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Dendrostoma covidicola sp. nov. (*Erythrogloeaceae*, *Diaporthales*) on *Fagus sylvatica* from Sichuan Province, China

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Abstract

Dendrostoma covidicola sp. nov. is described from a dead branch of *Fagus sylvatica* (*Fagaceae*) collected from Sichuan Province, China. The taxon has 5–20 perithecia per pseudostroma and small, ellipsoidal to subfusiform ascospores with polar appendages. Phylogenetic analyses of a combined ITS–LSU–*rpb2*–*tef1* sequence matrix confirmed its placement in *Dendrostoma* where it is close to *D. qinlingense*, *D. osmanthi* and *D. quercus*, but representing a distinct lineage. The new taxon is compared with other *Dendrostoma* species and comprehensive descriptions and illustrations are provided. An ascospore comparison for relevant species is also provided, as well as an updated phylogenetic tree (ITS–LSU–*rpb2*–*tef1*) including all the species of *Dendrostoma*.

Keywords: 1 new species, *Diaporthales,* Multi-gene, Phylogeny, Taxonomy

Introduction

Dendrostoma X.L. Fan & C.M. Tian was introduced by Fan *et al*. (2018) to accommodate three species and placed in *Erythrogloeaceae*, *Diaporthales* (Hyde *et al.* 2020a). *Dendrostoma mali* X.L. Fan & C.M. Tian as the type, *D. osmanthi* X.L. Fan & C.M. Tian and *D. quercinum* X.L. Fan & C.M. Tian were discovered on *Malus spectabilis* (Aiton) Borkh., *Osmanthus fragrans* Lour. and *Quercus acutissima* Carruth respectively. *Dendrostoma* species are characterized by typical diaporthalean perithecia with clavate asci and fusoid to cylindrical, bi-celled ascospores. Additionally, host association is suggested as an important character for reliable identification (Fan *et al.* 2018).

Senanayake *et al*. (2018) accepted *Dendrostoma leiphaemia* (Fr.: Fr.) Senan. & K.D. Hyde based on LSU–ITS–*rpb2*–*tef1* phylogenies. Jaklitsch & Voglmayr (2019) documented European *Dendrostoma* species occurring on *Castanea sativa* Mill. and *Quercus* sp., while introducing *D. atlanticum* Voglmayr & Jaklitsch, *D. castaneum* (Tul. & C. Tul.) Voglmayr & Jaklitsch, *D. creticum* Voglmayr & Jaklitsch and *D. istriacum* Voglmayr & Jaklitsch with evidence from morphology and multi-gene phylogenies. Extensive sampling of *Castanea mollissima* Blume and *Quercus* trees was carried out by Jiang *et al*. (2019) and ten new species (namely as *D. aurorae* C.M. Tian & N. Jiang, *D. castaneae* C.M. Tian & N. Jiang, *D. castaneicola* C.M. Tian & N. Jiang, *D. chinense* C.M. Tian & N. Jiang, *D. dispersum* C.M. Tian & N. Jiang, *D. parasiticum* C.M. Tian & N. Jiang, *D. qinlingense* C.M. Tian & N. Jiang, *D. quercus* C.M. Tian & N. Jiang, *D. shaanxiense* C.M. Tian & N. Jiang and *D. shandongense* C.M. Tian & N. Jiang) were introduced based on morphological and molecular (ITS–LSU–*rpb2*–*tef1*) data. A study focusing on diaporthalean fungi associated with canker and dieback symptoms from Mount Dongling in Beijing, China, introduced *D. donglingense* H.Y. Zhu & X.L. Fan (Zhu *et al*. 2019). Crous *et al*. (2020) introduced *Dendrostoma luteum* L.A. Shuttlew. *et al.* on branch lesions of *Castanea sativa*.

During investigations of microfungi in Sichuan Province, China, we found branches of *Fagus sylvatica* L. with abundant pseudostromata. Since there are few taxonomic studies on microfungi in Sichuan Province (Yang *et al.* 2019a,b,c; Samarakoon *et al*. 2020), exhaustive morpho-molecular studies were carried out. In this study, morphological

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and phylogenetic analyses indicate that our specimen belonged to *Dendrostoma* but not known before. Therefore, this collection introduces as *Dendrostoma covidicola* and justified with the morpho-molecular evidence, a detailed description, illustrations and comparisons with related taxa.

