX573916**	**KX573931**
Halodiatrype salinicola^{T}	MFLUCC 15-1277	KX573915	KX573932
Kretzschmaria deusta	CBS 826.72	KU683767	KU684190
Monosporascus cannonballus^{T}	CMM3646	JX971617	-
***Monosporascus cannonballus*^{T}**	**ATCC 26931**	**FJ430598**	**-**
Neoeutypella baoshanensis^{T}	EL51C, CBS 274.87	AJ302460	-
Neoeutypella baoshanensis^{T}	HMAS 255436	NR_164038	MH822888
Paraeutypella citricola	HVGRF01	HQ692579	HQ692512
Paraeutypella citricola	HVVIT07	HQ692589	HQ692521
Paraeutypella vitis	UCD2291AR	HQ288224	HQ288303
Paraeutypella vitis	UCD2428TX	FJ790851	GU294726
Pedumispora rhizophorae^{T}	BCC44877	KJ888853	-
Pedumispora rhizophorae^{T}	BCC44878	KJ888854	-
Peroneutypa alsophila	EL58C, CBS 250.87	AJ302467	-
Peroneutypa comosa	BAFC 393	KF964568	-
Peroneutypa curvispora	HUEFS 136877	KM396641	-
Peroneutypa diminutiasca	**MFLUCC 17-2144**	**MG873479**	**-**
Peroneutypa diminutispora	**HUEFS 192196**	**KM396647**	**-**
Peroneutypa kochiana	EL53M	AJ302462	-
Peroneutypa longiasca	**MFLUCC 17-0371**	**MF959502**	**MG334558**
Peroneutypa mackenziei	**MFLUCC 16-0072**	**KY283083**	**KY706363**
Peroneutypa mangrovei	NFCCI-4246	MG844286	MH094409
Peroneutypa rubiformis	**MFLUCC 17-2142**	**MG873477**	**-**
Peroneutypa scoparia	DFMAL100	GQ293962	GQ294029
Peroneutypa scoparia	IRAN 2345C	KR605646	KY352452
Quaternaria quaternata	CBS 278.87	AJ302469	-
Quaternaria quaternata	IRAN 2348C	KR605645	KY352464
***Xylaria hypoxylon*^{T}**	**CBS 122.620**	**AM993141**	**KX271279**

Results

Phylogenetic analyses

Phylogenetic analyses of a combined ITS and β-tubulin sequence dataset comprised 131 ingroup taxa and two outgroup taxa, namely *Kretzschmaria deusta* and *Xylaria hypoxylon (*Xylariaceae*)*. The combined matrix contained 1240 nucleotide sites (ITS: 1–515; β-tubulin: 516–1240). The ML analysis yielded a best-scoring tree with a final ML optimization likelihood value of -18323.132499. The matrix had 887 distinct alignment patterns, with 38.83% of gaps and completely undetermined characters. The ML and BI analyses yielded trees with similar topologies. The clades recovered in the phylogenetic tree are similar in topologies to previous studies (Acero et al. 2004, Dayarathne et al. 2020, Konta et al. 2020). *Diatrypella* species clustered in five distinct clades as A1, A2, B, C1 and D1 (Fig. 1). Our newly sequenced strain grouped separately from the type species *Diatrypella verruciformis* in clade D1. It clustered with the type of *D. macrospora* (IRAN 2344C) and with *Diatrype* sp. (H2/4b) with statistical support, MLBS 84%, BYPP 1.00 (Fig. 1).

