



Article

Two New Species of *Diatrype* (Xylariales, Ascomycota) with Polysporous Asci from China

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Abstract: Two new species of *Diatrype* collected in northeast China are described and illustrated based on morphological and molecular evidence. *Diatrype larissae* from Heilongjiang Province is characterised by having 3–6 perithecia in a stroma, asci polysporous, ascospores allantoid, aseptate, slightly or moderately curved, subhyaline. *Diatrype betulaceicola* from Inner Mongolia has large stroma with 5–14 perithecia, perithecium immersed, asci polysporous, long-stalked, ascospores allantoid, aseptate, slightly curved, subhyaline. The phylogenies inferred from the data set of nrDNA ITS1-5.8S-ITS2 (ITS) and beta-tubulin (β -tubulin) supported the two new species both as members in the genus *Diatrype* and distinct species. The morphological similarities and dissimilarities of the new species with phylogenetically close relatives are discussed. A dichotomous identification key to the *Diatrype* spp. known from China is proposed.

Keywords: Diatrypaceae; taxonomy; morphology; phylogenetic analyses



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1. Introduction

Diatrypaceae Nitschke, an important family of Xylariales, was introduced by Nitschke (1869) with *Diatrype* Fr. as the type genus [1–3]. Members of Diatrypaceae are world-wide in distribution, and, some species parasitize plants and cause plant diseases, which are plant pathogens [4–11].

The genus *Diatrype* Fr. was established by Fries (1849) with *Diatrype disciformis* (Hoffm.) Fr. as the type, which has often been identified as saprobe on rotting wood [3]. The known distribution of *Diatrype* covers Asia, Europe, North America, Oceania and South Africa [3,5,6,11–18]. Traditionally, the genus is characterised by stromata widely effuse or verrucose, flat or slightly convex, with discoid or sulcate ostioles at the surface, eight-spored and long-stalked asci and hyaline or brownish, allantoid ascospores [3,19–21].

The molecular phylogenetic analysis of Diatrypaceae based on ITS including five genera, viz. *Cryptosphaeria* Ces. and De Not., *Diatrype*, *Diatrypella* (Ces. and De Not.) De Not., *Eutypa* Tul. and C. Tul., and *Eutypella* (Nitschke) Sacc., were performed, and suggesting a polyphyletic origin for the five genera [4]. More recently, many diatrypaceous taxa were described and illustrated based on morphological characters and multi-gene phylogenetic analyses [8–11,18,22–24]. The taxonomy and molecular phylogeny of *Diatrype* have not been so well studied in China. A new species of *Diatrype subundulata* Lar. N. Vassiljeva and H.X. Ma is described, mainly based on morphological data from northeastern China [17]. Recently, two new species of *Diatrype* were studied based on anamorph and molecular data,

and recognised *Diatrype quercicola* H.Y. Zhu and X.L. Fan as a new species with polysporous asci by combining morphological and phylogenetic analyses [24]. In addition, a new species *Diatrype lancangensis* S.H. Long and Q.R. Li was introduced from Yunnan province of China by studying morphological characteristics and multi-gene phylogenetic analyses.

During investigations on the diversity of diatrypaceous fungi in northeast China, two undescribed species of *Diatrype* were identified based on morphological criteria and molecular genetic analyses. The primary purposes of the present paper were to study these specimens by using morphological and multi-gene DNA datasets and discuss the phylogeny of the family Diatrypaceae, based on expanded sampling.

2. Materials and Methods

2.1. Morphological Examination

The specimens studied are deposited at the Fungarium of Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences (FCATAS). Microscopical structures of the sexual morph were measured from fresh material mounted in distilled water, 10% KOH and Melzer's iodine reagent, respectively [25]. The photographs of stromata were taken using a Canon G16 camera. Each specimen measured over 30 ascus, 30 ascospores, 15 stromata, 15 perithecia. The photographs of stromatal surface were taken with a VHX-600E microscope of the Keyence Corporation. Microscopic observations and measurements were taken by an Olympus IX73 inverted fluorescence microscope at magnifications up to 1000×. The following abbreviations are used in the text: n = the quantity of a morphological feature measured from a specified number of specimens, \bar{x} = the arithmetic mean of the measured values.

2.2. DNA Amplification and Sequencing

A CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to obtain genomic DNA from dried specimens, according to the manufacturer's instructions. The internal transcribed spacer (ITS) region and β -tubulin (TUB2) was amplified with primer pairs ITS5/ITS4 and T1/T22, respectively [26,27]. Polymerase chain reaction (PCR) was carried out in a volume of 40 μ L, which contained 16 μ L of ddH₂O, 20 μ L of 2× PCR Master Mix (2× HSTM Mix), 1 μ L of DNA template and 1 μ L of forward and reverse primers in each reaction. The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. With regard to TUB2, the amplification reaction followed: 3 min at 95 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 52 °C, and 1.5 min at 72 °C, with a final extension of 72 °C for 10 min. DNA sequencing was performed at BGI tech, Guangzhou, China. All newly generated sequences were deposited at GenBank (<http://www.ncbi.nlm.nih.gov/genbank> (accessed on 13 January 2022); Table 1).

