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New Host Record of *Neoeutypella baoshanensis* (Diatrypaceae) from China

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Abstract

During an ongoing fungal diversity survey in Yunnan Province, China, an interesting species was collected from a dead branch of *Trachycarpus* species. Morphology, coupled with combined gene analysis of an internal transcribed spacer (ITS) and beta-tubulin (*tub*2) DNA sequence data, showed that it belongs to *Neoeutypella* in Diatrypaceae. Maximum likelihood and Bayesian analyses were performed to clarify the phylogenetic affinities of the species. *Neoeutypella baoshanensis* is introduced as a new host record and is compared with other similar species, and comprehensive descriptions and micrographs are provided.

Keywords – Diatrypaceous fungi, *Diatrypales, Trachycarpus,* Yunnan Province

Introduction

The Diatrypaceae (Xylariales, Sordariomycetes) was typified by *Diatrype* (Nitschke 1905), and it currently consists of 22 accepted genera and more than 1500 species (Kirk et al 2001, Phookamsak et al. 2019, Hyde et al. 2020, Wijayawardene et al. 2022, Yang et al. 2022). Members of this family are common in worldwide, typically occurring on a broad range of live, dead or decaying woody angiosperms as saprobes, pathogens, or endophytes (Acero et al. 2004, Trouillas et al. 2004, 2010, 2011, Trouillas & Gubler 2010, Grassi et al. 2014, Paolinelli-Alfonso et al. 2015). This family can mostly be found in terrestrial habitats but can also be found in marine habitats (Hyde & Rappaz 1993, Chalkley et al. 2010, Abdel-Wahab et al. 2014, Jones et al. 2015, Dayarathne et al. 2016, Li et al. 2016). The sexual morph is characterized by having perithecial ascomata, with poor or well-developed ascostromata, ostiolate with short to

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long necks, cylindric-clavate to clavate or spindle-shaped asci with long pedicel and allantoid ascospores (Trouillas et al. 2010, Mehrabi et al. 2015, Dayarathne et al. 2016, de Almeida et al. 2016, Li et al. 2016). The asexual morph is characterized by cylindrical or filiform or allantoid, hyaline, unicellular and slightly curved conidia (Glawe & Rogers 1982, 1984, Glawe 1983, Mostert et al. 2004, Luque et al. 2006, Wijayawardene et al. 2012, Liu et al. 2015, Mehrabi et al. 2015, Dayarathne et al. 2016, de Almeida et al. 2016). Members of this family have been reported as coelomycetes or hyphomycetes asexual morph (Glawe & Rogers 1982, 1984, Glawe 1983, Mostert et al. 2004, Luque et al. 2006, Wijayawardene et al. 2012, Liu et al. 2015, Mehrabi et al. 2015, Dayarathne et al. 2016, de Almeida et al. 2016). However, the asexual morph was not considered useful in identifying species of Diatrypaceae (Glawe & Roger 1986, de Almeida et al. 2016). For example, *Eutypella parasitica* produces both pycnidia and acervuli on both natural substrata and agar media (Glawe 1983). Also, conidial ontogenesis differs highly in the group and different types of conidiogenesis (sympodial and percurrent) have been reported in the same strain (Glawe and Rogers 1982a, b). In addition, Diatrypaceae species identification based only on morphological characteristics is difficult, because most of the characteristics overlap among the genus (Glawe & Rogers 1984, Rappaz 1987, Mehrabi et al. 2016). Thus, morphology together with the DNA sequence data of ITS and *tub*2 are used for the identification of Diatrypaceae species (Acero et al. 2004, Trouillas & Gubler 2010, Trouillas et al. 2011, Dayarathne et al. 2016, de Almeida et al. 2016, Shang et al. 2017). The latest treatments and updated accounts of Diatrypaceae in Phookamsak et al. (2019), Konta et al. (2020), and Yang et al. (2022) used multi-gene analyses (ITS and *tub*2).

Neoeutypella was introduced by Phookamsak et al. (2019) as a monotypic genus to accommodate *N. baoshanensis*. The morphology of this genus resembles *Eutypella* by forming spindle-shaped asci and allantoid ascospores, with a libertella-like asexual morph. *Neoeutypella* is characterized by entostromatic, carbonaceous ascostromata, immersed to semi-immersed, papilla filled with periphyses, 8-spored, spindle-shaped sexual morph (Phookamsak et al. 2019). Asexual morph is observed in culture as a hyphae-like, filiform, hyaline or pale brown conidia (Phookamsak et al. 2019). According to Phookamsak et al. (2019), a combined ITS and *tub*2 phylogenetic tree showed that type species of *Neoeutypella* grouped with *Eutypella caricae* (strains EL51C and GL08362). However, these two strains were unable to make morphology comparison as they do not have a clear morphological description. Consequently, *Eutypella caricae* was synonymized under *Neoeutypella baoshanensis* by Phukhamsakda et al. (2020).

In this paper, we used multi-gene sequence analyses (ITS and *tub*2) and morphological examinations for the identification and delimitation of fungal isolates. *Neoeutypella baoshanensis* was collected from *Trachycarpus* sp. in China. A comprehensive description, photomicrographs of *Neoeutypella baoshanensis*, and a phylogenetic tree to confirm the placement of the new isolate are provided.