Taxonomy

Diatrypella macrospora Mehrabi, Hemmati, Vasilyeva & Trouillas, Phytotaxa 252(1): 47 (2016)

Fig. 2

Index Fungorum number: IF813001; Facesoffungi number: FoF01891

Saprobic on dead land branch of *Quercus cerris.* Sexual morph: *Stromata* 1.2–2.8 mm ($\bar{x}$ = 1.8 mm, n = 10) wide, discoid to irregular, scattered or aggregated, immersed to erumpent arising from cracks in the bark, black, separated from host tissue by a black zone, perithecia arranged in groups of 3–8, entostroma well-developed, white to yellow to brown. *Perithecia* 260–490 µm ($\bar{x}$ = 375 µm, n = 10) in diameter, globoid to flask-shaped, sometimes deformed by compression. *Perithecial neck* about 110–350 µm ($\bar{x}$ = 240 µm, n = 10), ostiolar canals sulcate, compressed, converge together at the apex, black, ostiole brown, opening separately through host bark, periphysate. *Peridium* composed of light brown to brown, somewhat flattened cells of *textura angularis*, becoming hyaline towards the inner region. *Paraphyses* elongate, filiform, aseptate, unbranched. *Asci* spore-bearing part 72–120 µm × 8–14.5 µm ($\bar{x}$ = 94 × 11 µm, n = 20), basal part, filiform, 24–53 µm ($\bar{x}$ = 40 µm, n = 20), polysporous, unitunicate, elongate, cylindrical to clavate, obtuse apex, with a J-apical ring. *Ascospores* 7.5–12 µm × 2–3.1 µm ($\bar{x}$ = 10 × 2.5 µm, n = 50), allantoid, subhyaline, yellowish in mass, aseptate, usually bi-guttulate, thin, smooth-walled. Asexual morph: For morphological description see Mehrabi et al. (2016).

Culture characteristics – Colonies on MEA reaching 75 mm diam. after 2 weeks at room temperature. Colonies circular, slightly dense, flat, with fimbriate margin, white to light brown to black from above, similar colour from below.

Material examined – Italy, Province of Forlì-Cesena, Forlì, Farazzano, on a dead land branch of *Quercus cerris* (Fagaceae), 22 July 2019, E. Camporesi, IT 4432 (MFLU 19-2401, new record, dried culture), living culture MFLUCC 21-0010.

GenBank accession numbers – ITS: MW647094, β-tubulin: MW677962

Notes – Our new collection of *Diatrypella macrospora* resembles the holotype (IRAN 2344C) as it has subhyaline and allantoid ascospores, elongate asci, overlap in the number of perithecia as well as sizes of stromata, perithecia and asci (Table 2) (Mehrabi et al. 2016). However, our collection differs from *D. macrospora* (IRAN 2344C) in having smaller ascospores which are considerably more curved and shorter perithecial necks. Based on curvature and spore size, *D. macrospora* from our collection shows more resemblance with *D. quercina* (Croxall 1950). In the combined ITS and β-tubulin phylogeny, our new isolate MFLUCC 21-0010 clusters with *D. macrospora* (IRAN 2344C) and with *Diatrype* sp. (H2/4b), with high statistical support (MLBS 84%, BYPP 1.00) (Fig. 1, Clade D, D1). There are 2/510 (0.39%) and 9/349 (2.28%) base pair differences between our isolate and *D. macrospora*

(IRAN 2344C) for ITS and β-tubulin sequences respectively. For the ITS sequence of our isolate and *Diatrype* sp. (H2/4b) there are 2/510 (0.39%) base pair differences. The two strains of *Diatrypella macrospora* formed a clade with *D. iranensis*, *D.* sp. (C6, ENQ55 and MNQ75B), *D. quercina*, *Diatrype bullata*, *D. disciformis*, *D.* sp. (H2/5c and H3/2b), and *D. spilomea*, with 78% MLBS and 0.99 BYPP statistical support (Fig. 1, Clade D). Previously, *Diatrypella macrospora* had been reported only in Iran on *Quercus brantii*. Our new isolate represents a new host and geographical record of *D. macrospora* on *Quercus cerris* (Fagaceae) in Italy.

