

Diatrypella tectonae and *Peroneutypa mackenziei* spp. nov. (Diatrypaceae) from northern Thailand

Qiu-Ju Shang^{1,2,3,4} · Kevin D. Hyde^{2,3,4} · Rungtiwa Phookamsak^{2,3} · Mingkwan Doilom^{2,3,4} · Darbhe J. Bhat^{5,6} · Sajeewa S. N. Maharachchikumbura⁷ · Itthayakorn Promputtha¹

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Abstract Two novel species of Diatrypaceae, *Diatrypella tectonae* and *Peroneutypa mackenziei*, were collected from dead wood in northern Thailand. The new species are introduced in this paper, with evidence from morphology and phylogenetic analyses. The new taxa are described, illustrated and compared with related taxa. Phylogenetic analyses of combined ITS and partial β -tubulin (β -tub) sequence data support their natural placements in the genera *Diatrypella* and *Peroneutypa* as new species.

Keywords New species · Phylogeny · Sordariomycetes · Taxonomy · Xylariales

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✉ Itthayakorn Promputtha
itthayakorn.p@cmu.ac.th

¹ Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

² World Agroforestry Centre, East and Central Asia, Heilongtan, Kunming 650201, Yunnan, People’s Republic of China

³ Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, People’s Republic of China

⁴ Centre of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

⁵ Department of Botany, Goa University, Goa, India

⁶ Azad Housing Society, No. 128/1-J, Curca, P.O., Goa Velha 403108, India

⁷ Department of Crop Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, P.O. Box 34, Alkhoud 123, Oman

Introduction

Diatrypaceae is a large family in Xylariales, Sordariomycetes, with 16 genera and more than 1500 taxa (Maharachchikumbura et al. 2015, 2016; Senanayake et al. 2015; de Almeida et al. 2016; Dayarathne et al. 2016; Mehrabi et al. 2016). The family is characterised by perithecial ascomata, usually embedded in a black stroma, cylindrical-clavate to clavate, long pedicellate asci and allantoid ascospores (Glawe and Rogers 1984; Rappaz 1987; Mehrabi et al. 2015; de Almeida et al. 2016). Species of Diatrypaceae are cosmopolitan in distribution, and occur on a broad range of dead or dying wood of angiosperms (Carter 1991; Acero et al. 2004; Trouillas and Gubler 2004; Mehrabi et al. 2015). Most taxa in the family are saprobes, while some are pathogens and/or endophytes (Acero et al. 2004; de Errasti et al. 2014). The asexual morphs of Diatrypaceae have been reported as coelomycetous or hyphomycetous genera and include *Cytosporina* Sacc., *Libertella* Desm. and *Phaeoisaria* von Höhn. (Glawe and Rogers 1984; Wijayawardene et al. 2012; de Almeida et al. 2016; Mehrabi et al. 2016). Previous phylogenetic studies in Diatrypaceae have been based on combined ITS and β -tubulin (β -tub) sequence data (Acero et al. 2004; Trouillas and Gubler 2004; Carmaran et al. 2009; Trouillas et al. 2010b, 2011; Luque et al. 2012; Chacon et al. 2013; Mehrabi et al. 2015; Dayarathne et al. 2016; de Almeida et al. 2016; Mehrabi et al. 2016). The natural placements of some genera could not be resolved and, therefore, more taxon sampling and molecular data of selected genes are needed to clarify their natural affinities.

Diatrypella was introduced by Cesati and De Notaris (1863) to accommodate members of stromatic Sphaeriales (Rao 1964). Kirk et al. (2008) estimated that there were 33 worldwide species in the genus, which are mostly described from dead wood. There are 138 epithets listed in Index Fungorum (2016). *Diatrypella* is characterised by conical or

truncate, cushion-like or discoid stromata, which are usually delimited by a black zone on host tissues, perithecial ascomata, umbilicate or sulcate ostioles, and numerous small ovoid to allantoid ascospores (Simmons 1948; Rao 1964; Vasilyeva and Stephenson 2005; Mehrabi et al. 2015). The asexual morph of *Diatrypella* was reported as the hyphomycetous genus *Libertella* (Kirk et al. 2008).