Table 1. List of species, specimens, and GenBank accession numbers of sequences used in this study.

Species	Strain	Host/Substrate	Origin	GenBank Accession Numbers	
				ITS	TUB2
<i>Allocryptovalsa elaeidis</i>	MFLUCC 15-0707	<i>Elaeis guineensis</i>	Thailand	MN308410	MN340296
<i>A. rabenhorstii</i>	WA07CO	<i>Vitis vinifera</i>	Australia	HQ692620	HQ692522
<i>A. rabenhorstii</i>	WA08CB	<i>Vitis vinifera</i>	Australia	HQ692619	HQ692523
<i>Allodiatrype arengae</i> ^{TS}	MFLUCC 15-0713	<i>Arenga pinnata</i>	Thailand	MN308411	MN340297
<i>A. elaeidicola</i>	MFLUCC 15-0737a	<i>Elaeis guineensis</i>	Thailand	MN308415	MN340299
<i>A. elaeidis</i>	MFLUCC 15-0708a	<i>Elaeis guineensis</i>	Thailand	MN308412	MN340298
<i>Anthostoma decipiens</i> ^{TS}	JL567	<i>Vitis vinifera</i>	Spain	JN975370	JN975407
<i>Cryptosphaeria ligniota</i>	CBS 273.87	<i>Populus tremula</i>	Switzerland	KT425233	KT425168
<i>C. subcutanea</i>	CBS 240.87	NA	Norway	KT425232	KT425167
<i>C. subcutanea</i>	DSUB100A	NA	Norway	KT425189	KT425124

Table 1. Cont.