Materials & Methods

Collection, isolation and morphological studies

Dead branches attached to the host of *Fagus sylvatica* were collected from the University of Electronic Science and Technology of China (UESTC) campus (Qingshuihe), Chengdu, Sichuan Province, China. Macro-micro morphologies were observed as detailed in Samarakoon *et al.* (2020). Measurements were made with the Tarosoft (R) Image Frame Work program v. 0.9.7 following in Liu *et al.* (2010) and images used for figures were processed with Adobe Photoshop CS6 software (Adobe Systems, USA).

Pure cultures were obtained from single ascospore isolation on potato dextrose agar (PDA) in double-distilled water (Senanayake *et al.* 2020). Cultures were incubated at 25–30 °C for 3–4 weeks with frequent observations. The type specimens were deposited in the herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS), Chinese Academy of Sciences, Kunming, China and the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand. Ex-type cultures were deposited in the Guizhou Culture Collection (GZCC), Guizhou, China and the Culture Collection at Mae Fah Luang University (MFLUCC). The new taxon was linked with Facesoffungi and MycoBank databases as explained in Jayasiri *et al.* (2015) and MycoBank (https://www.mycobank.org).

DNA extraction, PCR amplification and sequencing

Genomic DNA extraction was carried out from fresh mycelium and fruiting bodies by using total DNA extraction kits (Sangon Biotech (Shanghai) Co. Ltd. China). ITS5/ITS4, LR0R/LR5 (Vilgalys & Hester 1990), EF1-728F/EF1-986R (Carbone & Kohn 1999), fRPB2-5F/fRPB2-7cR (Liu *et al.* 1999) and β-tubulin Bt2a/Bt2b (Glass & Donaldson 1995) were used to amplify the DNA sequence of the internal transcribed spacers (ITS), the partial 28S large subunit rDNA (LSU), partial translation elongation factor-1α (*tef1*), partial DNA-directed RNA polymerase II subunit (*rpb2*) and partial β-tubulin (*tub*) respectively. The PCR mixture contained 12.5 µl of 2× PCR Master Mix with dye (0.1 U Taq Polymerase/µl, 500 µM dNTP each, 20 mM Tris-HCl (pH 8.3), 100 mM KCl, 3 mM MgCl2), 1 µl of each primer, 9.5 µl of double-distilled water and 1 µl (100–500 ng) of DNA template with the total volume of 25 µl.

Initial denaturation and final extension of PCR thermal cycler programs for all gene regions were at 94 °C for 5 min and at 72 °C for 10 min respectively. ITS, LSU and *tef1* gene amplifications were followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 50 seconds, extension at 72 °C for 1 min. The PCR thermal cycles for *rpb2* were followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 2 min, extension at 72 °C for 90 seconds. The PCR thermal cycles for *tub* were followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 56 °C for 50 seconds, extension at 72 °C for 1 min. All the PCR products were immediately subjected to 4 °C and were visualized on 1 % agarose gel stained using GoldView I nuclear staining dye (1 µL/10 mL of agarose) with D2000 DNA ladder (Realtimes Biotech, Beijing, China). PCR products were sent for DNA sequencing using the same primers in an Applied Biosystem 3730 DNA analyser at Sangon Biotech (Shanghai) Co. Ltd., China.

TABLE 1. Taxa used in this study and GenBank accession numbers.

Species	Code	Accession numbers			
		ITS	LSU	*rpb2*	*tef1*
Dendrostoma atlanticum	CBS 145804*	MN447223	MN447223	MN432160	MN432167
D. atlanticum	WU 37025	MN447224	MN447224	MN432161	MN432168
D. aurorae	CFCC 52753*	MH542498	MH542646	MH545405	MH545447
D. aurorae	CFCC 52754	MH542499	MH542647	MH545406	MH545448
D. castaneae	CFCC 52745*	MH542488	MH542644	MH545395	MH545437
D. castaneae	CFCC 52749	MH542492	MH542645	MH545399	MH545441

.....continued on the next page

TABLE 1. (Continued)