Peroneutypa was introduced by Berlese (1902) for three species, viz. *P. bellula* (Desm.) Berl., *P. corniculata* (Ehrb.) Berl. and *P. heteracantha* (Sacc.) Berl., without designating a type (Saccardo 1905; Carmaran et al. 2006; Radu 2013). Von Hohnel (1917) selected *P. corniculata* as the type species of *Peroneutypa*, but this was rejected by Rappaz (1987) due to its taxonomic confusion. Later, Clements and Shear (1931) proposed *P. cylindrica* (Kalch. and Cooke) Berl. as the type species, but this was also rejected by Rappaz (1987) based on the fact that this species was treated in *Peroneutypella* by Berlese (1902). Rappaz (1987) proposed *P. bellula* as the type species, because the original material was easily found in herbaria from the Netherlands, Belgium and the UK, and the description of perithecia were compatible with the diagnosis of *Peroneutypa*. This species had also been listed together with *P. heteracantha* as a synonym of *Eutypella scoparia* (Schwein.) Ellis & Everh. and *Peroneutypa* was considered to be a synonym of *Eutypella* by Rappaz (1987).

The genus *Peroneutypa* was reinstated by Carmaran et al. (2006) based on morphological characteristics and phylogenetic data presented in a study by Acero et al. (2004). The genus has been designated to accommodate seven species transferred from *Eutypella* and one species from *Echinomyces* in Diatrypaceae (Carmaran et al. 2006). *Peroneutypa* is characterised by long prominent necks, asci with small, truncated apices and allantoid ascospores (Saccas 1954; Carmaran et al. 2006; Vasilyeva and Rogers 2010; Radu 2013; Raymundo et al. 2014; Carmaran et al. 2014). Presently, there are 50 epithets in Index Fungorum (2016), and no asexual morph has been reported for this genus (Saccas 1954; Carmaran et al. 2006, 2014; Vasilyeva and Rogers 2010; Radu 2013; Raymundo et al. 2014; de Almeida et al. 2016).

In the present study, two new species, *Diatrypella tectonae* and *Peroneutypa mackenziei*, which were collected from decaying woody substrates, are introduced. Evidence for the new species is based on their unique morphological characters, as well as the support from phylogenetic analyses of combined ITS and β -tub sequence data.

Materials and methods

Isolation and morphology

Decaying wood was collected from Chiang Rai Province, Thailand, and returned to the laboratory using plastic Ziploc

bags and maintained at room temperature. The specimens were examined following the methods described by Phookamsak et al. (2015) and Dai et al. (2016). Macro-morphological characters were examined using a Motic SMZ-140 dissecting microscope and photographed using a Discovery.V8 stereomicroscope. Ascomata were cut horizontally and vertically by hand under the microscope, using a razor blade, and the hymenium containing paraphyses, asci and ascospores were transferred to a drop of sterile water onto a clean slide. A Canon EOS 600D camera connected to a Nikon Eclipse 80i compound microscope was used to examine and capture the fungal micro-morphology. The fungal structures were measured using the Image Frame Work software. Pure cultures were obtained by single-spore isolation as described by Chomnunti et al. (2014) and are deposited in the Mae Fah Luang University Culture Collection (MFLUCC) and duplicated in the Kunming Culture Collection (KUMCC). Herbarium material is deposited in the herbarium of Mae Fah Luang University (MFLU) and duplicated in the herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (KUN-HKAS). Faces of Fungi and MycoBank numbers are registered (Crous et al. 2004; Jayasiri et al. 2015).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh fungal mycelium scraped from the margin of a colony grown on potato dextrose agar (PDA) for 1–4 weeks at room temperature (25 °C). Genomic DNA was obtained by using a DNA extraction kit (Biospin Fungus Genomic DNA Extraction Kit, BioFlux®, China), following the protocols in the manufacturer's instructions (Hangzhou, P.R. China).

The DNA amplification was obtained by polymerase chain reaction (PCR). The amplification was carried out as follows: the final volume of the PCR reaction was 25 μ L, which contained 1 μ L of DNA template, 1 μ L of each forward and reverse primers, 12.5 μ L of 2 \times Power Taq PCR Master Mix [mixture of EasyTaq™ DNA Polymerase, dNTPs and optimised buffer; Beijing BioTeke Corporation (BioTeke), P.R. China] and 9.5 μ L distilled-deionised water. Primers ITS4 and ITS5, Bt2a and Bt2b were used to amplify the internal transcribed spacer (ITS) region and a portion of the β -tub gene (Trouillas et al. 2011). The PCR thermal cycle program for ITS and β -tub loci was amplified at initially 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 50 s, elongation at 72 °C for 1 m and a final extension at 72 °C for 10 min. The quality of PCR products was observed on 1% agarose gel electrophoresis stained with ethidium bromide. Purification and sequencing of PCR products were carried out at Shanghai Sangon Biological Engineering Technology and Services Co., Shanghai, P.R. China.