Material and methods

Sample collection, morphological observations, and isolation

Decaying branches with black fungal fruiting bodies were collected at the Kunming Institute of Botany Garden and brought to the mycology laboratory. Morphological characteristics of the fungus were observed using an Olympus SZ61 (Japan) series stereomicroscope and photographed with an OLYMPUS SZ2-ILST and Industrial Digital Camera 16NP USB3.0 (Panasonic, Japan) microscope imaging system. Senanayake et al. (2020) was followed to carry out single spore isolation and to get a pure culture on potato dextrose agar (PDA). Morphological structures of the ascospores were examined and photographed with scanning electron microscopy (SEM) using ZEISS GeminiSEM and ZEISS Sigma 300 apparatus (Figueras & Guarro 1998). Tarosoft Image Framework program v.0.9.0.7. was used to measure all microscopic structures of fungi. Adobe Photoshop CS3 Extended version 10.0 (Adobe Systems, USA) was used to process and edit the images used in the figures. Herbarium specimen was deposited in Kunming Institute of Botany Academia Sinica (HKAS), while living culture was deposited in Kunming Institute of Botany Culture Collection (KUMCC).

DNA extraction, PCR amplification and sequencing

Actively growing mycelium was scraped using a sterile scalpel from a 7–10 days old PDA plate and put into a 1.5 ml centrifuge tube for DNA extraction. The Biospin Fungus Genomic DNA Extraction Kit–BSC14S1 (BioFlux, P.R. China) was used to extract DNA, following the manufacturer’s instructions. Extracted DNA was maintained at -20 °C for long-term storage. The polymerase chain reaction (PCR) conditions for ITS and *tub2* genes amplification are provided in Table 1. The total volume of PCR mixtures for amplifications was 25 μL following Tibpromma et al. (2018). The quality of PCR products was checked on 1% agarose gel electrophoresis stained with 4S green nucleic acid (Life Science Products and Services, Cat. No: A616694). Purification and sequencing of PCR products were carried out in Beijing Kinco Biotechnology Co., Ltd. Kunming Branch, P.R. China.

Table 1. Details of genes/loci with PCR primers and PCR conditions.

Genes/loci	PCR primers (forward/reverse)	PCR conditions	References
ITS	ITS5/ITS4	^{a}; 95 °C: 30 s, 55 °C: 50 s, 72°C: 30 s (35 cycles); ^{b}	White et al. (1990), O’Donnell & Cigelnik (1997)
tub2	T1/ Bt2b	^{a}; 94 °C: 1 min, 55 °C: 50 s, 72 °C: 1 min (35 cycles); ^{b}	Glass & Donaldson (1995)

^{a} Initiation step of 95 °C: 3 min; ^{b} Final elongation step of 72 °C: 10 min and final hold at 4 °C.

Phylogenetic analyses

Forward and reverse raw sequences generated were assembled with the Genious program (9.1.2) and initial identification was performed using a standard BLAST search in GenBank. The sequences similar to our strains were downloaded from the

GenBank nucleotide database based on recently published papers (Phukhamsakda et al. 2020, Yang et al. 2022, Table 2). Multiple alignments were constructed with the online tool MAFFT v.7 (http://mafft.cbrc.jp/alignment/ server) (Katoh & Standley 2013). Manual alignment was done by using BioEdit v.7.0.5.2 (Hall 1999) and alignments were converted in ALTER (https://www.sing-group.org/ALTER/) (Glez-Peña et al. 2010). Phylogenetic analyses were conducted on the CIPRES Science Gateway platform: https://www.phylo.org/portal2/ home.action, using the tool RAxML-HPC v.8 on XSEDE (8.2.12) for maximum likelihood (ML) and MrBayes on XSEDE (3.2.7a) for Bayesian inference (BI). ML analysis was done using GTR + GAMMA swap model with 1,000 bootstrap repetitions. Evolutionary models for Bayesian analysis were selected independently for each locus using MrModeltest v. 2.3 (Nylander et al. 2004) under the Akaike Information Criterion (AIC) implemented. MrBayes analysis was performed setting GTR + I + G, 5 M generations, sampling every 1,000th generations, ending the run automatically when the standard deviation of split frequencies dropped below 0.01 with a burn-in fraction of 0.25. ML bootstrap values equal to or greater than 70% and BYPP equal to or greater than 0.90 are given above each node of the phylogenetic tree. Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2014) and reorganized in Microsoft Powerpoint (2007). The generated new sequences were uploaded to GenBank (https://submit.ncbi.nlm.nih.gov/) to obtain accession numbers.

Table 2. Names, voucher numbers, host information, and corresponding GenBank accession numbers of the sequences used in this study.