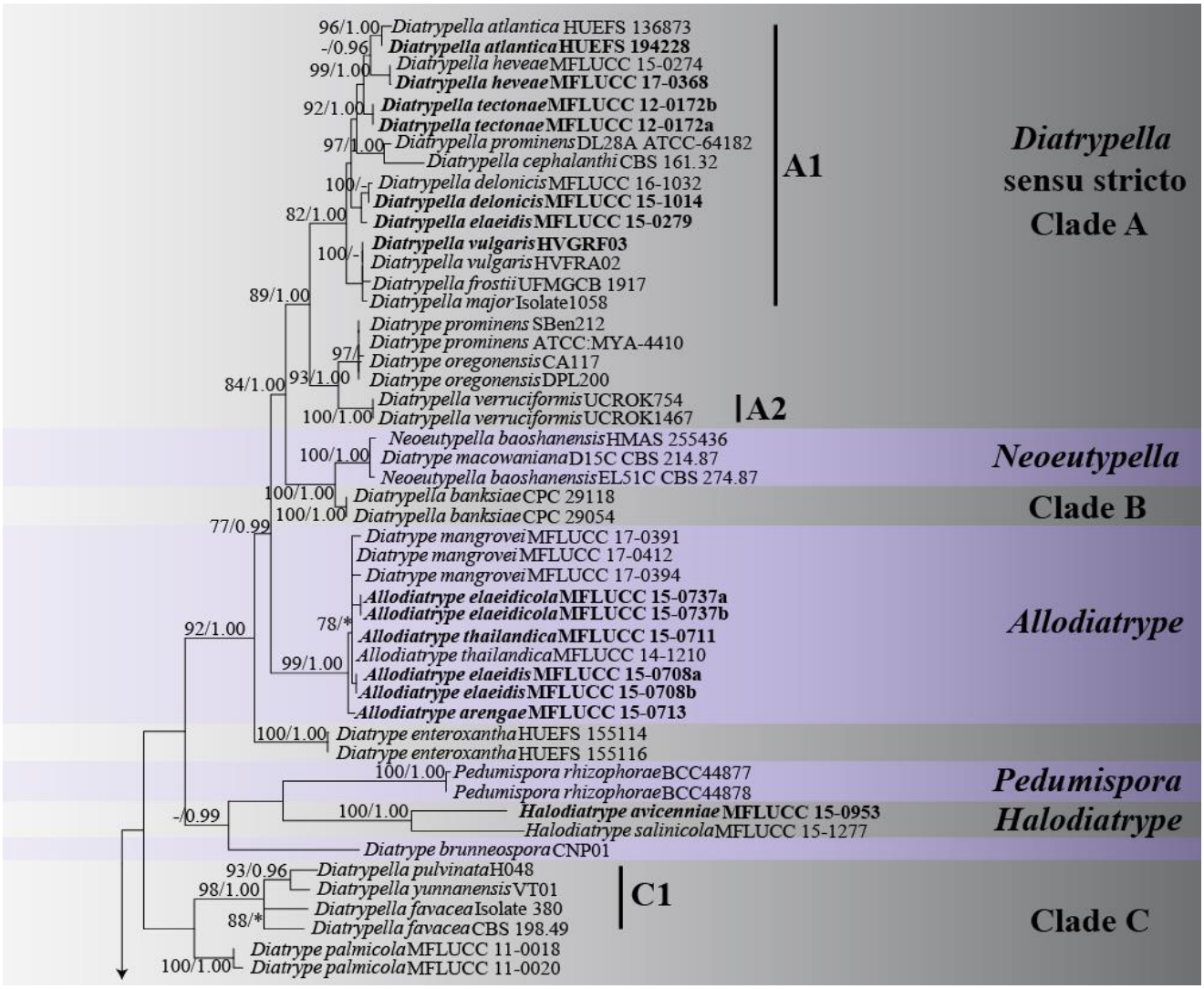

Fig. 1 – Maximum likelihood phylogenetic tree generated from combined ITS and β-tubulin sequence data of 131 Diatrypaceae taxa and 1240 sites. For each node maximum likelihood bootstrap support values (MLBS) are given first, followed by Bayesian posterior probabilities (BYPP). ML bootstrap support values ≥ 75% and BYPP ≥ 0.95 are indicated at the nodes. Lower values are indicated by a dash (-). Nodes that were not recovered are indicated by asterisks (*). The new isolate is in red bold font. Ex-type sequences are in black bold font. The tree is rooted to *Kretzschmaria deusta* (CBS 826.72) and *Xylaria hypoxylon* (CBS 122.620).