Species	Strain	Host/Substrate	Origin	GenBank Accession Numbers	
				ITS	TUB2
<i>Cryptovalsa ampelina</i>	A001	NA	Australia	GQ293901	GQ293972
<i>C. ampelina</i>	DRO101	NA	America	GQ293902	GQ293982
<i>Diatrype betulae</i>	CFCC 52416	<i>Betula davurica</i>	China	MW632943	NA
<i>D. betulaceicola</i> ^{TS}	FCATAS 2725	<i>Betula</i> sp.	China	OM040386	OM240966
<i>D. betulaceicola</i> ^{TS}	FCATAS 2726	<i>Betula</i> sp.	China	OM040387	OM240967
<i>D. decorticata</i>	1056	NA	NA	KU320621	NA
<i>D. brunneospora</i>	CNP01	<i>Acacia longifolia</i> subsp. <i>sophorae</i>	Australia	HM581946	HQ692478
<i>D. bullata</i>	UCDDCh400	NA	America	DQ006946	DQ007002
<i>D. castaneicola</i>	CFCC 52425	<i>Castanea mollissima</i>	China	MW632941	NA
<i>D. castaneicola</i>	CFCC 52426	<i>Castanea mollissima</i>	China	MW632942	NA
<i>D. disciformis</i> ^{TS}	CBS 205.87	<i>Fagus sylvatica</i>	Switzerland	AJ302437	NA
<i>D. disciformis</i> ^{TS}	GB 5815	<i>Fagus grandifolia</i>	America	KR605644.1	KY352434.1
<i>D. enteroxantha</i>	HUEFS155114	NA	Brazil	KM396617	KT003700
<i>D. enteroxantha</i>	HUEFS155116	NA	Brazil	KM396618	KT022236
<i>D. iranensis</i> (<i>Diatrypella iranensis</i>)	IRAN 2280C	<i>Quercus brantii</i>	Iran	KM245033	NA
<i>D. lancangensis</i>	GMB0045	NA	China	MW797113	MW814885
<i>D. lancangensis</i>	GMB0046	NA	China	MW797114	MW814886
<i>D. larissae</i> ^{TS}	FCATAS 2723	NA	China	OM040384	OM240964
<i>D. larissae</i> ^{TS}	FCATAS 2724	NA	China	OM040385	OM240965
<i>D. lijiangensis</i>	MFLU 19-0717	NA	China	MK852582	MK852583
<i>D. macrospora</i> (<i>Diatrypella macrospora</i>)	IRAN 2344C	<i>Quercus brantii</i>	Iran	KR605648	NA
<i>D. oregonensis</i>	DPL200	<i>Quercus kelloggii</i>	America	GQ293940	GQ293999
<i>D. palmicola</i>	MFLUCC 11-0018	<i>Caryota urens</i>	Thailand	KP744438	NA
<i>D. palmicola</i>	MFLUCC 11-0020	<i>Caryota urens</i>	Thailand	KP744439	NA
<i>D. quercicola</i>	CFCC 52418	<i>Quercus mongolica</i>	China	MW632938	NA
<i>D. quercicola</i>	CFCC 52419	<i>Quercus mongolica</i>	China	MW632939	NA
<i>D. quercina</i> (<i>Diatrypella quercina</i>)	F-091966	<i>Quercus faginea</i>	Spain	AJ302444	NA
<i>D. spilomea</i>	CBS 212.87	<i>Acer campestre</i>	Switzerland	AJ302433	NA
<i>D. stigma</i>	DCASH200	<i>Quercus</i> sp.	America	GQ293947	GQ294003
<i>D. stigma</i>	UCD23-Oe	NA	NA	JX515704	JX515670
<i>D. undulata</i>	CBS 271.87	<i>Betula</i> sp.	Switzerland	AJ302436	NA
<i>D. virescens</i>	CBS 128344	NA	NA	MH864890	NA
<i>D. whitmanensis</i>	CDB011	<i>Vitis vinifera</i>	America	GQ293954	GQ294010
<i>Diatrypella atlantica</i>	HUEFS 136873	unidentified plant	Brazil	KM396614	KR259647
<i>D. atlantica</i>	HUEFS 194228	unidentified plant	Brazil	KM396615	KR363998
<i>D. banksiae</i>	CPC 29054	<i>Banksia coccinea</i>	Australia	KY173401	NA
<i>D. banksiae</i>	CPC 29118	<i>Banksia formosa</i>	Australia	KY173402	NA
<i>D. delonicis</i>	MFLU 16-1032	<i>Delonix regia</i>	Thailand	MH812995	MH847791
<i>D. delonicis</i>	MFLUCC 15-1014	<i>Delonix regia</i>	Thailand	MH812994	MH847790
<i>D. elaeidis</i>	MFLUCC 15-0279	<i>Elaeis guineensis</i>	Thailand	MN308417	MN340300
<i>D. heveae</i>	MFLUCC 17-0368	<i>Hevea brasiliensis</i>	Thailand	MF959501	MG334557
<i>D. pulvinata</i>	H048	<i>Salix alba</i>	Czech Republic	FR715523	FR715495
<i>D. verruciformis</i> ^{TSQ}	UCROK1467	<i>Quercus agrifolia</i>	America	JX144793	JX174093
<i>D. verruciformis</i> ^{TSQ}	UCROK754	<i>Quercus agrifolia</i>	America	JX144783	JX174083
<i>D. vulgaris</i>	HVFRA02	<i>Fraxinus angustifolia</i>	Australia	HQ692591	HQ692503
<i>D. vulgaris</i>	HVGRF03	<i>Citrus paradisi</i>	Australia	HQ692590	HQ692502
<i>D. yunnanensis</i>	JZBH3380001	unidentified plant	China	MN653008	MN887112
<i>Eutypa cremea</i>	STEU 8082	<i>Vitis vinifera</i>	South Africa	KY111656	KY111598
<i>E. cremea</i>	STEU 8410	<i>Prunus armeniaca</i>	South Africa	KY752765	KY752789
<i>E. flavovirens</i>	CBS 272.87	<i>Quercus ilex</i>	France	AJ302457	DQ006959
<i>E. laevata</i>	CBS 291.87	<i>Salix</i> sp.	Switzerland	HM164737	HM164771
<i>E. lata</i> ^{TS}	EP18	<i>Vitis vinifera</i>	NSW, Australia	HQ692611	HQ692501
<i>E. lata</i> ^{TS} (<i>Eutypa armeniaca</i>)	CBS 622.84	<i>Vitis vinifera</i>	Italy	AJ302446	DQ006964
<i>E. lejoplaca</i>	020202-3	<i>Acer pseudoplatanus</i>	Switzerland	AY684238	AY684197
<i>E. lejoplaca</i>	020202-5	<i>Acer pseudoplatanus</i>	Switzerland	AY684221	AY684196
<i>E. sparsa</i>	3802-3b	<i>Populus</i> sp.	Switzerland	AY684220	AY684201
<i>E. tetragona</i>	CBS 284.87	<i>Sarothamnus scoparius</i>	France	DQ006923	DQ006960
<i>Eutypella citricola</i>	HVGRF01	<i>Citrus sinensis</i>	Australia	HQ692589	HQ692521
<i>E. citricola</i>	HVVIT07	<i>Vitis vinifera</i>	Australia	HQ692579	HQ692512
<i>E. leprosa</i>	STEU 8189	NA	South Africa	MF359637	MF359672
<i>E. leprosa</i>	STEU 8190	NA	South Africa	MF359638	MF359673

Table 1. Cont.

Species	Strain	Host/Substrate	Origin	GenBank Accession Numbers	
				ITS	TUB2
<i>E. microtheca</i>	ADEL200	<i>Ulmus procera</i>	Australia	HQ692559	HQ692527
<i>E. microtheca</i>	BCMX01	<i>Vitis vinifera</i>	Mexico	KC405563	KC405560
<i>E. vitis</i>	UCD2291AR	<i>Vitis vinifera</i>	America	HQ288224	HQ288303
<i>E. vitis</i>	UCD2428TX	<i>Vitis vinifera</i>	TX, America	FJ790851	GU294726
<i>Halodiatrype avicenniae</i>	MFLUCC 15-0953	<i>Avicennia</i> sp.	Thailand	KX573916	KX573931
<i>H. salimicola</i> ^{TS}	MFLUCC 15-1277	submerged marine wood	Thailand	KX573915	KX573932
<i>Kretzschmaria deusta</i>	CBS 826.72	NA	NA	KU683767	KU684190
<i>Monosporascus cannonballus</i> ^{TS}	ATCC 26931	NA	NA	FJ430598	NA
<i>M. cannonballus</i> ^{TS}	CMM 3646	<i>Boerhavia</i> sp.	Brazil	JX971617	NA
<i>Neoeutypella baoshanensis</i> ^{TS}	BAP101	<i>Pinus armandii</i>	China	MH822887	MH822888
<i>N. baoshanensis</i> ^{TS}	CBS 274.87	<i>Ficus carica</i>	France	AJ302460	NA
<i>Pedumispora rhizophorae</i> ^{TS}	BCC44877	<i>Rhizophora apiculata</i>	Thailand	KJ888853	NA
<i>P. rhizophorae</i> ^{TS}	BCC44878	<i>Rhizophora apiculata</i>	Thailand	KJ888854	NA
<i>Quaternaria quaternata</i>	GNF13	<i>Fagus</i> sp.	Iran	KR605645	KY352464
<i>Q. quaternata</i>	CBS 278.87	<i>Fagus sylvatica</i>	Switzerland	AJ302469	NA
<i>Xylaria hypoxylon</i>	CBS 122620	NA	Sweden	AM993141	KX271279