Species	**Strain/Voucher**	**GenBank accession number**		**Host/Substrate**	**Countries**
		ITS	***tub2***		
Allocryptovalsa elaeidis	MFLUCC 15-0707	MN308410	MN340296	*Elaeis guineensis*	Thailand
A. rabenhorstii	WA07CO	HQ692620	HQ692522	*Vitis vinifera*	Australia
A. rabenhorstii	WA08CB	HQ692619	HQ692523	*Vitis vinifera*	Australia
Allodiatrype arengae^{T}	MFLUCC 15-0713	MN308411	MN340297	*Arenga pinnata*	Thailand
A. elaeidicola	MFLUCC 15-0737a	MN308415	MN340299	*Elaeis guineensis*	Thailand
A. elaeidis	MFLUCC 15-0708a	MN308412	MN340298	*Elaeis guineensis*	Thailand
Anthostoma decipiens^{T}	JL567	JN975370	JN975407	*Vitis vinifera*	Spain
Cryptosphaeria ligniota	CBS 273.87	KT425233	KT425168	*Populus tremula*	Switzerland
C. subcutanea	CBS 240.87	KT425232	KT425167	NA	Norway
C. subcutanea	DSUB100A	KT425189	KT425124	NA	Norway
Cryptovalsa ampelina	A001	GQ293901	GQ293972	NA	Australia
C. ampelina	DRO101	GQ293902	GQ293982	NA	America
Diatrype betulae	CFCC 52416	MW632943	NA	*Betula davurica*	China
D. betulaceicola^{T}	FCATAS 2725	OM040386	OM240966	*Betula* sp.	China
D. betulaceicola^{T}	FCATAS 2726	OM040387	OM240967	*Betula* sp.	China
D. decorticata	1056	KU320621	NA	NA	NA
D. brunneospora	CNP01	HM581946	HQ692478	*Acacia longifolia* sub sp. *sophorae*	Australia
D. bullata	UCDDCh400	DQ006946	DQ007002	NA	America
D. castaneicola	CFCC 52425	MW632941	NA	*Castanea mollissima*	China
D. castaneicola	CFCC 52426	MW632942	NA	*Castanea mollissima*	China

D. disciformis^{T}	CBS 205.87	AJ302437	NA	*Fagus sylvatica*	Switzerland
D. disciformis^{T}	GB 5815	KR605644.1	KY352434	*Fagus grandifolia*	America
D. enteroxantha	HUEFS155114	KM396617	KT003700	NA	Brazil
D. enteroxantha	HUEFS155116	KM396618	KT022236	NA	Brazil
D. lancangensis	GMB0045	MW797113	MW814885	NA	China
D. lancangensis	GMB0046	MW797114	MW814886	NA	China
D. larissae^{T}	FCATAS 2723	OM040384	OM240964	NA	China
D. larissae^{T}	FCATAS 2724	OM040385	OM240965	NA	China
D. lijiangensis	MFLU 19-0717	MK852582	MK852583	NA	China
D. macrospora	IRAN 2344C	KR605648	NA	*Quercus brantii*	Iran
D. oregonensis	DPL200	GQ293940	GQ293999	*Quercus kelloggii*	America
D. palmicola	MFLUCC 11-0018	KP744438	NA	*Caryota urens*	Thailand
D. palmicola	MFLUCC 11-0020	KP744439	NA	*Caryota urens*	Thailand
D. quercicola	CFCC 52418	MW632938	NA	*Quercus mongolica*	China
D. quercicola	CFCC 52419	MW632939	NA	*Quercus mongolica*	China
D. quercina	F-091966	AJ302444	NA	*Quercus faginea*	Spain
D. spilomea	CBS 212.87	AJ302433	NA	*Acer campestre*	Switzerland
D. stigma	DCASH200	GQ293947	GQ294003	*Quercus* sp.	America
D. stigma	UCD23-Oe	JX515704	JX515670	NA	NA
D. undulata	CBS 271.87	AJ302436	NA	*Betula* sp.	Switzerland
D. virescens	CBS 128344	MH864890	NA	NA	NA
D. whitmanensis	CDB011	GQ293954	GQ294010	*Vitis vinifera*	America
Diatrypella atlantica	HUEFS 136873	KM396614	KR259647	unidentified plant	Brazil
D. atlantica	HUEFS 194228	KM396615	KR363998	unidentified plant	Brazil

D. banksiae	CPC 29054	KY173401	NA	*Banksia coccinia*	Australia
D. banksiae	CPC 29118	KY173402	NA	*Banksia formosa*	Australia
D. delonicis	MFLU 16-1032	MH812995	MH847791	*Delonix regia*	Thailand
D. delonicis	MFLUCC 15-1014	MH812994	MH847790	*Delonix regia*	Thailand
D. elaeidis	MFLUCC 15-0279	MN308417	MN340300	*Elaeis guineensis*	Thailand
D. heveae	MFLUCC 17-0368	MF959501	MG334557	*Hevea brasiliensis*	Thailand
D. iranensis	IRAN 2280C	KM245033	NA	*Quercus brantii*	Iran
D. pulvinata	H048	FR715523	FR715495	*Salix alba*	Czech Republic
D. verruciformis	UCROK1467	JX144793	JX174093	*Quercus agrifolia*	America
D. verruciformis^{T}	UCROK754	JX144783	JX174083	*Quercus agrifolia*	America
D. vulgaris	HVFRA02	HQ692591	HQ692503	*Fraxinus angustifolia*	Australia
D. vulgaris	HVGRF03	HQ692590	HQ692502	*Citrus paradisi*	Australia
D. yunnanensis	JZBH3380001	MN653008	MN887112	unidentified plant	China
Eutypa cremea	STEU 8082	KY111656	KY111598	*Vitis vinifera*	South Africa
E. cremea	STEU 8410	KY752765	KY752789	*Prunus armeniaca*	South Africa
E. flavovirens	CBS 272.87	AJ302457	DQ006959	*Quercus ilex*	France
E. laevata	CBS 291.87	HM164737	HM164771	*Salix* sp.	Switzerland
E. lata^{T}	EP18	HQ692611	HQ692501	*Vitis vinifera*	NSW, Australia
E. lata (*Eutypa armeniacae*)	CBS 622.84	AJ302446	DQ006964	*Vitis vinifera*	Italy
E. lejoplaca	020202-3	AY684238	AY684197	*Acer pseudoplatanus*	Switzerland
E. lejoplaca	020202-5	AY684221	AY684196	*Acer pseudoplatanus*	Switzerland
E. sparsa	3802-3b	AY684220	AY684201	*Populus* sp.	Switzerland