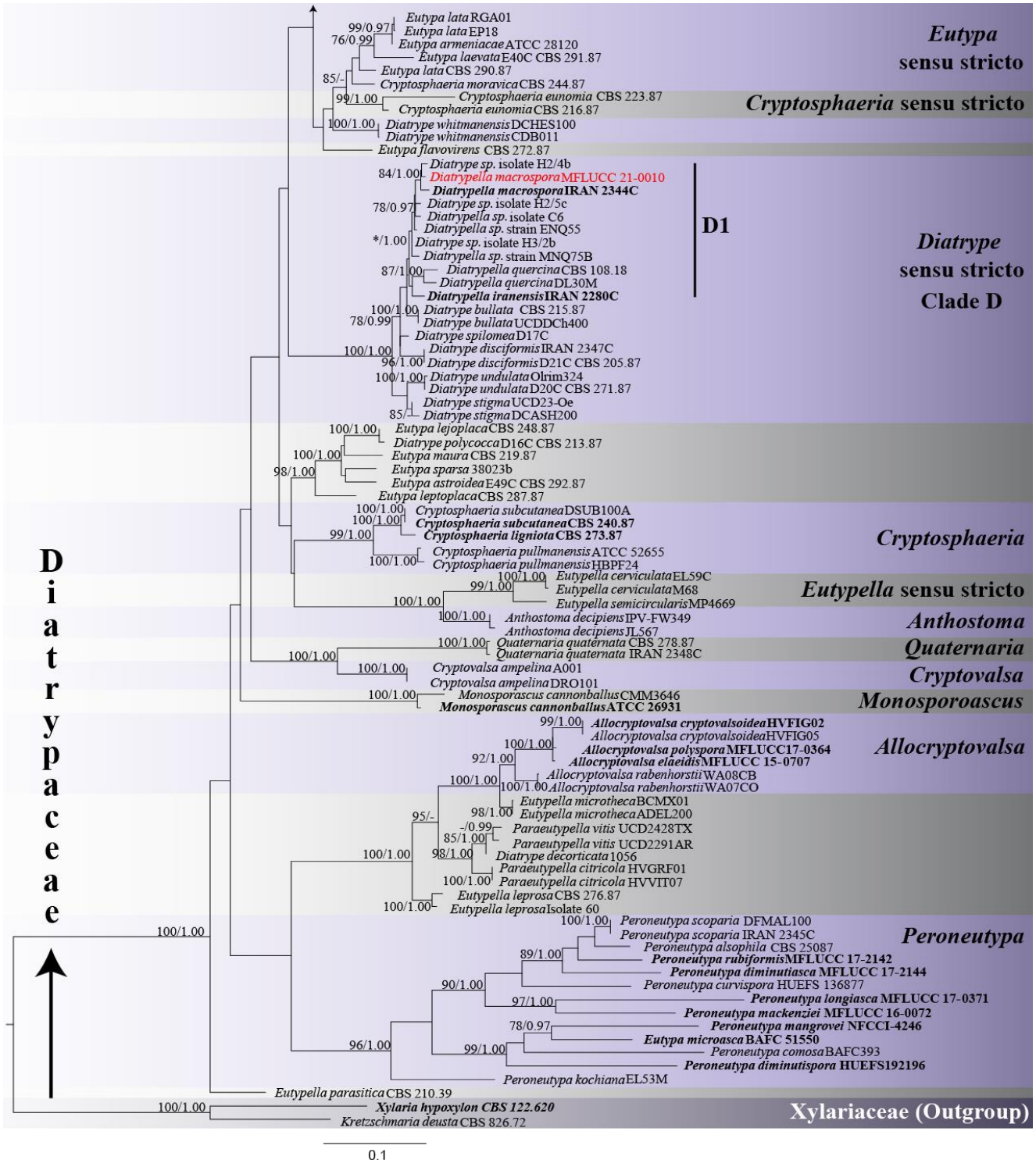

Fig. 1 – Continued.