Phylogenetic analyses

The sequences generated from this study were analysed with sequence data from related taxa in Diatrypaceae as obtained from GenBank (Table 1) and recent relevant publications (Maharachchikumbura et al. 2015; Dai et al. 2016; Dayarathne et al. 2016; de Almeida et al. 2016; Li et al. 2016). The consensus sequences were initially aligned by using MAFFT v.7 (Kato and Standley 2013; <http://mafft.cbrc.jp/alignment/server/>) and further improved where necessary by using BioEdit v.7.0.9.1 (Hall 1999) and MEGA6 (Tamura et al. 2013).

A maximum likelihood analysis was performed using raxmlGUI v.1.3 (Silvestro and Michalak 2012). The optimal ML tree search was conducted with 1000 replicates, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTR+GAMMAI substitution model.

Maximum parsimony (MP) analysis was performed by PAUP (Phylogenetic Analysis Using Parsimony) v.4.0b10 (Swofford 2002). Trees were inferred using the heuristic search option with 1000 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight, and gaps were treated as missing data. Maxtrees was set as 1000, branches of zero length were collapsed and all multiple equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with ten replicates of random stepwise addition of taxa (Hillis and Bull 1993). Descriptive tree statistics for parsimony tree length (TL), consistency index (CI), retention index (RI), relative consistency index (RC) and homoplasy index (HI) were calculated. The Kishino–Hasegawa tests (Kishino and Hasegawa 1989) were performed to determine whether the trees inferred under different optimality criteria were significantly different.

Bayesian analyses were performed by using MrBayes v.3.0b4 (Ronquist and Huelsenbeck 2003). The model of evolution was estimated using MrModeltest 2.3 (Nylander 2008). Posterior probabilities (PP) (Rannala and Yang 1996) were performed by Markov chain Monte Carlo (MCMC) sampling in MrBayes v.3.0b4. Six simultaneous Markov chains were run for 10,000,000 generations and trees were sampled every 100th generation. The first 25,000 trees representing the burn-in phase of the analyses were discarded and the remaining 75,000 (post-burning) trees used for calculating PPs in the majority rule consensus tree (Cai et al. 2006; Liu et al. 2012).

Trees were shown in TreeView (Page 1996) and FigTree v.1.4.2 (Rambaut 2012) and annotated in Microsoft PowerPoint 2013. Sequences derived in this study were deposited in GenBank (Table 1). The finalised alignment and tree were deposited in TreeBASE, submission ID: 20687 (TreeBASE 2016).

Results

Phylogeny

Phylogenetic analyses of combined ITS and β -tub sequence data were used to determine the taxonomic placement of our strains. The dataset comprised 63 diatrypaceous taxa, with *Xylaria hypoxylon* (CBS 122620) and *Kretzschmaria deusta* (CBS 826.72) as the outgroup taxa. The maximum parsimony dataset consists of 1126 characters, including 475 constant characters, 201 variable parsimony-uninformative characters and 450 parsimony-informative characters. The most parsimonious tree showed TL = 2144, CI = 0.519, RI = 0.754, RC = 0.391 and HI = 0.481. Phylogenetic analyses obtained from maximum likelihood (RAxML), maximum parsimony (MP) and Bayesian inference (BI) analyses showed similar topologies and were not significantly different. The best scoring RAxML tree was selected to represent the relationships among taxa, with the final ML optimisation likelihood value of -1596.00000 (ln) and is shown in Fig. 1. Genera in Diatrypaceae form well-resolved clades and give a similar result to previous studies (de Almeida et al. 2016). Some genera such as *Eutypella* and *Cryptosphaeria* appear polyphyletic.

Taxonomy

Diatrypella tectonae M. Doilom, Q.J. Shang & K.D. Hyde, sp. nov.