E. tetragona	CBS 284.87	DQ006923	DQ006960	*Sarothamnus scoparius*	France
Eutypella citricola	HVGRF01	HQ692589	HQ692521	*Citrus sinensis*	Australia
E. citricola	HVVIT07	HQ692579	HQ692512	*Vitis vinifera*	Australia
E. leprosa	STEU 8189	MF359637	MF359672	NA	South Africa
E. leprosa	STEU 8190	MF359638	MF359673	NA	South Africa
E. microtheca	ADEL200	HQ692559	HQ692527	*Ulmus procera*	Australia
E. microtheca	BCMX01	KC405563	KC405560	*Vitis vinifera*	Mexico
E. vitis	UCD2291AR	HQ288224	HQ288303	*Vitis vinifera*	America
E. vitis	UCD2428TX	FJ790851	GU294726	*Vitis vinifera*	TX, America
Halodiatrype avicenniae	MFLUCC 15-0953	KX573916	KX573931	*Avicennia* sp.	Thailand
H. salinicola^{T}	MFLUCC 15-1277	KX573915	KX573932	submerged marine wood	Thailand
Kretzschmaria deusta	CBS 826.72	KU683767	KU684190	NA	NA
Monosporascus cannonballus^{T}	ATCC 26931	FJ430598	NA	NA	NA
M. cannonballus^{T}	CMM 3646	JX971617	NA	*Boerhavia* sp.	Brazil
Neoeutypella baoshanensis^{T}	LC 12111/ HMAS 255436	MH822887	MH822888	*Pinus armandii*	China
N. baoshanensis (*Eutypella caricae*)	GL08362	JX241652	NA	*Malus pumila*	China
N. baoshanensis (*Eutypella caricae*)	CBS 274.87 (EL51C)	AJ302460	NA	*Ficus carica*	France
N. baoshanensis	**KUMCC 21-0050**	**OP420774**	**OP604393**	***Trachycarpus* sp.**	**China**
N. baoshanensis	MFLUCC 16–1002	MT310662	NA	*Clematis vitalba*	UK
Pedumispora rhizophorae^{T}	BCC44877	KJ888853	NA	*Rhizophora apiculata*	Thailand
P. rhizophorae^{T}	BCC44878	KJ888854	NA	*Rhizophora apiculata*	Thailand
Quaternaria quaternata	GNF13	KR605645	KY352464	*Fagus* sp.	Iran

Q. quaternata	CBS 278.87	AJ302469	NA	*Fagus sulvatica*	Switzerland
Xylaria hypoxylon	CBS 122620	AM993141	KX271279	NA	Sweden

Note: Newly generated sequences are in bold; Type species are denoted with the superscript "T". NA = Not Available in GenBank accession numbers.

