Phylogenetic and morphological appraisal of *Diatrype lijiangensis* sp. nov. (*Diatrypaceae*, Xylariales) from China

Thiyagaraja V1,2,3,5, Senanayake IC2,3,4, Wanasinghe DN2,3,5, Karunarathna SC2,3,5, Worthy FR3,6, To-Anun C1*

1Department of Biology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50002, Thailand
2Centre of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand
3Key Laboratory for Plant Biodiversity and Biogeography of East Asia (KLPB), Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, People’s Republic of China
4Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Science and Oceanography, Shenzhen University, 3688, Nanhai Avenue, Nanshan, Shenzhen 518055, China
5World Agroforestry Centre East and Central Asia, Kunming 650201, Yunnan, People’s Republic of China
6Centre for Mountain Futures, Kunming Institute of Botany, Kunming 650201, Yunnan, People’s Republic of China

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Abstract

The majority of *Diatrype* species are saprobes, while a few species are pathogens which form cankers on forest trees. The placement of several species in the genus *Diatrype* is uncertain, as the phylogeny has yet to be well-resolved with extensive taxon sampling and authentic ex-type cultures. In this study, a diatrype-like taxon was found on decaying wood in Lijiang, Yunnan Province, China. Morphological characteristics and phylogenetic analyses of combined ITS and β-tubulin sequences, supported the conclusion that the taxon is a new species, which is named as *Diatrype lijiangensis*. The new species differs from other species of *Diatrype* in asci and ascospore characteristics. The morphological similarities and dissimilarities of the new species with phylogenetically close alliances are discussed. Micro-morphological characteristics of this novel taxon are illustrated with descriptions.

Key words – 1 new species – Phylogeny – Sordariomycetes – Taxonomy

Introduction

*Diatrypaceae* Nitschke, a family in Xylariales, comprises 17 genera (Index Fungorum 2019) with *Diatrype* Fr. as the type genus (Maharachchikumbura et al. 2015, 2016, Senanayake et al. 2015, de Almeida et al. 2016, Dayarathne et al. 2016, Mehrabi et al. 2016, Senwanna et al. 2017, Shang et al. 2017, Wijayawardene et al. 2018). Species of *Diatrypaceae* are mostly saprobes on decaying wood (Carter 1991, Acero et al. 2004, Trouillas & Gubler. 2004, Mehrabi et al. 2015, de Almeida et al. 2016, Shang et al. 2017), however several species are pathogens and endophytes (Acero et al. 2004, de Errasti et al. 2014, Shang et al. 2017). The taxa of *Diatrypaceae* have perithecial ascomata, a poor or well-developed stroma with an ostiole, short to long neck, clavate or spindle-shaped asci and allantoid ascospores (Trouillas et al. 2010, Mehrabi et al. 2015, Dayarathne et al. 2016, de Almeida et al. 2016, Senwanna et al. 2017, Shang et al. 2017). However, the intraspecific variations in stromatal characteristics make the delineation of the genera in
Diatrypaceae challenging (Vasilyeva & Stephenson 2004, Dayarathne et al. 2016). Asexual morphs have been reported to be either coelomycetous, ex: *Cytosporina* Sacc and *Libertella* Desm. (de Almeida et al. 2016, Senwanna et al. 2017, Shang et al. 2017) or hypomycetous, ex: genus *Phaeoisaria* which has recently been linked to the family *Pleurotheciaceae* (Luo et al. 2018). The asexual morph has not been used to identify species in *Diatrypaceae* (de Almeida et al. 2016, Senwanna et al. 2017).

Recent studies have provided updated phylogenetic analyses of *Diatrypaceae* (Mehrabi et al. 2015, Dayarathne et al. 2016, de Almeida et al. 2016, Mehrabi et al. 2016, Senwanna et al. 2017, Shang et al. 2017). However, phylogenetic placement of *Eutypa*, *Diatrype* and *Diatrypella* in this family remains unresolved (de Almeida et al. 2016, Senwanna et al. 2017, Shang et al. 2017). Therefore, more taxon sampling and extended molecular data are needed to elucidate their natural placements and close alliances (Shang et al. 2017).

In this study, morphological characteristics and molecular phylogenetic analyses showed that the new fungus groups within *Diatrype* and forms a strongly supported clade. The objectives of this study are to 1) introduce a new species of *Diatrype* and 2) strengthen its taxonomic placement using both morphological characteristics and phylogenetic analyses results of maximum likelihood and Bayesian analyses, based on combined ITS and β-tubulin sequences.