NA: not applicable. Type species are denoted with the superscript “^{TS}” and the disputable type species are denoted with the superscript “^{TSQ}”.

2.3. Phylogenetic Analyses

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 7 (<http://mafft.cbrc.jp/alignment/server/>) (accessed on 6 December 2021) using the “G-INS-i” strategy and manually adjusted in BioEdit [28]. The sequence alignment was deposited in TreeBase (submission ID 29299). *Xylaria hypoxylon* (L.) Grev. and *Kretzschmaria deusta* (Hoffm.) P.M.D. Martin obtained from GenBank were used as outgroups.

Sequences were analysed using Maximum Likelihood (ML) through the raxmlGUI 2.0 and Cipres Science Gateway (www.phylo.org) (accessed on 6 December 2021) [29]. Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 [30] was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes 3.1.2 with a general time reversible GTR+I+G model of DNA substitution and a gamma distribution rate variation across sites [31]. Four Markov chains were run for 2 runs from random starting trees for 1.2 million generations, and trees were sampled every 100 generations. The first 1/4 generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap (BS) > 70%, Bayesian posterior probabilities (BPP) > 0.95.

3. Results

3.1. Molecular Phylogeny

The contribution of the molecular phylogenetic tree based on 151 sequences of two DNA loci (87 ITS and 64 β -tubulin sequences) was composed of 59 strains including two newly described species, two species of *Allocryptovalsa*, three species of *Allodiatrype*, one species of *Anthostoma*, two species of *Cryptosphaeria*, one species of *Cryptovalsa*, 20 species of *Diatrype*, nine species of *Diatrypella*, seven species of *Eutypa*, four species of *Eutypella*, two species of *Halodiatrype*, one species of *Monosporascus*, one species of *Neoeutypella*, one species of *Pedumispora*, one species of *Quaternaria* and the *X. hypoxylon* and *K. deusta* as outgroups. The dataset had an aligned length of 881 characters, of which 419 characters are constant, 54 are variable and parsimony-uninformative, and 408 are parsimony-informative. Bayesian analysis resulted in a similar topology with an average standard deviation of split frequencies = 0.007642 (BI).

The phylogeny (Figure 1) inferred from ITS and β -tubulin sequences demonstrated that two new taxa were nested in *Diatrype* and are clearly separated from other sampled species of *Diatrype*. *Diatrype larissae* was sister to *Diatrype betulaceicola* with a weakly supported lineage in the phylogenetic tree.