Species	Code	Accession numbers			
		ITS	LSU	*rpb2*	*tef1*
D. castaneicola	CFCC 52743*	MH542496	N/A	MH545403	MH545445
D. castaneicola	CFCC 52744	MH542497	N/A	MH545404	MH545446
D. castaneum	CBS 145803	MN447225	MN447225	MN432162	MN432169
D. castaneum	WU 37030	MN447226	MN447226	N/A	MN432170
D. castaneum	WU 37029	MN447227	MN447227	N/A	N/A
D. chinense	CFCC 52755*	MH542500	MH542648	MH545407	MH545449
D. chinense	CFCC 52756	MH542501	MH542649	MH545408	MH545450
D. creticum	CBS 145802*	MN447228	MN447228	MN432163	MN432171
D. dispersum	CFCC 52730*	MH542467	MH542629	MH545374	MH545416
D. dispersum	CFCC 52731	MH542468	MH542630	MH545375	MH545417
D. donglingense	CFCC 53148*	MN266206	MN265880	MN315491	MN315480
D. donglingense	CFCC 53149	MN266207	MN265881	MN315492	MN315481
D. donglingense	CFCC 53150	MN266208	MN265882	MN315493	MN315482
D. istriacum	CBS 145801*	MN447229	MN447229	MN432164	MN432172
D. leiphaemia	CBS 145800*	MN447230	MN447230	MN432165	MN432173
D. leiphaemia	WU 37041	MN447231	MN447231	MN432166	MN432174
D. leiphaemia	CBS 187.37	MH855882	MH867393	N/A	N/A
D. luteum	IMI 506898*	MN648726	MN648728	N/A	MN812768
D. mali	CFCC 52102*	MG682072	MG682012	MG682032	MG682052
D. osmanthi	CFCC 52106*	MG682073	MG682013	MG682033	MG682053
D. osmanthi	CFCC 52108	MG682074	MG682014	MG682034	MG682054
D. covidicola	**GZCC 20–0355***	**MW261327**	**MW261325**	**MW262892**	**MW262894**
D. covidicola	**MFLU 21–0001**	**MW261328**	**MW261326**	**MW262893**	**N/A**
D. parasiticum	CFCC 52761	MH542480	MH542636	MH545387	MH545429
D. parasiticum	CFCC 52762*	MH542482	MH542638	MH545389	MH545431
D. parasiticum	CFCC 52765	MH542484	MH542640	MH545391	MH545433
D. parasiticum	CFCC 52766	MH542485	MH542641	MH545392	MH545434
D. qinlingense	CFCC 52732*	MH542471	MH542633	MH545378	MH545420
D. qinlingense	CFCC 52733	MH542472	MH542634	MH545379	MH545421
D. quercinum	CFCC 52103*	MG682077	MG682017	MG682037	MG682057
D. quercinum	CFCC 52104	MG682078	MG682018	MG682038	MG682058
D. quercinum	CFCC 52105	MG682079	MG682019	MG682039	MG682059
D. quercus	CFCC 52738	MH542477	N/A	MH545384	MH545426
D. quercus	CFCC 52739*	MH542476	MH542635	MH545383	MH545425
D. quercus	CFCC 52740	MH542479	N/A	MH545386	MH545428
D. shaanxiense	CFCC 52741*	MH542486	MH542642	MH545393	MH545435
D. shaanxiense	CFCC 52742	MH542487	MH542643	MH545394	MH545436
D. shandongense	CFCC 52759*	MH542504	MH542652	MH545411	MH545453
D. shandongense	CFCC 52760	MH542505	MH542653	MH545412	MH545454
Disculoides eucalypti	CBS 132183*	JQ685517	JQ685523	MH545413	MH545455
D. eucalyptorum	CBS 132184*	JQ685518	JQ685524	MH545414	MH545456

Types are indicated with *, newly generated sequences in this study are shown in bold. "N/A" sequence is unavailable.