**Material and Methods**

Decaying woody material were collected from Lijiang, Yunnan Province, China, N 27° 00' 30.8", E 100° 11' 26.1", 3234 m in September 2018 and brought to the laboratory in a Zip-lock plastic bag. Samples were examined under a Motic SMZ 168 Series microscope and photographed using a Carl Zeiss Discovery V8 stereo-microscope fitted with Axiocam. Hand sections of the ascomata, were mounted on 5% KOH. Sections of ascomata and other micro-morphological characteristics were photographed using a Nikon ECLIPSE 80i compound microscope fitted with a Canon 550D digital camera. All microscopic measurements were made with Tarosoft Image Frame Work (v.0.9.0.7).

Images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software (Adobe Systems, USA). The holotype specimen was deposited in the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Faces of Fungi and Index Fungorum numbers were provided as outlined in Jayasiri et al. (2015) and Index Fungorum (2019) respectively. The new species was established based on the recommendations of Jeewon & Hyde (2016). We could not obtain a pure culture of this fungus and all the morphological characteristics and phylogenetic data were obtained from fresh fruiting structures.

**DNA extraction, PCR amplification and gene sequencing**

DNA was extracted directly from fruiting bodies of the fungus as outlined by Wanasinghe et al. (2018). An E.Z.N.A. ® Forensic DAT (D3591 – 01, Omega Bio – Tek) DNA extraction kit was used to extract DNA by following the manufacturer’s instructions. DNA samples that were intended for use as a template for PCR were stored at 4°C for use in regular work and duplicated at -20 °C for long-term storage. DNA sequence data were obtained from internal transcribed spacers (ITS) and β-tubulin. The internal transcribed spacers (ITS) were amplified with primers ITS4 and ITS5 (White et al. 1990) while the β-tubulin was amplified with primers Bt2a and Bt2b (Glass & Donaldson 1995).

The components for the PCR amplification are described below. The final volume of the PCR mixture was 25 µl with 2.0 µl of DNA template, 1 µl of each forward and reverse primers, 12.5 µl of 2 × Easy Taq PCR Super Mix (mixture of Easy Taq TM DNA Polymerase, dNTPs, obtained buffer (Beijing Trans Gen Biotech Co., Chaoyang District, Beijing, PR China) and 8.5 µl sterilized water. The PCR thermal cycle program for ITS gene was used as: initial denaturation at 94°C for 3 mins, followed by 35 cycles of 30 sec at 94°C, 50 sec at 55°C, and 1 min at 72°C, with a final extension of 10 mins at 72°C. Amplification of β-tubulin was accomplished by an initial denaturation at 94°C for 3 mins, followed by 35 cycles consisted of denaturation at 94°C for 30 sec,
annealing at 55°C for 50 sec, elongation at 72°C for 1 min with a final extension for 10 mins at 72°C.

PCR products were observed on 1% agarose electrophoresis gels stained with ethidium bromide. Purification and DNA sequencing were performed by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China). The nucleotide sequence data acquired were deposited in GenBank and alignments and the trees were submitted to TreeBASE under submission ID 24320.

**Phylogenetic analyses and species recognition**

Sequence homologies were searched by using the NCBI BLAST search engine for the preliminary identification (https://www.ncbi.nlm.nih.gov). Phylogenetic analyses were constructed based on ITS and β-tubulin sequence data. Sequences of available closely related taxa from the family Diatrypaceae were obtained from GenBank and following Senwanna et al. (2017) (Table 1). *Kretzschmaria deusta* (CBS 826.72) and *Xylaria hypoxylon* (CBS 122620) were selected as the outgroup taxa. Multiple sequence alignments were generated with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html; Katoh et al. 2017), and where necessary were manually adjusted using Bioedit v. 7.0.5.2 (Hall 1999).