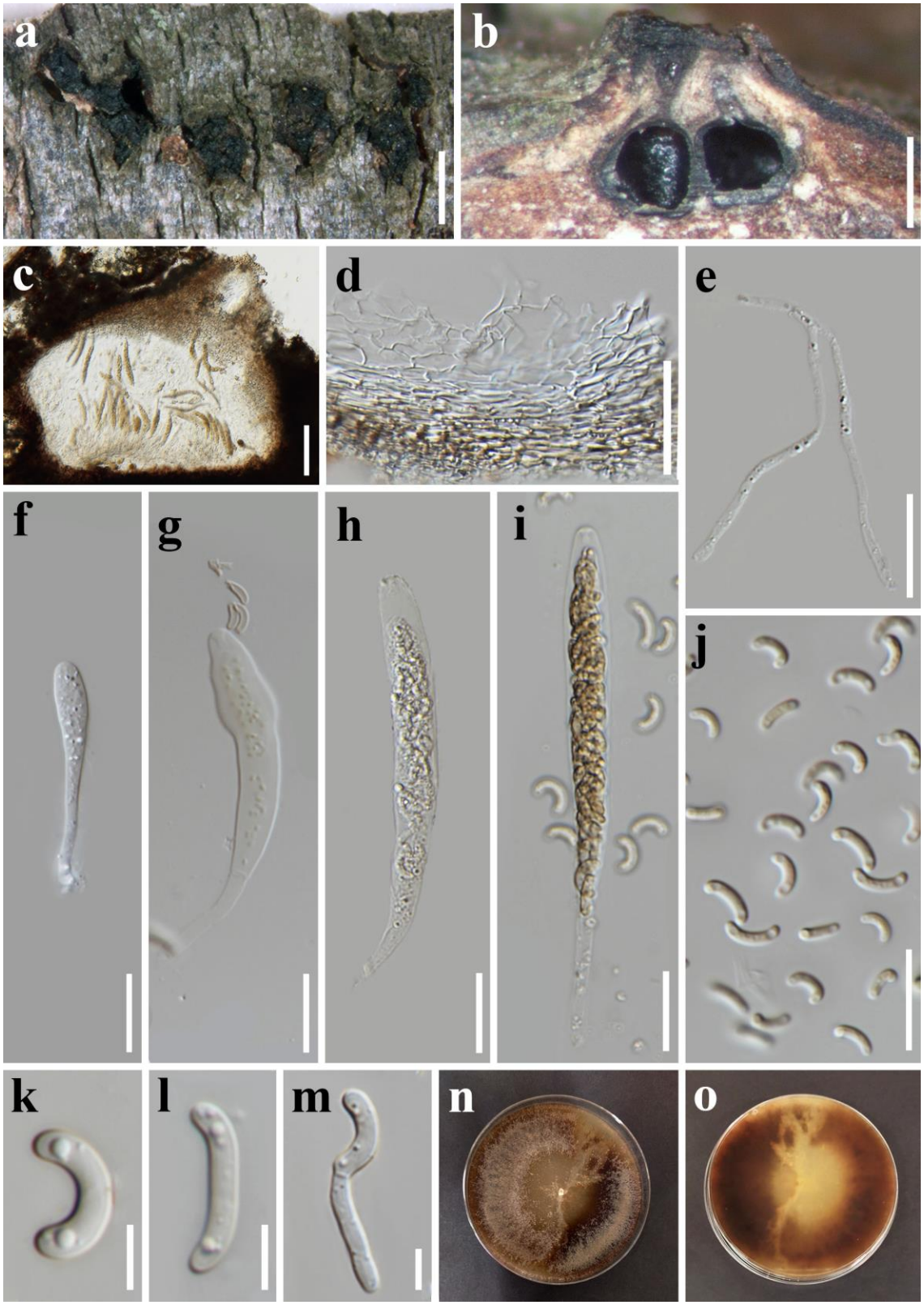

Fig. 2 – *Diatrypella macrospora* (MFLU 19-2401, new record). a Stromata erupting from bark. b Longitudinal section through stroma. c Section through a perithecium. d Peridium. e Paraphyses. f-i Asci. j-l Ascospores. m Germinated ascospore. n, o Top and bottom view of culture. Scale bars: a = 2 mm, b = 500 µm, c = 100 µm, d = 50 µm, e-j = 20 µm, k-m = 5 µm.

Table 2 Comparative morphology of *Diatrypella macrospora* and its relative species

Species	**Shape of stromata**	**Entostroma colour**	**Perithecial neck (µm)**	**Asci**				**Ascospore Shape, colour, length × width (µm)**	**Reference**
				Shape	**Number of spores**	**Length (p. sp. µm)**	**Width (µm)**		
Diatrype bullata (representative strain)	ovoid	white	short neck	cylindrical	8-spored	25–35	5–7	allantoid, pale yellow, 5–7.5 × 1.2	Rappaz (1987), Vasilyeva & Ma (2014)
Diatrype disciformis (reference specimen)	orbicular, disc-like	yellowish white	-	cylindrical	8-spored	30–40	5–6	allantoid, pale yellow, 5–9 × 1.5–2	Senanayake et al. (2015)