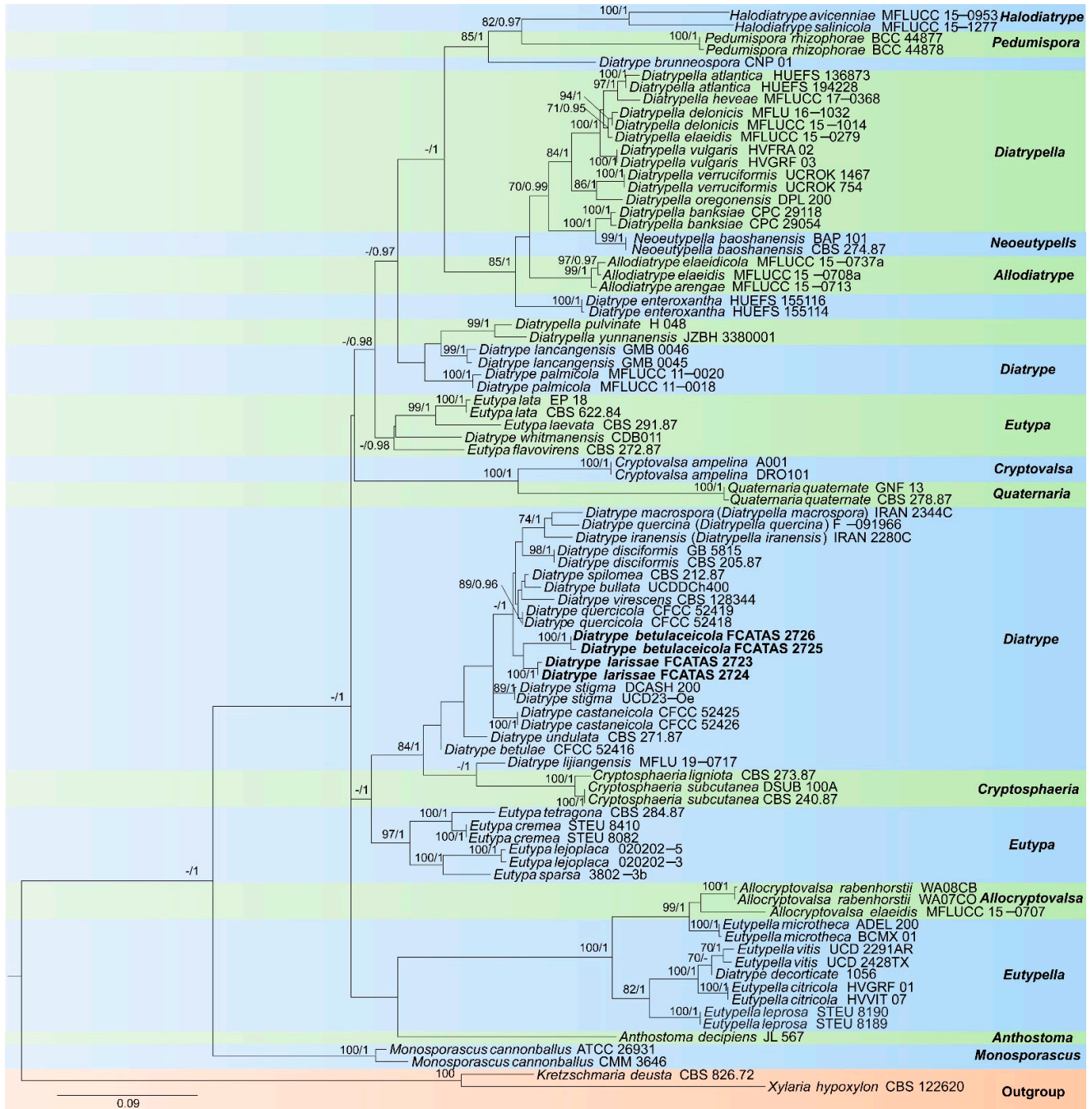


Figure 1. Phylogram generated from Maximum Likelihood (ML) analyses, based on ITS- β -tubulin matrix. Branches are labelled with maximum likelihood bootstrap $\geq 70\%$, Bayesian posterior probabilities ≥ 0.95 . New species are labelled in bold.

3.2. Taxonomy

Diatrype betulaceicola Z.E. Yang and Hai X. Ma, sp. nov. Figure 2.