Mycobank number: MB 820327 *Facesoffungi number*: FoF 02403; Fig. 2

Etymology: The specific epithet “*tectonae*” refers to the host from which the holotype was isolated.

Holotype: MFLU 15-3430

Saprobic on bark of *Tectona grandis*. **Sexual morph**: *Stromata* 1–1.3 mm wide, with well-developed interior, solitary to gregarious, immersed to erumpent in the bark, mostly superficial, black, glabrous, circular to irregular in shape, pustulate, bi- to multi-ascoma, with umbilicate ostioles appearing on the surface of stroma. *Ascomata* (excluding necks) 240–440 μ m high, 255–390 μ m diam. (\bar{x} = 339 \times 322 μ m, n = 33), perithecial, immersed in stroma, delimited by a black zone in host tissues, globose to subglobose, glabrous, ostioles individual, with short neck. *Ostiolar canal* 105–185 μ m high, 55–120 μ m diam. (\bar{x} = 145 \times 88 μ m, n = 20), cylindrical, sulcate, periphysate. *Peridium* 20–37 μ m wide, composed of two section layers, with outer part comprising 3–6 layers of relatively small, brown to dark brown, thick-walled cells, arranged in *textura angularis*, inner layer comprising 3–4 layers, hyaline cells of *textura angularis*. *Hamathecium* composed of 2–2.4 μ m wide, dense, filiform, septate, hyaline, paraphyses. *Asci* (107–)120–150(–173) \times (13.5–)15.5–21.5(–30.5) μ m (\bar{x} = 138 \times 19 μ m, n = 87),

Table 1 Isolates used in this study and their GenBank accession numbers

Taxon	Strain no.	Provenance	Collector	GenBank accession no.		Reference
				ITS	β -tub	
<i>Anthostoma decipiens</i> Ts	IPV-FW349	Italy	Unknown	AM399021	AM920693	Unpublished
<i>Anthostoma decipiens</i> Ts	JL567	Spain	Unknown	JN975370	JN975407	Luque et al. (2012)
<i>Cryptosphaeria eunomia</i> var. <i>eunomia</i> Ts	C1C (CBS 216.87)	Switzerland	F. Rappaz	AJ302417	–	Acero et al. (2004)
<i>Cryptosphaeria eunomia</i> var. <i>fraxini</i> Ts	CBS 223.87	Switzerland	F. Rappaz	AJ302421	–	Acero et al. (2004)
<i>Cryptosphaeria ligniota</i>	CBS 273.87	Switzerland	F. Rappaz	KT425233	KT425168	Acero et al. (2004)
<i>Cryptosphaeria pullmanensis</i>	HBPF24	USA	F.P. Trouillas	KT425202	GQ294014	Trouillas et al. (2010b)
<i>Cryptosphaeria pullmanensis</i>	ATCC 52655	Washington, USA	D.A. Glawe	KT425235	KT425170	Trouillas et al. (2015)
<i>Cryptosphaeria subcutanea</i>	DSUB100A	Norway	Geir Mathiassen	KT425189	KT425124	Trouillas et al. (2015)
<i>Cryptosphaeria subcutanea</i>	CBS 240.87	Norway	F. Rappaz	KT425232	KT425167	Trouillas et al. (2015)
<i>Cryptovalsa ampelina</i>	A001	Australia	M.L. Creaser	GQ293901	GQ293972	Trouillas et al. (2010b)
<i>Cryptovalsa ampelina</i>	DRO101	California, USA	F.P. Trouillas	GQ293902	GQ293982	Trouillas et al. (2010b)
<i>Diatrype bullata</i>	D6C (CBS 215.87)	Switzerland	F. Rappaz	AJ302422	–	Acero et al. (2004)
<i>Diatrype bullata</i>	UCDDCh400	USA	Unknown	DQ006946	DQ007002	Rolshausen et al. (2006b)
<i>Diatrype disciformis</i> Ts	D21C (CBS 205.87)	Switzerland	F. Rappaz	AJ302437	–	Acero et al. (2004)
<i>Diatrype disciformis</i> Ts	D7M (GB 5815)	New Jersey, USA	G.F. Bills	AJ302423	–	Acero et al. (2004)
<i>Diatrype stigma</i>	D19M (F-091,970)	Segovia, Spain	J. Checa	AJ302435	–	Acero et al. (2004)
<i>Diatrype stigma</i>	DCASH200	USA	F.P. Trouillas	GQ293947	GQ294003	Trouillas et al. (2010b)
<i>Diatrype spilomea</i>	D17C (CBS 212.87)	Switzerland	F. Rappaz	AJ302433	–	Acero et al. (2004)
<i>Diatrype undulate</i>	D20C (CBS 271.87)	Switzerland	F. Rappaz	AJ302436	–	Acero et al. (2004)
<i>Diatrypella atlantica</i>	HUEFS 194228	Brazil	D.A.C. de Almeida	KM396615	KR363998	de Almeida et al. (2016)
<i>Diatrypella atlantica</i>	HUEFS 136873	Brazil	D.A.C. de Almeida	KM396614	KR259647	de Almeida et al. (2016)
<i>Diatrypella frostii</i>	UFMGCB 1917	Brazil	M.L.A. Vieira	HQ377280	–	Vieira et al. (2011)
<i>Diatrypella tectonae</i>	<u>MFLUCC 12-0172a</u>	<u>Thailand</u>	<u>M. Doilom</u>	<u>KY283084</u>	–	<u>This study</u>
<i>Diatrypella tectonae</i>	<u>MFLUCC 12-0172b</u>	<u>Thailand</u>	<u>M. Doilom</u>	<u>KY283085</u>	<u>KY421043</u>	<u>This study</u>
<i>Diatrypella verruciformis</i> Ts	UCROK1467	USA	S.C. Lynch	JX144793	JX174093	Lynch et al. (2013)