Diatrype spilomea (representative strain)	effuse	white	short neck	-	8-spored	20–30	3–6	allantoid, pale yellow, 4.5–7 × 1–1.2	Rappaz (1987)
Diatrype stigma (representative strain)	effuse	white	short neck -	cylindrical	8-spored	25–50	5–6	allantoid, pale yellow, 5.8–10.5 × 1.2–2	Rappaz (1987), Vasilyeva & Ma (2014)
Diatrype undulata (representative strain)	effuse	white	short neck	cylindrical	8-spored	25–40	4–7	allantoid, pale yellow, 5–8 × 1.2–1.8	Rappaz (1987), Vasilyeva & Ma (2014)
Diatrypella hevea (Holotype)	rounded to irregular	white	-	clavate to cylindric-clavate	multispored	80–113	10–21	hyaline to pale yellowish to pale brown oblong to allantoid, aseptate, slightly curved, 5–9 × 1–3	Senwanna et al. (2017)
Diatrypella iranensis (Holotype)	circular to ovoid	whitish yellow	-	elongate, subcylindr-ical to clavate	multispored	-	6–9	allantoid, subhyaline 6–7 × 1–1.3	Mehrabi et al. (2015)
Diatrypella macrospora (Holotype)	circular	white to yellow to light brown	relatively long neck, 200–500, converge together	elongate, more or less cylindrical	8-spored	110–150	10–15	allantoid, subhyaline 12–20 × 1.7–3	Mehrabi et al. (2016)

Table 2 Continued.