***Abbreviations**: **CBS**: CBS-KNAW Collections, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **CFCC**: China Forestry Culture Collection Centre, China; **IMI**: CABI-IMI Culture Collection; **HKAS**: Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica, Chinese Academy of Sciences, Kunming, China; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **WU**: Fungarium of the Department of Botany and Biodiversity Research, University of Vienna*

Phylogenetic analyses

Contig sequences were used for BLASTn searches. Related sequences were downloaded from GenBank following recent publications and blast similarities (Fan *et al.* 2018; Jaklitsch & Voglmayr 2019; Jiang *et al.* 2019; Crous *et al.* 2020) (Table 1). Individual loci were aligned using FFT-NS-2 Tree-based progressive method in MAFFT V.7.036 (http://mafft.cbrc.jp/alignment/server/) (Katoh *et al.* 2019) and manually improved when necessary in BioEdit v. 7.0 (Hall 2004). Characters were assessed to be unordered and equally weighted. MrModeltest 2.3 was performed for each gene to estimate the best-fit evolutionary models under the Akaike Information Criterion (AIC) (Nylander 2004) and each resulted SYM+I+G (ITS) and GTR+I+G (LSU, *rpb2*, *tef1*) models. Phylogenies were generated using maximum parsimony (MP), maximum-likelihood (ML) and Bayesian inference (BI) analyses using combined ITS–LSU–*rpb2*–*tef1* and *tub* was excluded due to lack of sequence data. All the newly generated sequences were deposited in GenBank for future studies (Dissanayake *et al*. 2020).