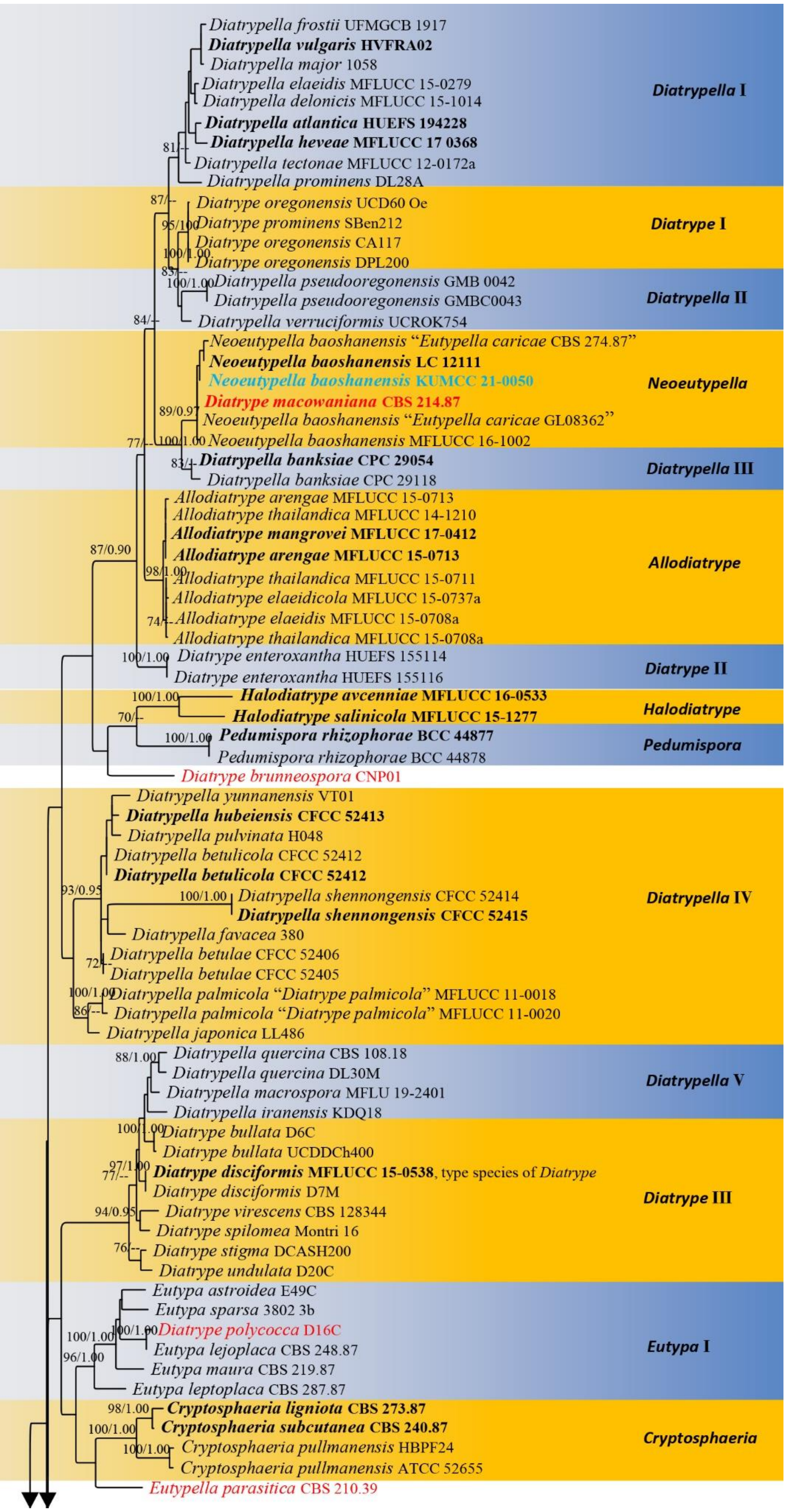
Results

Phylogenetic analyses

Phylogenetic analyses of combined ITS and *tub*2 sequence data based on ML and BYPP analyses indicate that the two tree topologies are similar. The dataset consists of 120 taxa for representative strains of species in Diatrypaceae (Yang et al. 2022, Table 2). Phylogram generated in RAxML analysis represented 29 clades (Fig. 1), and the tree was rooted to *Kretzschmaria deusta* (CBS 826.72) and *Xylaria hypoxylon* (CBS 122620). The final alignment contained 1006 characters for the phylogenetic analyses, including alignment gaps. Single gene analyses were performed to compare the topology and clade stability with combined gene analyses (data not shown).

The phylogenetic trees obtained from RAxML and BI analyses gave essentially similar topologies. The RAxML analysis of the combined dataset yielded the best-scoring tree (Fig 1), with a final ML optimization likelihood value of -14360.051494. The matrix had 681 distinct alignment patterns, with 31.88% of undetermined characters or gaps. Parameters for the GTR+I+G model of the combined ITS and β-tubulin were as follows: estimated base frequencies A = 0.226421, C = 0.263353, G = 0.234300, T = 0.275926; substitution rates AC = 1.064327, AG = 3.202603, AT = 1.246982, CG = 0.839398, CT = 4.365896, GT = 1.000000, and gamma distribution shape parameter α = 0.399161.

NCBI BLASTn search of the ITS and *tub*2 sequences of our new isolate closely matched with *Neoeutypella baoshanensis* type strain (ITS: MH822887.1 and TUB2: MH822888.1), with 100% similarity. The final RAxML (Fig 1) inferred from ITS and *tub*2 sequences also demonstrated that our new taxon was nested in strains of *N. baoshanensis* (LC 12111, CBS 274.87, MFLUCC 16-1002 and GL08362). Therefore, the new isolate is identified as a new strain of *N. baoshanensis*. Strains of *N. baoshanensis* were sister to *Diatrypella banksiae* (CPC 29054 and CPC 29118) with a strongly supported lineage (99% MLBS/1.00 BYPP).