Species	Shape of stromata	Entostroma colour	Perithecial neck (µm)	Asci				Ascospore Shape, colour, length × width (µm)	Reference
				Shape	Number of spores	Length (p. sp. µm)	Width (µm)		
Diatrypella macrospora (representative strain)	discoid to irregular in shape	white to yellow to brown	short neck, 110–350, converge together	elongate, cylindrical to clavate	multispored	72–120	8–14.5	allantoid, strongly curved, subhyaline 7.5–12 × 2–3.1	This study
Diatrypella quercina (representative strain)	Subregularrounded or angular	-	-	cylindrical to clavate	multispored	80–120	10–12	allantoid, strongly curved 8–12 × 2–3	Croxall (1950), Saccardo (1882)
Diatrypella tectonea (Holotype)	circular to irregular	white to yellow	short neck	clavate	multispored	100–128	15.5–21.5	yellowish to brown, ellipsoidal to cylindrical or elongate-allantoid, 7–9 × 2–2.5	Shang et al. (2017)
Diatrypella verruciformis (representative strain)	circular, subconical	white	- converge together	spindle-shaped	multispored	100–132	11–11.5	allantoid, moderately or variously curved subolivaceous 6–8 × 1–2	Glawe & Rogers (1984)

Discussion

In this study, we identify our collection as *Diatrypella macrospora* because it is morphologically similar to the holotype, has a short phylogenetic distance, and little ITS base pair differences with the type strain. It also occurs on the same host genus as the holotype. Previously, *D. macrospora* had only been reported on *Quercus brantii* in Iran (Mehrabi et al. 2016). Our collection occurs on *Quercus cerris*, commonly known as Turkey oak. The tree is widespread in the Italian Peninsula (Taffetani et al. 2012) and it is similar to other oak trees but its wood is more prone to cracking and splitting (Vidrinskas & Deveikis 2016). Nonetheless, there are some differences between our strain and the type specimen. The observed differences in morphology may be attributed to differences in host and geographical location. Alternatively, the morphological distinctions along with differences in the nucleotide sequence of β-tubulin also indicate the possibility of a new species rather than a new strain of *D. macrospora*. However, there is only a single collection of *D. macrospora* (as well as *D. iranensis*) and it is therefore difficult to determine species boundaries given the lack of data. Ideally, several differences in more than one locus are required to introduce a new species. With the data currently available, we take a conservative approach and list our isolate as a new record of *D. macrospora.*

Our analysis also provides insights into *Diatrypella*. The phylogenetic tree (Fig. 1) clearly shows that the genus is not monophyletic, confirming previous studies (Acero et al. 2004). *Diatrypella* sequences formed five distinct clades in the tree (Clades A1, A2, B, C1, D1). The newly identified strain along with *D. macrospora* (IRAN 2344C) formed a clade with *D. iranensis* and *D. quercina*, both of which are closely associated with oak trees (Croxall 1950, Farr & Rossman 2020). Further collections are required to determine host-specificity of *Diatrypella* species in Clade D1. In the phylogenetic tree, the above three species group together in the same clade, distant from the type species, *D. verruciformis* (Fig. 1), a consistent finding across studies *(*Mehrabi et al. 2015, 2016, 2019, Konta et al. 2020*)*. *Diatrypella verruciformis* clusters as sister to *Diatrype* sequences separately from other *Diatrypella*. *Diatrypella iranensis*, *D. macrospora* and *D. quercina* could be considered as part of *Diatrype*, since they form a strongly supported clade with the type *D. disciformis*. This placement is congruent with previous studies (Acero et al. 2004, Mehrabi et al. 2019, Konta et al. 2020). In these investigations, the three species are treated as part of *Diatrype.* However, the *D. disciformis* sequence data used in these studies are not from ex-type strains, thus they might be misidentified. Senanayake et al. (2015) proposed a reference specimen for *D. disciformis* (MFLU 15-0722, MFLUCC 15-0538), for which LSU and ITS sequences are available. However, these data were not included in the present analysis. In fact, only a few taxa in our dataset have available LSU sequences. Moreover, the ITS sequence for *D. disciformis* (MFLUCC 15-0538) appears to be problematic. When the strain was subjected to BLAST search, the closest match were members of *Nectriaceae*, a different family. Therefore, the taxonomic placement of the three *Diatrypella* species cannot be conclusively confirmed based on molecular data. Similarly, *D. favacea*, *D. pulvinata*, *D. yunnanensis* also form a distinct clade (Clade C1). Clade C1 and D1 could actually represent two distinct and novel genera. However, it is difficult to separate them with certainty from *Diatrypella*, since there is no sequence from the ex-type of *D. verruciformis*.