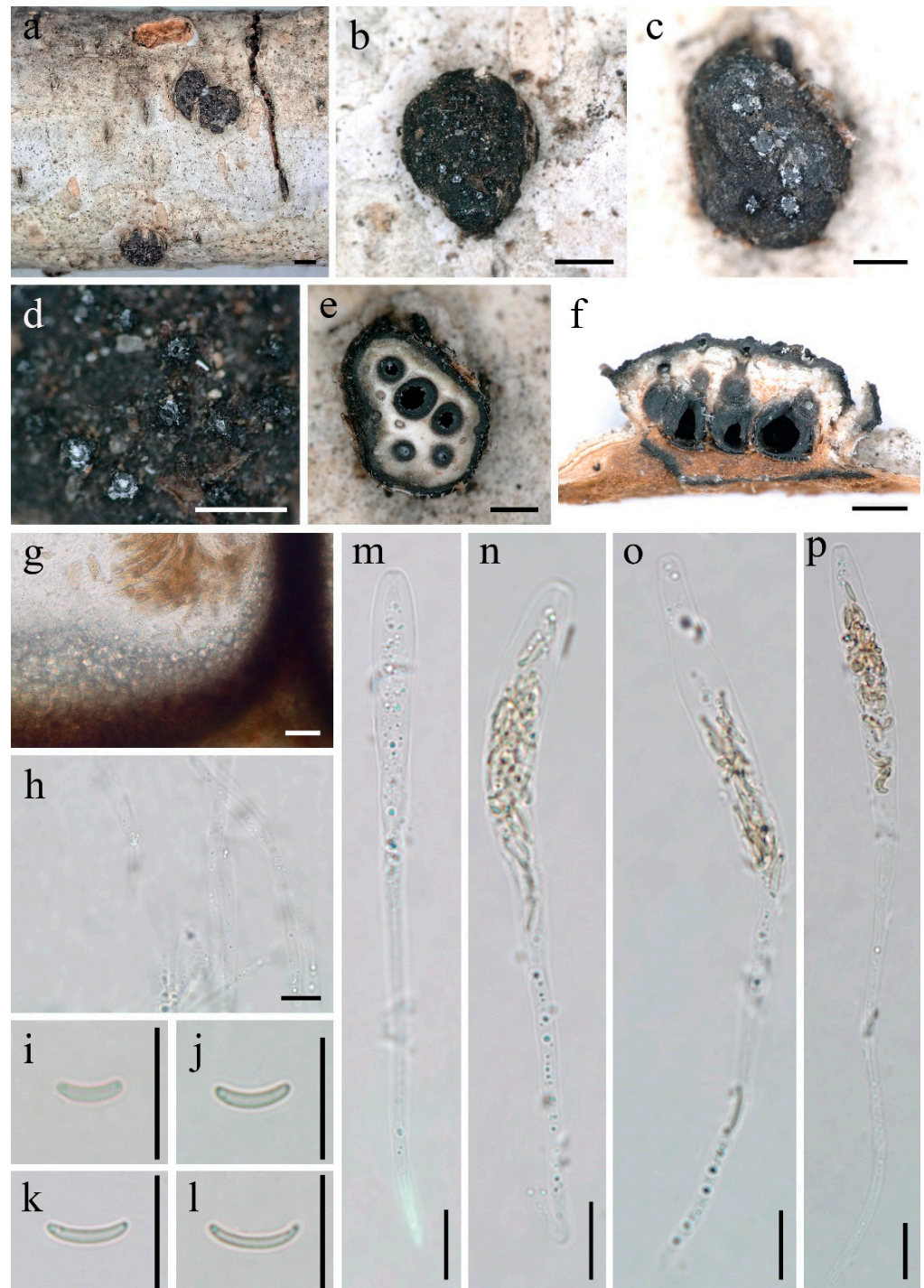


Figure 2. *Diatrype betulaceicola* (FCATAS 2725, Holotype). (a–d) Stromata on substrate; (e) cross section of a stroma; (f,g) vertical section through stroma showing ostiole and perithecia; (h) paraphyses in water; (i–l) ascospore in water; (m–p) ascus in water; scale bars ((a,b) = 1 mm; (c–f) = 500 μm; (g–p) = 10 μm).

MycoBank: MB 842591.

Holotype—CHINA. Inner Mongolia Autonomous Region, Genhe City, forest maintenance station, saprobic on decaying branches of *Betula* L., 3 September 2021, Zhang Bo, FCATAS 2725.

Etymology—betulaceicola lat.: Refers to the host family Betulaceae which the fungus inhabits.

Description—**Sexual morph**: Stromata scattered on the host, erumpent through bark, pustulate, solitary, semi-immersed, 1.9–2.6 mm long \times 1.2–2.4 mm broad (\bar{x} = 2.3 \times 1.7 mm, n = 15), ca. 1.1 mm thick, circular to oblong, upper surface nearly flat; surface black; with 5–14 perithecia immersed in per stroma. Endostroma consists of an outer dark brown layer, dense, thin parenchymatous cell layer and an inner white, thick, parenchymatous cell layer, with residual light orange host tissue. Perithecia immersed in stroma, globose to subglobose, 395–588 μ m high \times 340–516 μ m diam (\bar{x} = 499 \times 420 μ m, n = 15), individual ostiole with a long neck. Peridium composed of an outer dark brown to black, thin-walled cells, inner layer of hyaline thin-walled cells. Ostiole papillate or apapillate, separately, dark brown to black, 110–191 μ m (\bar{x} = 142 μ m, n = 10) in diam. Paraphyses elongate, hyaline, long, filiform, unbranched, septate, guttulate. Asci 88–153 \times 7–10 μ m (\bar{x} = 110.4 \times 8 μ m, n = 30), polysporous, unitunicate, clavate, long-stalked (50–81 μ m), apically rounded, with a J-apical ring. Ascospores 4.8–8.6 \times 0.9–1.4 μ m (\bar{x} = 6 \times 1.2 μ m, n = 30), polyseriate, allantoid, aseptate, slightly curved, subhyaline, smooth-walled.

Asexual morph: Undetermined.

Additional specimen examined—CHINA. Inner Mongolia Autonomous Region, Genhe City, forest maintenance station, on fallen branch of *Betula* L., 3 September 2021, Zhang Bo, FCATAS 2726.

Diatrype larissae Z.E. Yang and Hai X. Ma, sp. nov. Figure 3.

MycoBank: MB 842592.

Holotype—CHINA. Heilongjiang Province, Wudalianchi City, Wudalianchi National Natural Reserve, saprobic on decaying branches of an unidentified plant, 10 September 2021, Zhang Bo, FCATAS 2723.

Etymology—larissae lat.: Refers to honour Russian mycologist Prof. Dr. Larissa N. Vasilyeva, who greatly contributed to studies of pyrenomycetous fungi.

Description—**Sexual morph**: Stromata scattered on the host, erumpent through bark, pustulate, semi-immersed, solitary, 0.7–1.4 mm long \times 0.5–1.1 mm broad, (\bar{x} = 1.1 \times 0.8 mm, n = 30), ca. 0.5 mm thick, circular to oval or rather angular, upper surface nearly flat to slightly convex; surface dark-brown to black, usually smooth, with 3–6 perithecia immersed in per stroma. Endostroma consists of an outer dark brown layer, small, dense, thin parenchymatous cell layer and an inner white, thick, parenchymatous cell layer. Perithecia immersed in stroma, globose to subglobose, 254–401 μ m high \times 211–362 μ m diam (\bar{x} = 320 \times 282 μ m, n = 15), individual ostiole with a neck, cylindrical. Peridium composed of outer layer of dark brown, thin-walled cells, inner layer of hyaline thin-walled cells. Ostiole papillate or apapillate, separately, black. Paraphyses elongate, hyaline, filiform, unbranched, septate, guttulate. Asci 83–123 \times 7–12 μ m (\bar{x} = 101 \times 10 μ m, n = 30), polysporous, unitunicate, clavate, long-stalked (26–67 μ m), apically truncate, with a J-apical ring. Ascospores 5.6–8.4 \times 0.8–1.4 μ m (\bar{x} = 7 \times 1.1 μ m, n = 30), polyseriate, allantoid, aseptate, slightly or moderately curved, subhyaline, smooth-walled.