**Table 1** Taxa used in the phylogenetic analyses and their GenBank accession numbers. The newly generated sequence from this study, and the ex-type isolates are given in bold.

| Taxon                        | Strain          | GenBank Accessions |
|------------------------------|-----------------|--------------------|
| Allocryptovalsa polyspora    | MFLU:17-1218    | NR153588           |
| Allocryptovalsa rabenhorstii | WA07CO          | HQ692620           |
| Allocryptovalsa rabenhorstii | WA08CB          | HQ692619           |
| Anthostoma decipiens        | IPVFW349        | AM399021           |
| Anthostoma decipiens        | JL567           | JN975370           |
| Cryptosphaeria ligniota     | CBS 273.87      | KT425233           |
| Cryptosphaeria pullmanensis | HBPF24          | KT425202           |
| Cryptosphaeria pullmanensis | ATCC 52655      | KT425235           |
| Cryptosphaeria subcutanea    | DSUB100A        | KT425189           |
| Cryptosphaeria subcutanea    | CBS 240.87      | KT425232           |
| Cryptovalsa ampelina        | A001            | GQ293901           |
| Cryptovalsa ampelina        | DRO101          | GQ293902           |
| Diatrype bullata            | UCDCh400        | DQ006946           |
| Diatrype disciformis        | MFLUCC 15-0538  | KRO92795           |
| Diatrype disciformis        | CBS 205.87      | AJ302437           |
| Diatrype lijiangensis       | MFLU:19-0717    | MK852582           |
| Diatrype spilomea           | D17C            | AJ302433           |
| Diatrype stigma             | DCASH200        | GQ293947           |
| Diatrype undulata           | D20C            | AJ302436           |
| Diatrype virescens          | 1057            | KU320619           |
| Diatrypella frostii         | UFMGCB 1917     | HQ377280           |
| Diatrypella heveae          | MFLU:17-1216    | NR154046           |
| Diatrypella major           | Isolate 1058    | KU320613           |
| Diatrypella tectonae        | MFLUCC 12-0172a | KY283084           |
| Diatrypella tectonae        | MFLUCC 12-0172b | KY283085           |
| Diatrypella verruciformis   | UCROK1467       | JX144793           |
| Diatrypella verruciformis   | UCROK754        | JX144783           |
| Diatypella vulgaris          | HVGRF03         | HQ692590           |
| Eutypa armeniacae           | ATCC 28120      | DQ006948           |
| Eutypa guttulata            | HUEFS 192075    | AJ302450           |
| Eutypa lata                 | EP18            | HQ692611           |
| Eutypa longa                | RGA01           | HQ692614           |
| Eutypella citricola         | HVVIT07         | HQ692579           |
| Eutypella citricola         | HVGRF01         | HQ692589           |
| Eutypella vitis             | UCD2428TX       | FJ790851           |
Table 1 Continued.

| Taxon                     | Strain                  | GenBank Accessions                      |
|---------------------------|-------------------------|----------------------------------------|
|                           |                         | ITS                                    | β -tubulin                             |
| Eutypella vitis           | UCD2291AR               | HQ288224                               | HQ288303                               |
| Halodiatrype avcenniae    | MFLUCC 15-0953          | KX573916                               | KX573931                               |
| Halodiatrype salinicola   | MFLUCC 15-1277          | KX573915                               | KX573932                               |
| Kretzschmaria deusta      | CBS 826.72              | KU683767                               | KU684190                               |
| Monosporascus cannonballus| CMM3646                 | JX971617                               | -                                      |
| Monosporascus cannonballus| ATCC 26931              | NR111370                               | -                                      |
| Peroneutypa alsophila     | EL58C                   | AJ302467                               | -                                      |
| Peroneutypa comosa        | BAF 393                 | KF964568                               | -                                      |
| Peroneutypa diminutispora | MFLUCC 17-2144          | MG873479                               | MH316765                               |
| Peroneutypa kochiana      | EL53M                   | AJ302462                               | -                                      |
| Peroneutypa rubiformis     | MFLUCC 17-2142          | MG873477                               | -                                      |
| Peroneutypa scoparia      | MFLUCC 11-0478          | KU940151                               | -                                      |
| Quaternaria quaternata    | GNF13                   | KR605645                               | -                                      |
| Quaternaria quaternata    | CBS 278.87              | AJ302469                               | -                                      |
| Xylaria hypoxylon         | CBS 122620              | AM993141                               | -                                      |

Phylogenetic analyses of both individual and combined aligned data were performed under maximum-likelihood (ML) and Bayesian (BI) criteria. Terminal ends of sequences and ambiguous regions were deleted manually and excluded from the dataset. The phylogeny web tool “ALTER” (Glez-Peña et al. 2010) was used to convert sequence alignment from FASTA to PHYLIP for RAxML analysis and FASTA to NEXUS format for Bayesian analysis. The estimated model of maximum likelihood and Bayesian analyses were performed independently for each locus using MrModeltest v.2.2 (Nylander 2004). Maximum likelihood trees were generated using RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis 2006, Stamatakis et al. 2008) in the CIPRES Science Gateway platform (Miller et al. 2010).