Transfer of *D. iranensis*, *D. macrospora* and *D. quercina* to *Diatrype* would need revision of the generic concepts or a new genus should be erected. Cesati & De Notaris (1863) separated *Diatrypella* and *Diatrype* solely based on the number of spores per ascus (Croxall 1950, Acero et al. 2004). In early taxonomic studies, it was recognized that, although convenient, this classification system is likely to be artificial and not a good reflection of evolutionary history of the two genera (Glawe & Rogers 1984, Rappaz 1987). In fact, phylogenetic analyses show that number of spores per ascus is highly variable throughout the evolution of Diatrypaceae and multispored asci appeared several times independently (Dayarathne et al. 2020, Acero et al. 2004). This explains the mixed clades (Clade A, C, D) comprising both *Diatrypella* and *Diatrype* species recovered in this study and previous studies (Acero et al. 2004, de Almeida et al. 2016, Dayarathne et al. 2020, Konta et al. 2020). Apart from the number of spores per ascus other unique characteristics should be used to delineate between genera (Carmarán et al. 2006). However, from the data available (Table 2) there is no unique common characteristic separating *Diatrypella* species in Clade C1 from those in Clade A.

As mentioned by Acero et al. (2004), several early taxonomists have highlighted the morphological distinction between *D. quercina* and other *Diatrypella* species (Acero et al. 2004). Specifically, although the species has polysporous asci it also has a well-developed ectostroma, which is unusual of *Diatrypella*, but common in *Diatrype* (Wehmeyer 1926). *Diatrypella macrospora* (MFLUCC 21-0010), which groups in the same clade as *D. quercina*, also displays a higher degree of ectostromatic development. No details were given for the ectostroma of *D. iranensis* and *D. macrospora* (IRAN 2344C). Thus, even though ectostroma could be used as a taxonomic character this cannot be conclusively determined due to lack of information. This could be because the ectostroma is normally not used as taxonomic characteristic for herbarium as it is present only on immature specimens (Rappaz 1987). Also, some taxonomists argue that microscopic characteristics are more appropriate for delineating diatrypaceous taxa as compared to the more variable macroscopic characters (de Almeida et al. 2016).

Another difference observed by Croxall (1950) in *D. quercina* is its strongly curved ascospores. However, curvature in ascospore appears only as a difference between species rather than between

genera (Table 2). Molecular analysis confirmed the distinction of *D. quercina* from other *Diatrypella* species (Acero et al. 2004). The morphological characteristics related to these molecular differences are not clear. A summary of recent morphological descriptions of species in Clade D is also provided by Thiyagaraja et al. (2019). The data presented is coherent with that reviewed in our study except for the colour of the entostroma which seems to have been mixed with the colour of stromata. Still, the additional details do not provide insights into a more reliable classification. From the morphological descriptions available, *D. iranensis*, *D. macrospora* and *D. quercina* show no clear common feature that separate them from other *Diatrypella* species or explain their phylogenetic position among *Diatrype* species (Table 2). Collectively, these results suggest that the currently used morphological features have a high degree of overlap and their taxonomic value might need to be reconsidered.

Missing data, inaccessibility of type specimens and inadequate original descriptions make resolving the taxonomic problems mentioned above challenging. The genus concept of *Diatrypella* and *Diatrype* as well as the classification of Diatrypaceae as a whole should be reviewed. Sequencing of the reference specimen for *Diatrype* should be checked and an epitype should be provided for *Diatrypella* (de Almeida et al. 2016). More collections of diatrypaceous taxa and a combination of reliable molecular and morphological information would greatly aid resolving the taxonomy of Diatrypaceae.

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