Asexual morph: Undetermined.

Additional specimen examined—CHINA. Heilongjiang Province, Wudalianchi City, Wudalianchi National Natural Reserve, on fallen branch of angiosperm, 10 September 2021, Zhang Bo, FCATAS 2724.

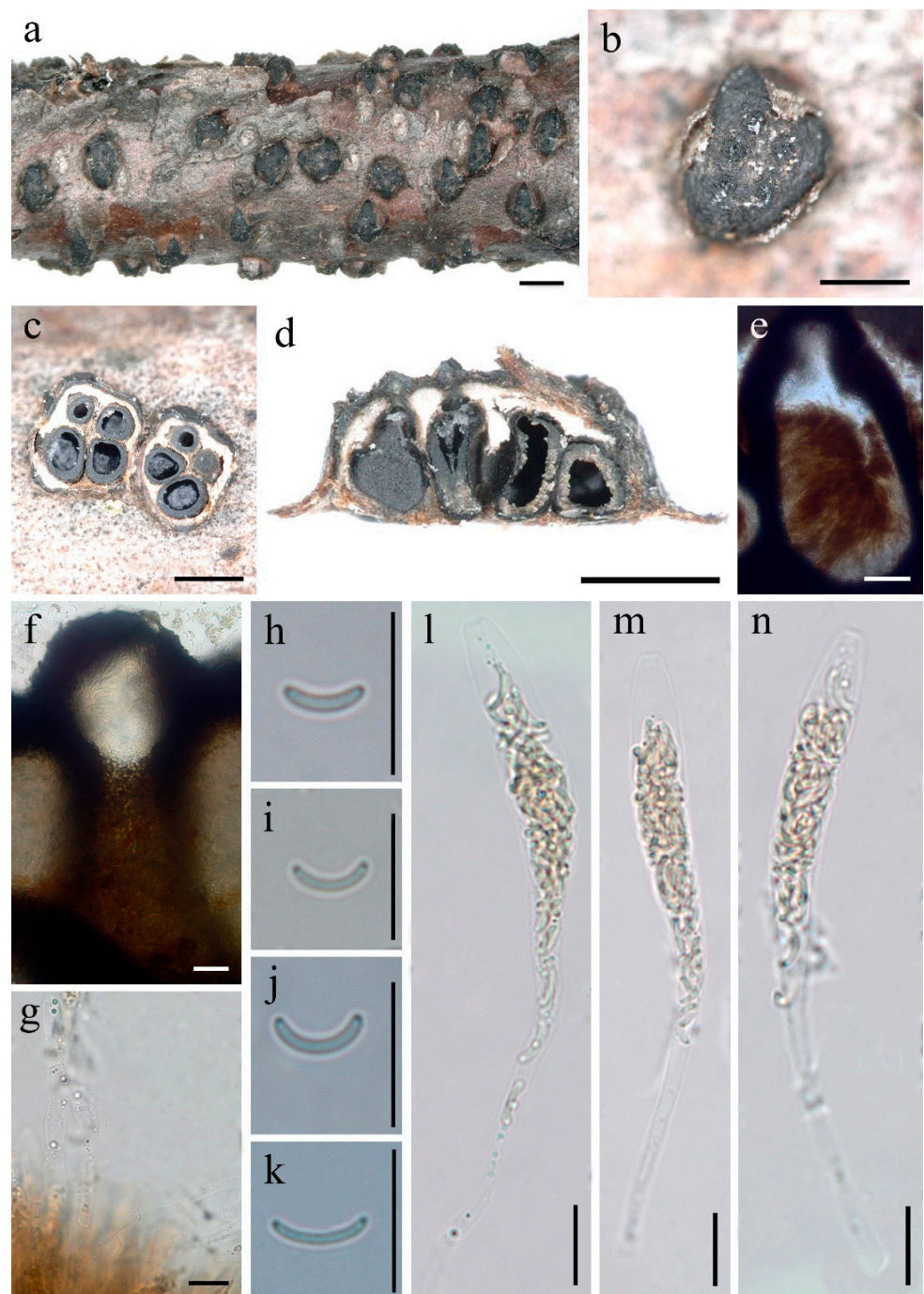


Figure 3. *Diatrype larissae* (FCATAS 2723, Holotype). (a,b) Stromata on substrate; (c) cross section of a stroma; (d,e) vertical section through stroma showing ostioles and perithecia; (f) ostiolar canal; (g) paraphyses in water; (h–k) ascospore in water; (l–n) ascus in water; scale bars ((a) = 1 mm; (b–d) = 500 μ m; (e) = 20 μ m; (f–n) = 10 μ m).