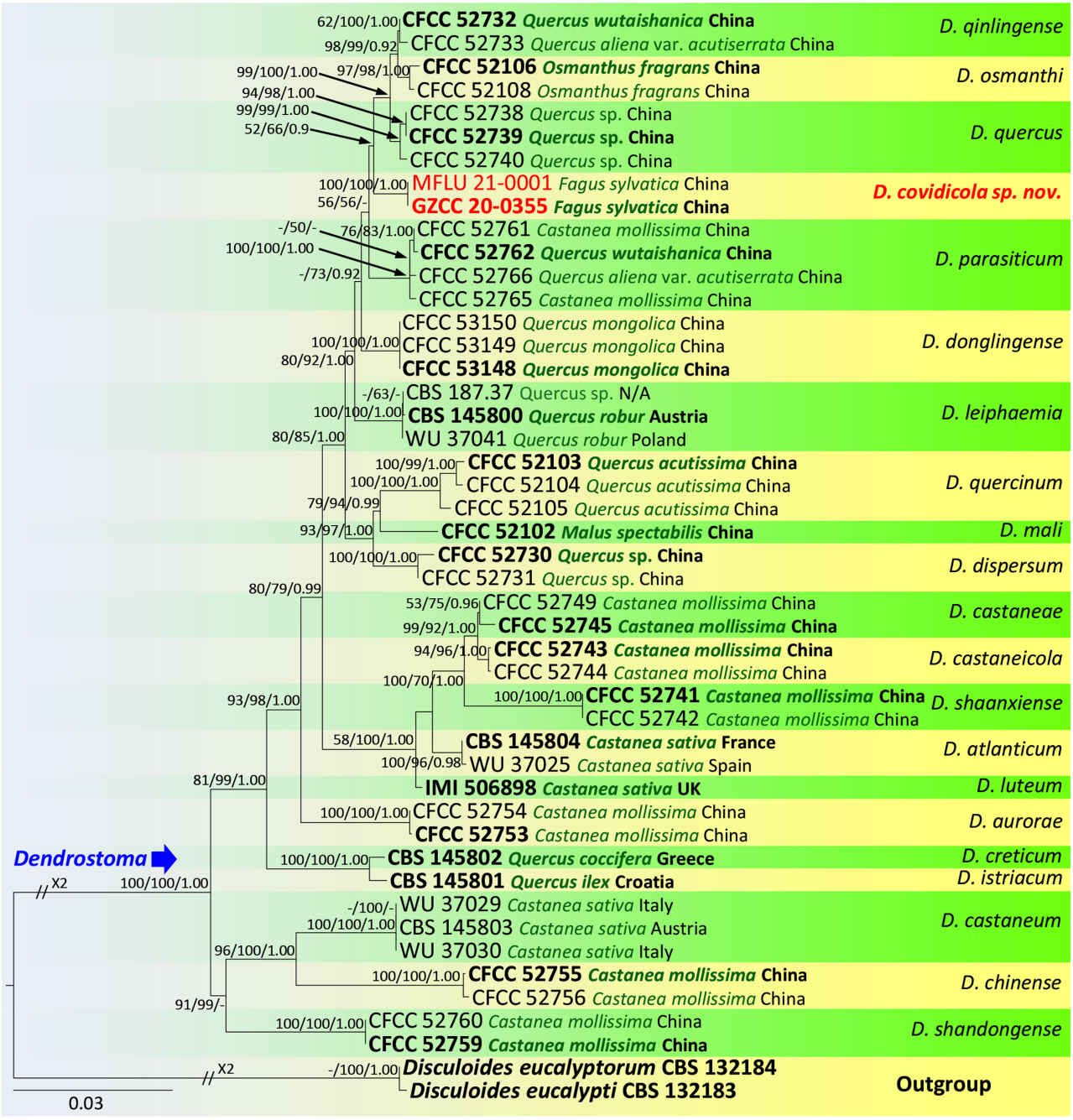

FIGURE 1. Phylogram generated from maximum likelihood (RAxML) based on an ITS–LSU–*rpb2*–*tef1* sequence matrix. The tree is rooted with *Disculoides eucalypti* (CBS 132183) and *D. eucalyptorum* (CBS 132184). MP, ML bootstrap supports (≥50%) and BI posterior probabilities (≥0.95 PP) supports are given above or below the branches respectively. Host and country are denoted in green and black fonts respectively. Types are in bold and the newly introduced taxon is in red.

The analyses of MP, ML and BI were carried out as detailed in Dissanayake *et al*. (2020) using PAUP v.4.0b 10 (Swofford 2002), CIPRES web portal (Miller *et al.* 2010; Stamatakis 2014), and MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001; Zhaxybayeva & Gogarten 2002). Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed in order to determine whether trees were significantly different. The final alignment and tree were registered in TreeBASE under the submission ID: 27335 (http://www.treebase.org). The resulting trees were viewed with FigTree v.1.4.0 (Rambaut 2012) and with Adobe Illustrator® CS5 (Adobe Systems, USA).

Results

Topology of phylogenetic analyses

The combined ITS–LSU–*rpb2–tef1* sequence matrix comprised 47 taxa including the new collections. The concatenated alignment comprised 2,907 characters including gaps (ITS: 1–537 bp, LSU: 538–1,380 bp, *rpb2*: 1,381–2,457 bp, *tef*1: 2,458–2,907 bp) with 618 distinct alignment patterns and 12 % proportion of gaps and completely undetermined characters, 2,318 constants, 50 parsimony uninformative and 539 parsimony informative characters. The MP analysis resulted a single most parsimonious tree (TL = 1065, CI = 0.687, RI = 0.856, RC = 0.588, HI = 0.313). The best ML phylogram (Figure 1) (InL = -9987.466409, α = 0.831334, invar = 0.580982) resulted with estimated base frequencies A = 0.240693, C = 0.267217, G = 0.269895, T = 0.222195, substitution rates AC = 1.624127, AG = 2.526890, AT = 0.820150, CG = 0.848594, CT = 6.554846, GT = 1.0000, proportion of variable sites and gamma distribution shape parameter. The tree topologies resulting from ML, MP and BI analyses are similar.

Our collections formed a basal mono clade to the clade comprising *Dendrostoma qinlingense*, *D. osmanthi* and *D. quercus* with poor statistical support (52% MP/66% ML/0.9 PP), and these four species are described from China.

Taxonomy

Dendrostoma covidicola Samarak. & Jian K. Liu, *sp. nov.* (FIGURE 2)
MycoBank number: MB838110; Facesoffungi number: FoF 09528

Etymology—the epithet "*covidicola*" referring to the COVID-19 pandemic and as a tribute to the battle against COVID-19