Diatrypella frostii UFMGCB 1917
Diatrypella vulgaris HVFRA02
Diatrypella major 1058
Diatrypella elaeidis MFLUCC 15-0279
Diatrypella delonicis MFLUCC 15-1014
Diatrypella atlantica HUEFS 194228
Diatrypella heveae MFLUCC 17 0368
81/--
Diatrypella tectonae MFLUCC 12-0172a
Diatrypella prominens DL28A
Diatrypella I
87/--
Diatrype oregonensis UCD60 Oe
95/100
Diatrype prominens SBen212
Diatrype oregonensis CA117
100/1.00
Diatrype oregonensis DPL200
Diatrype I
83/--
100/1.00
Diatrypella pseudooregonensis GMB 0042
Diatrypella pseudooregonensis GMBC0043
84/--
Diatrypella verruciformis UCROK754
Diatrypella II
Neoeutypella baoshanensis "Eutypella caricae CBS 274.87"
Neoeutypella baoshanensis LC 12111
Neoeutypella baoshanensis KUMCC 21-0050
Diatrype macowaniana CBS 214.87
89/0.97
Neoeutypella baoshanensis "Eutypella caricae GL08362"
77/--
100/1.00
Neoeutypella baoshanensis MFLUCC 16-1002
Neoeutypella
83/--
Diatrypella banksiae CPC 29054
Diatrypella banksiae CPC 29118
Diatrypella III
Allodiatrype arengae MFLUCC 15-0713
Allodiatrype thailandica MFLUCC 14-1210
Allodiatrype mangrovei MFLUCC 17-0412
87/0.90
Allodiatrype arengae MFLUCC 15-0713
98/1.00
Allodiatrype thailandica MFLUCC 15-0711
Allodiatrype elaeidicola MFLUCC 15-0737a
74/--
Allodiatrype elaeidis MFLUCC 15-0708a
Allodiatrype thailandica MFLUCC 15-0708a
Allodiatrype
100/1.00
Diatrype enteroxantha HUEFS 155114
Diatrype enteroxantha HUEFS 155116
Diatrype II
100/1.00
Halodiatrype avcenniae MFLUCC 16-0533
70/--
Halodiatrype salinicola MFLUCC 15-1277
Halodiatrype
100/1.00
Pedumispora rhizophorae BCC 44877
Pedumispora rhizophorae BCC 44878
Pedumispora
Diatrype brunneospora CNP01
Diatrypella yunnanensis VT01
Diatrypella hubeiensis CFCC 52413
Diatrypella pulvinata H048
Diatrypella betulicola CFCC 52412
Diatrypella betulicola CFCC 52412
93/0.95
100/1.00
Diatrypella shennongensis CFCC 52414
Diatrypella shennongensis CFCC 52415
Diatrypella favacea 380
Diatrypella betulae CFCC 52406
72/--
Diatrypella betulae CFCC 52405
100/1.00
Diatrypella palmicola "Diatrype palmicola" MFLUCC 11-0018
86/--
Diatrypella palmicola "Diatrype palmicola" MFLUCC 11-0020
Diatrypella japonica LL486
Diatrypella IV
88/1.00
Diatrypella quercina CBS 108.18
Diatrypella quercina DL30M
Diatrypella macrospora MFLU 19-2401
Diatrypella iranensis KDQ18
Diatrypella V
100/1.00
Diatrype bullata D6C
Diatrype bullata UCDDCh400
97/1.00
77/--
Diatrype disciformis MFLUCC 15-0538, type species of Diatrype
Diatrype disciformis D7M
94/0.95
Diatrype virescens CBS 128344
Diatrype spilomea Montri 16
76/--
Diatrype stigma DCASH200
Diatrype undulata D20C
Diatrype III
Eutypa astroidea E49C
Eutypa sparsa 3802 3b
100/1.00
100/1.00
Diatrype polycocca D16C
Eutypa lejoplaca CBS 248.87
96/1.00
Eutypa maura CBS 219.87
Eutypa leptoplaca CBS 287.87
Eutypa I
98/1.00
Cryptosphaeria ligniota CBS 273.87
100/1.00
Cryptosphaeria subcutanea CBS 240.87
100/1.00
Cryptosphaeria pullmanensis HBPF24
Cryptosphaeria pullmanensis ATCC 52655
Cryptosphaeria
Eutypella parasitica CBS 210.39