MrBayes v. 3.0b4 was used to perform Bayesian analysis (Ronquist & Huelsenbeck 2003). GTR + GAMMA + I nucleotide substitution best-fit model is determined with MrModeltest v. 2.2 (Nylander 2004). The Bayesian Markov Chain Monte Carlo (BMMC) sampling method in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001) was used to define Bayesian Posterior Probabilities (BP) (Rannala et al. 1998, Zhaxybayeva & Gogarten 2002). Six simultaneous Markov Chains were run for 5 million generations and the trees were sampled every 100th generation. The first 10% of trees that represented the burn-in phase were discarded, and only the remaining 90% of trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree. The resulting trees are displayed using FigTree v1.4.0 (Rambaut 2012) and converted to jpeg file in Adobe Photoshop CS6 version 13.0. (Adobe Systems. U.S.A.).

Results

Phylogenetic analyses

Our preliminary dataset comprised representatives of the family Diatrypaceae and our strain nested together with Diatrype sensu stricto based on combined ITS and β-tubulin data. We excluded other Diatrype species which were not cladded in Diatrype sensu stricto from the final analysis (Fig. 1). Based on megablast search of the NCBI nucleotide database using the ITS sequence, the highest similarities were found with D. spilomea [GenBank AJ302433; Identities = 587/600 (97%), Gaps = 75/600 (12%)], D. stigma [GenBank KX828152; Identities = 517/600 (97%), Gaps = 87/600(14%)] and D. virescens [GenBank MH864890; Identities = 584/607 (96%), (Gaps =7/607 (2%)]. Based on megablast search of the NCBI nucleotide database using the β-tubulin sequence, the highest similarities were found with undefined Diatrype species. The results obtained by both ML and BI analyses of the combined ITS and β-tubulin dataset comprised selected 50 taxa including the new strain.
Phylogenetic analyses obtained from maximum likelihood and Bayesian inference analysis showed similar topologies and were not significantly different. The best scoring RAxML tree was selected to represent the relationship analyses obtained among taxa, with the final ML optimization likelihood value of -8747.484409 and is shown in Fig. 1. Bayesian posterior probabilities from MCMC were evaluated with final average standard deviation of split frequencies = 0.006514. The new strain, MFLU 19-0717, was grouped in *Diatrype sensu sticto* within *Diatrypaceae* and formed a distinct clade (ML/BP=97/1.00) with high bootstrap support (Fig. 1). *Diatrype sensu sticto* comprises *D. stigma*, *D. undulate* and *D. bullata* and *D. spilomea* species with the type *D. disciformis* and this concur the phylogenetic studies of Dayarathne et al. 2016, de Almeida et al. 2016, Senwanna et al. 2017, Shang et al. 2017. We have defined *Diatrype sensu sticto* based on the published sequence data from reference stain of the type species *D. disciformis* (Senanayake et al. 2015).

**Taxonomy**

*Diatriy ljiangensis* Thiyagaraja & Wanas., sp. nov.

Index Fungorum Number: IF556377; Facesoffungi number: FoF06032

Etymology – The specific epithet “ljiangensis” refers to the name of the place, from which the type specimen of the species was collected.

Holotype – MFLU 19-0717

*Saprobic* on decaying woody bark. Sexual morph: *Ascostromata* 1 mm diam., black, superficial, solitary to gregarious, subglobose or ellipsoidal, carbonaceous. *Ascomata* 170–460 × 200–300 μm (x̅ = 300 × 250 μm, n = 10), perithecial, black, subglobose to ovoid, clustered, immersed in ascostroma, glabrous, 2–5 loculate, ostiolate. *Ostiole* papillate or apapillate, central, ostiolar canal filled with periphyses. *Peridium* 15–25 μm wide, composed of two layers, outer layer comprising several layers of thick-walled, dark brown to black cells of *textura angularis*, inner layer comprising 3–5 layers of thin-walled, hyaline cells of *textura angularis*. *Hamathecium* 140–165 μm wide, hyaline. *Paraphyses* 2–4 μm wide, arising from base of perithecia, composed of long, narrow, unbranched, septate, guttulate, narrowing and tapering towards the apex, with apex blunt. *Asci* 50–90 × 6–9 μm (x̅ = 65 × 8 μm, n = 20), 8-spored, unitunicate, thin-walled, clavate to cylindric clavate, long pedicillate, apically truncate. *Ascospores* 6–8 × 1–2 μm (x̅ = 7 × 1.5 μm, n = 30), overlapping bi-seriate, allantoid, aseptate, hyaline to pale-brown, with one to few small guttules, slightly to moderately curved and smooth-walled. Asexual morph: Undetermined.