Holotype—HKAS 107013

Saprobic on dead branches of *Fagus sylvatica* L. (*Fagaceae*). **Sexual morph** *Pseudostromata* 1.5–2.6 mm in their widest dimension in cross section, variable, flat subconical or lenticular, in outline circular, elliptic or elongate, scattered, gregarious or confluent, and forming elongate patches, lifting the periderm slightly and often becoming visible as a dark zone on the bark surface, causing bumps in the bark, splitting the periderm. *Ectostromatic discs* yellowish brown to dark brown, flat, surrounded by bark flaps, first present as a covering layer with ostiolar necks subsequently bursting through it, soon crumbling away. *Perithecia* 400–530 *μm* high × 290–425 *μm* diam. ($\overline{x}$ = 470 × 362 *μm*, n = 15), 5–20 per disc, aggregated, depressed subglobose to ellipsoid, with a flattened base. *Ostioles* eccentric, bluntly conical or cylindrical with black sides and yellowish, or brownish tip, often attenuated to a minute, periphysate. *Peridium* 13–26 μm ($\overline{x}$ = 19.4 μm, n =10) thick, *textura angularis* cells 11.5–19 × 2.4–4 μm ($\overline{x}$ = 15 × 3.3 μm, n = 20), hyaline, pale olivaceous to brown, thicker peridium near to ostioles. *Paraphyses* absent at maturity. *Asci* 45–55 × 8–14 μm ($\overline{x}$ = 51.5 × 10.8 μm, n = 25), 8-spored, unitunicate, narrowly clavate to subfusoid or oblong, floating freely in the centre, thick-walled at the apex containing a distinct apical ring. *Ascospores* 11–15 × 2.5–3.5 μm ($\overline{x}$ = 13.5 × 3.1 μm, n = 30), l/w 4.3, overlapping uni- or bi-seriate, ellipsoidal to subfusiform, hyaline, 2-celled, constricted at the septum, oblong to inequilaterally ellipsoid, straight to mostly curved, with the upper cell often slightly wider than the lower, broadly rounded at the ends, guttulate (mostly 2 large guttules per cell), smooth, with or without a hyaline, straight or curved filiform appendage 3.4–5.2 μm ($\overline{x}$ = 4.3 μm, n = 20) long. **Asexual morph**: Undetermined.