4. Discussion

In the present study, two *Diatrype* species with polysporous asci from China, *D. larissae* and *D. betulaceicola*, were described and illustrated as new species based on phylogenetic analyses and morphological characteristics.

Diatrype betulaceicola is characterized by larger stoma with 5–14 perithecia, pustulate, circular to oblong, perithecium immersed, ostiole with a long neck, asci polysporous, ascospores allantoid, aseptate, slightly curved, subhyaline (Figure 2). The new species were

found on branch of *Betula* sp., and, while five *Diatrype* taxa, *D. albopruinosa* (Schwein.) Cooke, *D. betulae* H.Y. Zhu and X.L. Fan, *D. oregonensis* (Wehm.) Rappaz, *D. stigma* (Hoffm.) Fr. and *D. undulata* (Pers.) Fr., were also reported from *Betula* sp. [6,15,17,24,32]. However, *D. betulaceicola* can be easily distinguished from the other five taxa by its polysporous asci and phylogenetic analyses. According to our phylogenetic tree based on a combined ITS-TUB2 dataset, and as shown in previous studies [4,11,23,24,33–37], the genus *Diatrype* as currently circumscribed is of polyphyletic origin within the family Diatrypaceae. *Diatrype betulaceicola* and *D. larissae* clustered together with a weakly supported sister branch, which were clearly separated from other sampled species of *Diatrype*. Morphologically, *D. larissae* is distinguished by its smaller stroma with 3–6 perithecia, pustulate, circular to oval, rather angular, asci polysporous, ascospores allantoid, slightly or moderately curved, subhyaline (Figure 3). The two new *Diatrype* are somewhat similar in morphology and size of asci and ascospores, but *D. betulaceicola* has larger stromata ($1.9\text{--}2.6 \times 1.2\text{--}2.4$ vs. $0.7\text{--}1.4 \times 0.5\text{--}1.1$ mm) and perithecia ($395\text{--}588 \times 340\text{--}516$ vs. $254\text{--}401 \times 211\text{--}362$ μm).

Diatrype quercicola H.Y. Zhu and X.L. Fan also has multispore asci, but it differs in having larger asci and ascospores ($172\text{--}183 \times 20\text{--}43$, $17\text{--}27 \times 4\text{--}6$ μm) [24], and is distinct from *D. betulaceicola* and *D. larissae* phylogenetic trait (Figure 1).

Traditionally, the number of ascospores per ascus (eight-spores vs. more than eight-spores) was morphological criterion for separating the two genera of *Diatrype* and *Diatrypella* [19]. Based on phylogenetic analysis of ITS and β -tubulin presented here, we agree with the previous concepts that the polysporous ascus feature cannot be used to distinguish *Diatrypella* from *Diatrype* [4,11,33,38,39] (Figure 1). The current phylogenetic analyses show the generic divisions are confusing, and cannot reflect the natural relationships of genera and species within the Diatrypaceae. Therefore, more collections, epitypification, and multi-gene sequences as well as the application of integrative (or polyphasic) taxonomic approach of Diatrypaceae are needed in the future.

Key to the species of *Diatrype* known from China

1. Sexual morph not known 2
1. Sexual morph present 3
2. Conidia $10\text{--}13 \times 1\text{--}2$ μm *D. betulae*
2. Conidia $4\text{--}6 \times 1\text{--}1.5$ μm *D. castaneicola*
3. Stromata solitary, pustulate, circular or elliptic 4
3. Stromata widely and indefinitely effuse 13
4. Ascus with more than 8 spores 5
4. Ascus with 8 spores 7
5. Ascospores $17\text{--}27 \times 4\text{--}6$ μm *D. quercicola*
5. Ascospores length less than 10 μm 6
6. Perithecia less than 6 *D. larissae*
6. Perithecia more than 6 *D. betulaceicola*
7. Ascus length more than 30 μm 8
7. Ascus length less than 30 μm 11
8. Ascospores 6–8 μm long, size of asci more than 65 μm *D. lijiangensis*
8. Ascospores 8–15 μm long, size of asci less than 65 μm 9
9. Ascospores brownish *D. albopruinosa*
9. Ascospores subhyaline to light yellow, or light yellow 10
10. Stromata pulvinate, hemispherical or forming linear stripes *D. oregonensis*
10. Stromata circular-polygonal or irregular *D. macowaniana*
11. Ascospores 4–6 μm long *D. macounii*
11. Ascospores 7–9 μm long 12
12. Stromata $1\text{--}2 \times 1\text{--}1.5$ mm *D. acericola*
12. Stromata 2–7 mm diam *D. bullata*
13. Ascospores 11–19 μm long *D. lancangensis*
13. Ascospores 4–9 μm long 14
14. Ascospores yellowish *D. subundulata*
14. Ascospores hyaline 15
15. Asci 20–25 μm long, Stromata chocolate-brown *D. hypoxyloides*
15. Asci 25–30 μm long, Stromata different shades of brownish tinges or black 16
16. Stromata black, perithecia more than 200 μm diam *D. spilomea*
16. Stromata various shades of brownish tinges, perithecia less than 200 μm diam *D. stigma*

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