Material examined – China, Yunnan Province, Lijiang, on dead wood of unidentified host, N 27° 00' 30.8'', E 100° 11' 26.1'', 3234m, 7 September 2018, V. Thiyagaraja (MFLU 19-0717, holotype).

Addition GenBank number – LSU (MK810546)

**Discussion**

The genus *Diatrype* was introduced by Fries (1849) with the type species *D. disciformis* (Hoffm.) (Tilak 1964). It comprises 60 species (Wijayawardene et al. 2017), of which the majority are saprobes, with a few species that are pathogens which form cankers on forest trees. Its asexual morph is reported as libertella-like (Senanayake et al. 2015, Dayarathne et al. 2016, Wijayawardene et al. 2017). The species of *Diatrype* possess characteristics that include perithecia embedded in discoid or widely effuse stromata that are erumpent from the bark (Vasilyeva & Stephenson. 2009). The young stromata are sometimes covered with a layer of sterile tissue that eventually peels off to expose a fertile surface extruded with papillate or stellate ostioles (Vasilyeva & Stephenson. 2009, Dayarathne et al. 2016). The polysporous ascus feature has been traditionally used to distinguish the species of *Diatrype* from those of *Diatrypella* (Liu et al. 2015).

The most recent phylogenetic analysis for the genera of *Diatrypaceae* is provided by Senwanna et al. (2017) with twelve genera. The genera *Diatrype*, *Diatrypella* and *Eutypa* are polyphyletic within the family as found in several previous studies (Acero et al. 2004, Trouillas et
In this study, we introduce a new species to the family Diatrypaceae based on molecular phylogenetic analyses of combined ITS and β-tubulin sequence data and morphological characteristics. According to our phylogenetic analyses, the genus Diatrype sensu stricto formed a distinct clade (ML/BP=97/1.00) with selected taxa including *D. disciformis*, *D. stigma*, *D. undulate*, *D. virescens*, *D. bullata*, *D. spilomea*, and our new species *D. lijiangensis*. However, further studies need to clarify the taxonomic placement of these taxa.

![RAxML tree based on analysis of combined ITS and β-tubulin partial sequence data.](image)

**Fig. 1** – RAxML tree based on analysis of combined ITS and β-tubulin partial sequence data. Bootstrap support values for ML equal or greater than 60%, and Bayesian posterior probabilities (BP) equal or greater than 0.90 are given as ML/BP above the nodes. The tree is rooted to *Kretzschmaria deusta* (CBS 826.72) and *Xylaria hypoxylon* (CBS 122620). All ex-type strains are displayed in bold and the new species that was found in this study is displayed in blue bold.
Our novel taxon *D. lijiangensis* exhibits distinct morphological characteristics: viz. long pedicellate asci (50–90 μm) with superficial stromata (Fig. 2) which contrast to those recorded for *D. acericola* (23–27 μm), *D. albopruinosa* (40–60 μm), *D. bullata* (25–30 μm), *D. hypoxylodes* (50–90 μm), *D. macounii* (25–30 μm), *D. stigma* (25–30 μm) and *D. subundulata* (35–40 μm) which were previously collected from China (Vasilyeva & Ma 2014) (Table 2).

**Fig. 2** – *Diatrype lijiangensis* (MFLU 19-0717, holotype). a-c Stromata on substrate. d, e Cross-section of stroma. f, g Vertical section through stroma showing ostioles and perithecia. h Peridium. i, j Ostiolar canals. k Paraphyses. l-r Asci. r-u Ascospores. Scale bars: b-e = 200 μm, f, g = 100 μm, h = 30 μm, k-r = 20 μm, s-v = 5 μm
ITS gene region and β-tubulin sequence data were not available in GenBank for comparisons. These two species exhibit distinct morphological differences in asci and ascospore characteristics whereas, asci and ascospores of *D. virescens* are 35–40 μm long and (10)12–14 μm long respectively (Vasiljeva & Stephenson 2004).