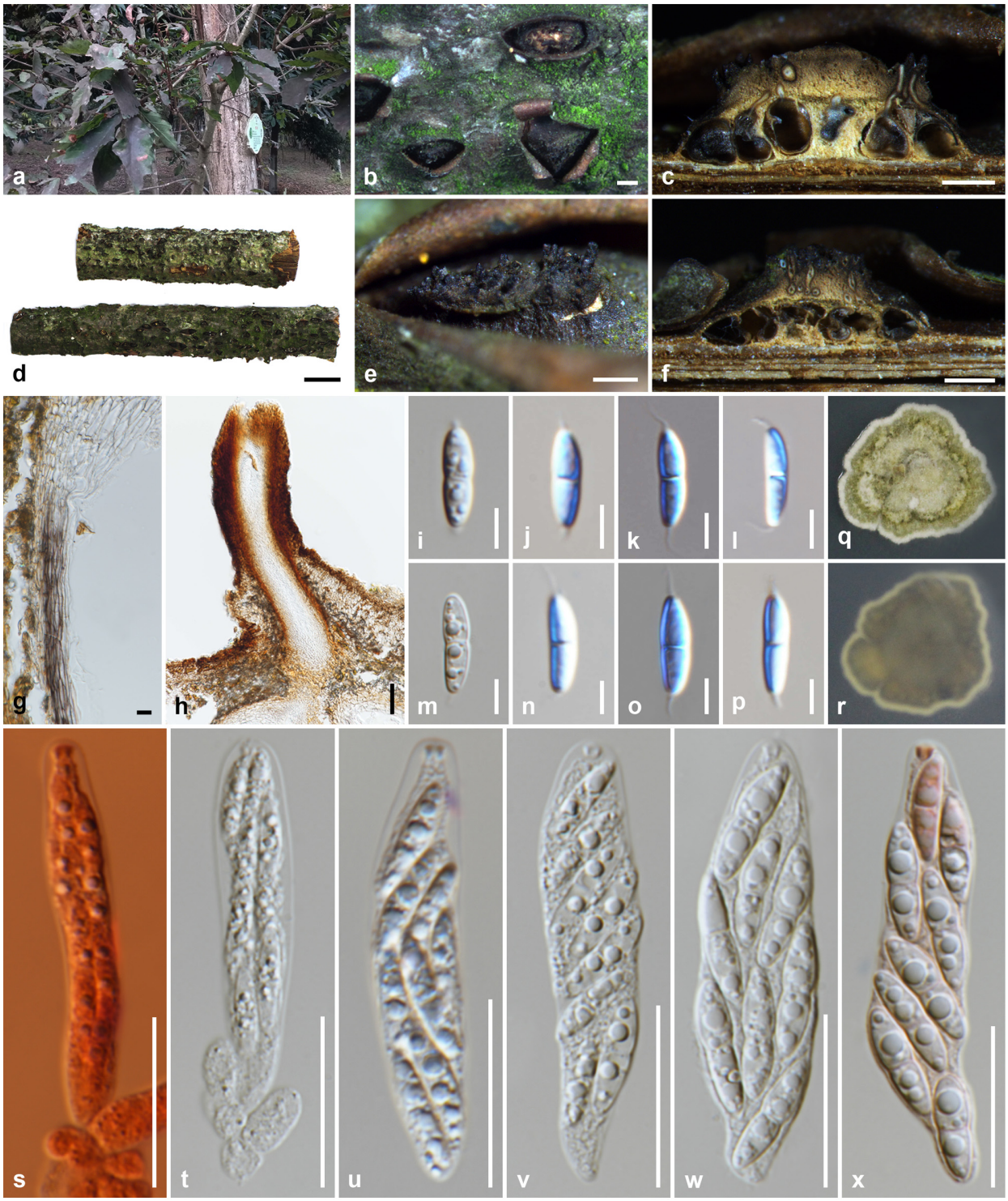

FIGURE 2. ***Dendrostoma covidicola*** (holotype, HKAS 107013) **a** Host. **b,d,e** Ascomata on the substrate. **c,f** Vertical section of pseudostroma. **g** Peridium. **h** Vertical section of ostioles. **i–p** Ascospores. **q,r** Upper and reverse view of the 2 weeks old colony on PDA respectively. **s–x** Asci (**s,x** in Congo Red). Scale bars: d = 1 cm, b,c,e,f = 500 µm, h = 50 µm, s–x = 20 µm, g = 10 µm, i–p = 5 µm.

Colony characteristics—on PDA, reaching 20–25 mm diam. after four weeks at 25 °C, dark, circular to irregular, dense, zonate, yellowish green to pale grayish brown from the top view with white margin; dark yellowish green with white margin from lower view.

Material examined—China, Sichuan Province, Chengdu, University of Electronic Science and Technology of China (UESTC) campus (Qingshuihe), on the dead branch of *Fagus sylvatica* L. (Fagaceae), 30 September 2019, MC. Samarakoon, SAMC245 (HKAS 107013, holotype; MFLU 21–0001, isotype), ex-type living cultures GZCC 20–0355 = MFLUCC. Additional sequence: *tub* MW262895.

Discussion

The morphology of our taxa has an affinity with *Dendrostoma* in its pseudostromata with embedded perithecia, 8-spored, sessile asci and hyaline, fusoid to cylindrical, bi-celled ascospores (Fan *et al.* 2018). *Dendrostoma covidicola* differs from other *Dendrostoma* species in having 5–20 perithecia per pseudostroma and smaller, ellipsoidal to subfusiform ascospores among appendage bearing ascospores. In BLASTn searches of NCBI GenBank, the closest matches of our sequences are *Dendrostoma*. The ITS sequence is 98 % similar to *D. leiphaemia* (CBS 145800) with 1.4 % nucleotide differences while LSU is 99 % similar to *D. creticum* (CBS 145802) and *D. atlanticum* (CBS 145804). The *rpb2* of *D. covidicola* is 97 % similar to *D. leiphaemia* (CBS 145800) with 2.6 % nucleotide difference. Here, we introduce *Dendrostoma covidicola* isolated from bark of senescent branches as a new species and also the first report of *Dendrostoma* on *Fagus sylvatica* following the guidelines of Jeewon & Hyde (2016).