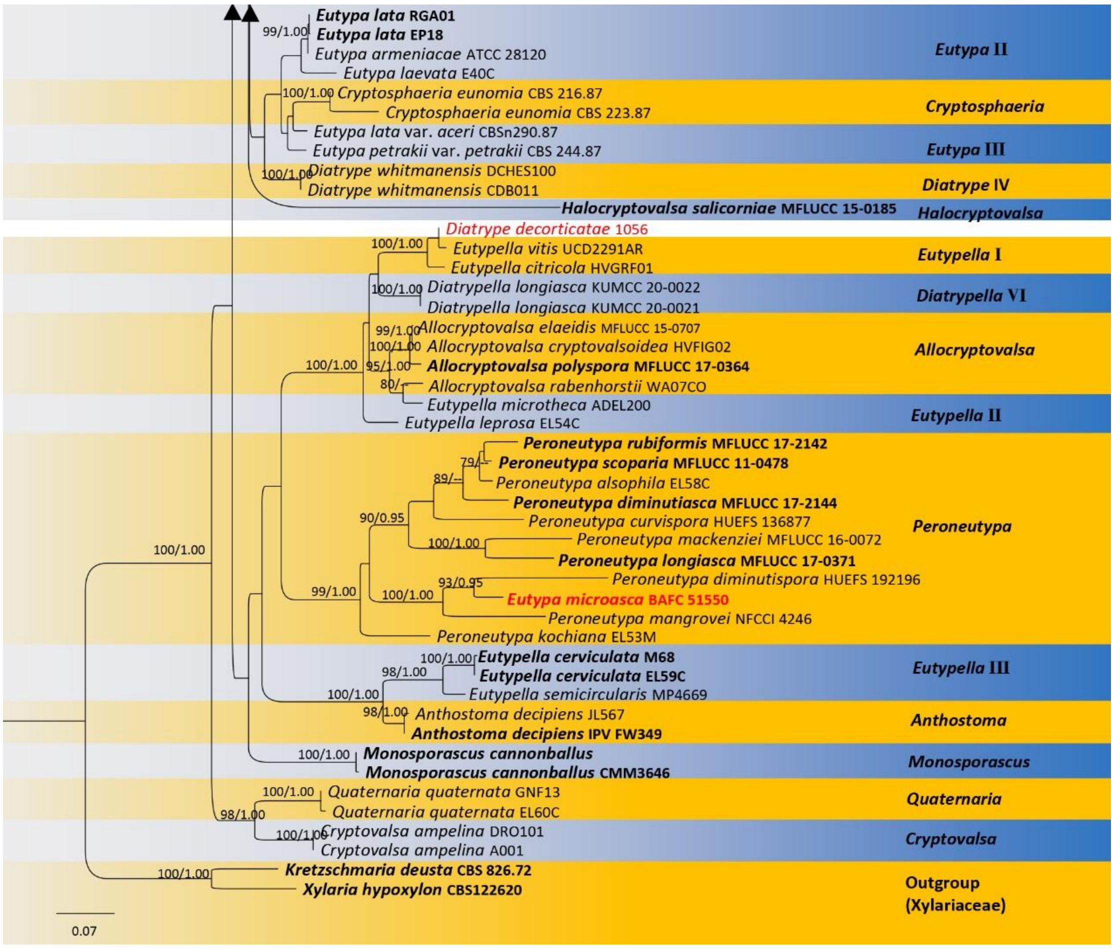


Figure 1. Phylogram generated from maximum likelihood analysis based on ITS and *tub*2 sequence data representing diatrypaceous species. Bootstrap values for ML equal to or greater than 70% and clade credibility values equal to or greater than 0.90 (the rounding of values to 2 decimal proportions) from BYPP analysis are labelled on the nodes. Strains of the newly described species are in bold blue, while type strains are in bold, and the species needed to clarify are in red.

Taxonomy

Diatrypaceae Nitschke [as 'Diatrypeae'], Verh. naturh. Ver. preuss. Rheinl. 26: 73 (1869)

Neoeutypella M. Raza, Q.J. Shang, Phookamsak & L. Cai (2019)

Neoeutypella baoshanensis M. Raza, Q.J. Shang, Phookamsak & L. Cai (2019), **Fig 2**

Saprobic on a dead branch of *Trachycarpus* sp. **Sexual morph**: *Ascostromata* entostromatic, carbonaceous, visible as black, solitary to gregarious, globose to long irregular in shape on host surface, erumpent through host epidermis, producing yellow pigments surrounding ascostroma. *Ascomata* perithecial, 450–520 µm high, 300–400 µm diam., black, immersed to semi-immersed in stromatic tissues, aggregated, globose or subglobose, ostiolate, papillate, slightly conspicuous, with periphyses, inner layers yellow to orange pigmented. *Peridium* 20–35 µm wide, thin, outer layer comprising 5–7 layers of thick-walled, brown to dark brown cells of *textura angularis*, inner layer comprising 3–5 layers of thin-walled, hyaline to brown cells of *textura prismatica*. *Hamathecium* 1.5–3 µm wide ($\bar{x}$ = 2.1 µm, n = 20), comprising aseptate, filamentous paraphyses, tapering toward the apex, embedded in hyaline gelatinous matrix. *Asci* 85–115 × 4–10 µm ($\bar{x}$ = 97.7 × 6 µm; n = 20), 8-spored, unitunicate, spindle-shaped, long pedicellate, subapical ring. *Ascospores* 8–12 × 1.5–2.5 µm ($\bar{x}$ = 9.6 × 1.9 µm; n = 30), overlapping 1–3-seriate, allantoid, slightly or moderately curved, initially hyaline, becoming pale brown at maturity, aseptate, mostly with small 1–2 guttules. **Asexual morph**: Undetermined.

Culture characters – Ascospores germinating on PDA within 24 h. *Colonies* on PDA reaching 90 mm diam. after 4 weeks at room temperature (22–25 °C), circular, edge irregular, white from above and below. *Mycelium* superficial to immersed in media, branched, septate, smooth.

Material examined – CHINA, Yunnan Province, Kunming Institute of Botany, on a dead branch of *Trachycarpus* sp. (Arecaceae), 28 May 2020, Tibpromma S, ST65 (HKAS 115569, **new host record**); living culture KUMCC 21-0050.

Addition GenBank number – LSU = OP420772, SSU = OP420783, *rpb*2 = OP604395, *tef*1 = OP604394

Hosts –*Pinus armandii* (Pinaceae) (Phookamsak et al. 2019); *Clematis vitalba* (Ranunculaceae) (Phukhamsakda et al. 2020); Branches of an unidentified plant (Long et al. 2021); *Trachycarpus* sp. (Arecaceae) (In this study).

Distribution – China (Phookamsak et al. 2019, Long et al. 2021, This study), the UK (Phukhamsakda et al. 2020).