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**Table 2** Synopsis of *D. lijiangensis* and related species discussed in this study

| Species name     | Colour of entostroma | Ascus length (μm) | Ascus width (μm) | Ascospore color | Ascospore length (μm) | Ascospore width (μm) | Reference                  |
|------------------|----------------------|-------------------|------------------|-----------------|-----------------------|----------------------|---------------------------|
| *D. lijiangensis*| Dark brown           | 50–90             | 6–9              | Hyaline to pale-brown | 6–8                  | 1–2                  | This study                |
| *D. acericola*   | Brownish or almost black | 23–27           | 5–7              | Very slightly yellowish | 7.5–9                | 0.9–1.1               | Vasiljeva & Ma 2014       |
| *D. albobruinosa*| Brownish or almost black | 40–60            | 10–15            | Brownish        | 12–15(–18)            | 3.5–4                | Vasiljeva & Ma 2014       |
| *D. atlantica*   | Chocolate colored    | 30–40             | 4–6              | Hyaline         | (6–)7–9(–10)          | –                    | Vasiljeva & Stephenson. 2009 |
| *D. bullata*     | Light to dark brown  | 25–30             | 5–7              | Slightly yellowish | 7.5–9                | –                    | Vasiljeva & Ma 2014       |
| *D. caryae*      | Light brown         | 28–33             | 4–5              | Hyaline         | 5–7                  | –                    | Vasiljeva & Stephenson. 2009 |
| *D. decoricata*  | Pallid-brown or brown | 30–40            | 4–6              | Hyaline         | 6–8                  | –                    | Vasiljeva & Stephenson. 2009 |
| *D. enteroxantha*| Brown or black       | 18–28.5           | 5–9              | Subhyaline      | 7–10                 | 1.5–2.5              | Almeida et al. 2014       |
| *D. hypoxyloides*| Chocolate-brown      | (15–)20–25        | 4–6              | Hyaline         | 4–6                  | –                    | Vasiljeva & Ma 2014       |
| *D. ilicina*     | Dark brown          | 25–35             | 4–6              | Hyaline         | 5–7                  | –                    | Vasiljeva & Stephenson. 2009 |
| *D. macounii*    | Dark brown          | 25–30             | 4–6              | Slightly yellowish | 4–6                  | 0.7–1               | Vasiljeva & Ma 2014       |
| *D. spilomea*    | Black                | 25–30             | 4–6              | –                | 5–7                  | –                    | Vasiljeva & Ma 2004       |
| *D. stigma*      | Brownish            | 25–30             | 5–7              | Hyaline         | 6–8                  | 1.5–2               | Vasiljeva & Ma 2014       |
| *D. stigmaoides* | Grey or dark-grey   | 20–30             | 5–6              | Hyaline         | 4–6                  | –                    | Vasiljeva & Stephenson. 2009 |
| *D. subundulata* | Dark brown          | 35–40             | 5–7              | Yellowish       | 7–9                  | 1.7–1.9             | Vasiljeva & Ma 2014       |

The comparatively longer asci (50–90 μm) of *D. lijiangensis* were observed to delineate from extant species such as *D. decoricata* (30–40 μm), *D. caryae* (28–33 μm), *D. atlantica* (30–40 μm), *D. ilicina* (25–35μm), *D. stigmaoides* (20–30 μm) (Vasiljeva & Stephenson 2009), *D. acerisrubrae* (35–50 μm) (Vasiljeva & Stephenson 2014), *D. Montana* (30–35 μm), *D. rappazii* (26–30 μm) and *D. subaffixa* (30–40 μm) (Vasiljeva & Stephenson 2004). Our taxon shares similar morphological characteristics with the type species *D. disciformis*, but differs in having aseptate paraphyses whereas *D. disciformis* has septate paraphyses (Senanayake et al. 2015). *D. disciformis* phylogenetically differs from our new taxon in 2.47 % (14/565) base pair differences present in ITS gene but β-tubulin sequence data were not available in GenBank for comparisons. *D. virescens* phylogenetically closely related to our taxon but, differs in 4.77 % (27/565) base pair differences in ITS gene region and β-tubulin sequence data were not available in GenBank for comparisons. These two species exhibit distinct morphological differences in asci and ascospore characteristics whereas, asci and ascospores of *D. virescens* are 35–40 μm long and (10)12–14 μm long respectively (Vasiljeva & Stephenson 2004).
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