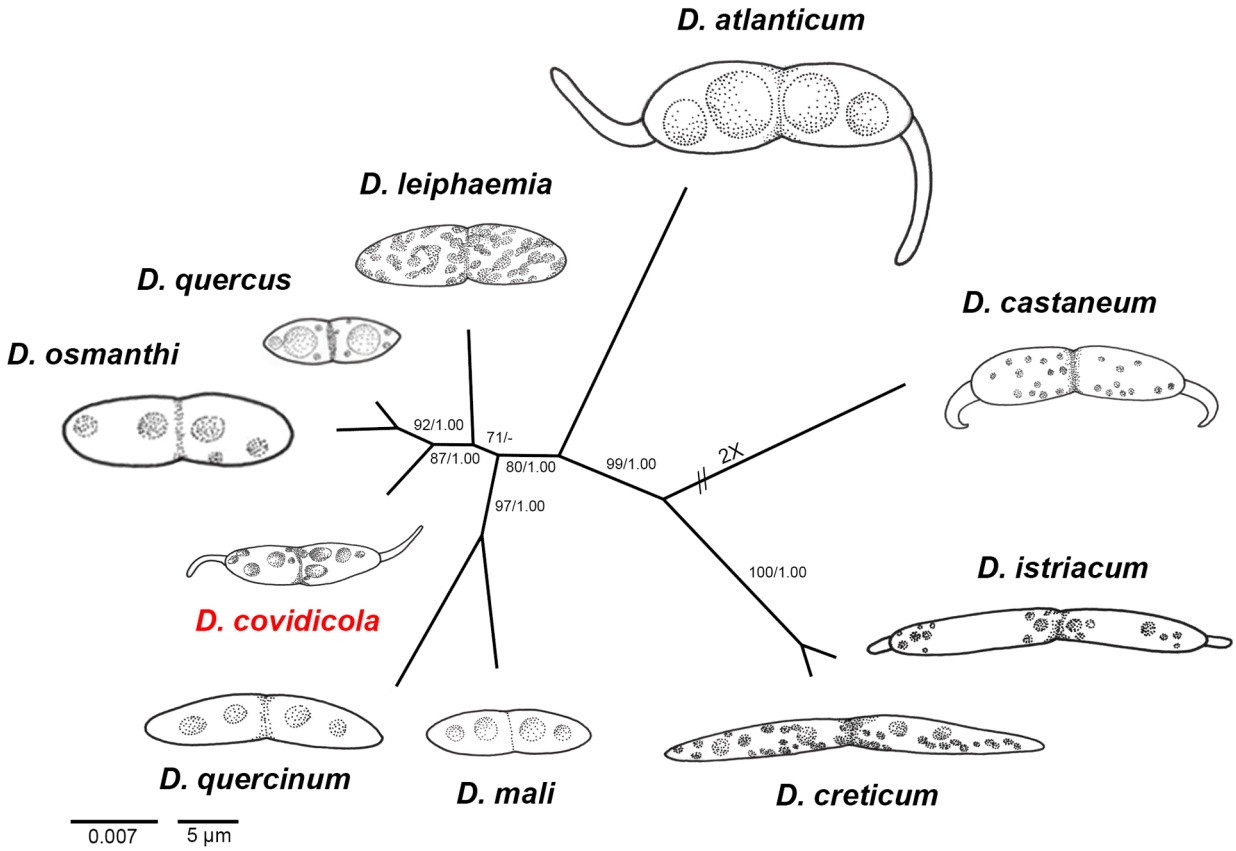

FIGURE 3. Phylogram generated from maximum likelihood (RAxML) based on ITS–LSU–*rpb2*–*tef1* matrix. ML bootstrap supports (≥50%) and BI posterior probabilities (≥0.95 PP) supports are given above or below the branches respectively. The new taxon is in red and the scale bars represent nucleotide substitutions per site and ascospore size (5 µm).

The number of taxa in this genus has rapidly increased since its introduction by Fan *et al*. (2018). This provides evidence that when a genus is studied in detail, numerous new taxa can be expected to be discovered (Hyde *et al*. 2020b). *Dendrostroma* ascospores vary in size, shape, guttules and presence or absence of appendages at ends (Figure 3). *Dendrostroma atlanticum*, *D. castaneum*, *D. covidicola* and *D. istriacum* have distinct appendages which are important for species delimitation. In addition, most species have been described from specific Fagales hosts which can provide additional evidence for species identification.

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Conflicts of Interest: The authors declare no conflict of interest.

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