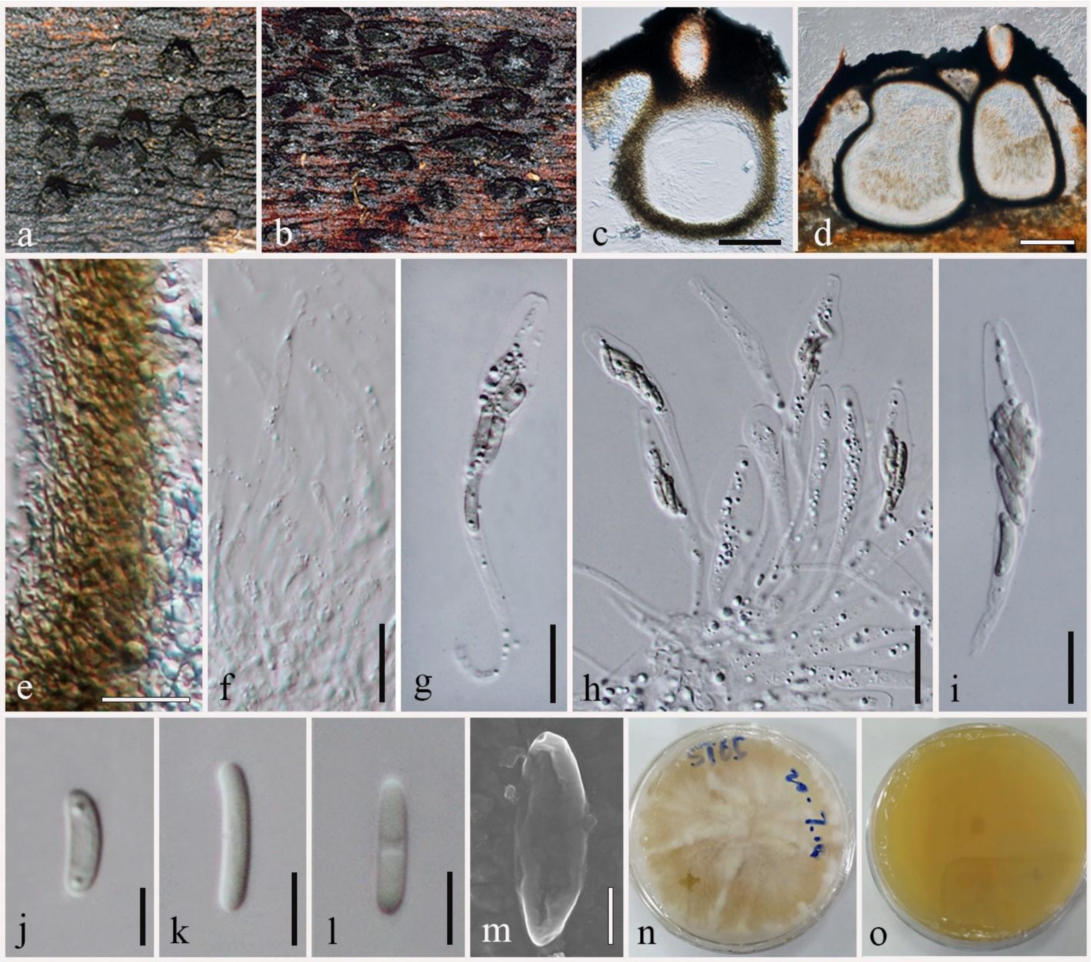


Figure 2. *Neoeutypella baoshanensis* (HKAS 115569). a, b Ascomata on host surface. c, d Vertical sections of ascomata. e Peridium. f Hamathecium. g–i Asci. j–m Ascospores (m = SEM photo). n Culture characteristics on PDA (n = from above, o = from below). Scale bars: c, d = 50 µm, e–i = 20 cm, j–l = 5 µm, m = 2 µm

Discussion

Phylogenetic analyses of ML and BI analyses based on the combined ITS and *tub*2 sequence dataset show that our new isolate groups with *Neoeutypella baoshanensis* (strains LC 12111) with week statistical support (< 70% MLBB/<0.90 BYPP). The morphology of our new isolate resembles *N. baoshanensis* in having entostroma, 8-spored, spindle-shaped asci and allantoid ascospores (Phookamsak et al. 2019). The comparison of ITS and *tub*2 sequences between type strain of *Neoeutypella baoshanensis* (LC 12111) and new isolate (KUMCC 21-0050) shows 9 gaps (within 524 base pairs) and 1 gap (within 375 base pairs) respectively, for each gene. Therefore, the new isolate is identified as *Neoeutypella baoshanensis* and this is the first record of *N. baoshanensis* from a *Trachycarpus* species. Previously, *N. baoshanensis* has been reported from *Clematis vitalba* (Ranunculaceae) (Phukhamsakda et al. 2020) and *Pinus armandii* (Pinaceae) (Phookamsak et al. 2019) as a saprobic species. In addition, we also provide the SEM image of ascospores of *N. baoshanensis* for the first time.

However, *Eutypella caricae* (strains CBS 274.87 and GL08362) and *Diatrype macowaniana* (CBS 214.87) claded with genus *Neoeutypella* (Fig. 1). Only ITS sequences of these three strains are available in GenBank. Thus, it is difficult to get a better phylogenetic separation of these strains and no clear morphological description is available for *Eutypella caricae* (strains CBS 274.87 and GL08362) for comparative studies. According to the description of Saccardo (1882) and Berlese (1902), *Eutypella caricae* differs from *N. baoshanensis* (LC 12111) in having smaller asci (35–45 × 6–7 µm vs. 75–85 × 6.5–7.5 µm). *Neoeutypella baoshanensis* has spindle-shaped asci and pale yellowish to pale brown ascospores, whereas *E. caricae* has clavate asci and hyaline ascospores (Saccardo 1882; Berlese 1902).

Diatrype macowaniana and *N. baoshanensis* differs in having smaller asci (32–36 × 6 µm vs. 75–85 × 8 µm) and ascospores (5–7×1.5–2 vs. 10–11.5 × 2.3–2.5 µm) and the color of ascospores (hyaline vs. hyaline to pale brown) (Saccardo 1882, Phookamsak et al. 2019). Therefore, we maintain the strains under the current name and more isolations and studies are needed to understand the genus *Neoeutypella*.

Generic delimitations in Diatrypaceae have not been resolved as the phylogeny has yet to be resolved using extensive taxon sampling and sequencing of ex-type cultures. For example, the placements of some species (marked in red) in Fig. 1 are needed to be clarified. Therefore, more sampling and sequencing of ex-type cultures are necessary to get a better picture of the family Diatrypaceae.

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