Table 1 (continued)

Taxon	Strain no.	Provenance	Collector	GenBank accession no.		Reference
				ITS	β -tub	
<i>Diatrypella verruciformis</i> Ts	UCROK754	USA	S.C. Lynch	JX144783	JX174083	Lynch et al.(2013)
<i>Diatrypella vulgaris</i>	HVFRA02	New South Wales	F.P. Trouillas/W.M. Pitt	HQ692591	HQ692503	Trouillas et al. (2011)
<i>Diatrypella vulgaris</i>	HVGRF03	New South Wales	F.P. Trouillas/W.M. Pitt	HQ692590	HQ692502	Trouillas et al. (2011)
<i>Eutypa armeniaca</i>	ATCC 28120	Australia	Unknown	DQ006948	DQ006975	Rolshausen et al. (2006)
<i>Eutypa lata</i> Ts	EP18	New South Wales	W.M. Pitt	HQ692611	HQ692501	Trouillas et al. (2011)
<i>Eutypa lata</i> Ts	RGA01	Adelaide Hills, South Australia	F.P. Trouillas	HQ692614	HQ692497	Trouillas et al. (2011)
<i>Eutypa lata</i> var. <i>aceri</i>	CBS 290.87	Switzerland	F. Rappaz	HM164736	HM164770	Trouillas and Gubler (2010)
<i>Eutypa laevata</i>	CBS 291.87	Switzerland	F. Rappaz	HM164737	HM164771	Trouillas and Gubler (2010)
<i>Eutypa laevata</i>	E40C (CBS 291.87)	Switzerland	F. Rappaz	AJ302449	–	Acero et al. (2004)
<i>Eutypa petrakii</i> var. <i>petrakii</i> (current name = <i>Cryptosphaeria moravica</i>)	E47C (CBS 245.87)	Switzerland	F. Rappaz	AJ302456	–	Acero et al. (2004)
<i>Eutypa petrakii</i> var. <i>petrakii</i> (current name = <i>Cryptosphaeria moravica</i>)	CBS 244.87	Switzerland	F. Rappaz	HM164735	HM164769	Trouillas and Gubler (2010)
<i>Eutypella cerviculata</i> Ts	M68	Latvia	Unknown	JF340269	–	Arhipova et al. (2012)
<i>Eutypella cerviculata</i> Ts	EL59C (CBS 221.87)	Switzerland	F. Rappaz	AJ302468	–	Acero et al. (2004)
<i>Eutypella citricola</i>	HVVIT07	New South Wales	F.P. Trouillas/W.M. Pitt	HQ692579	HQ692512	Trouillas et al. (2011)
<i>Eutypella citricola</i>	HVGRF01	New South Wales	F.P. Trouillas/W.M. Pitt	HQ692589	HQ692521	Trouillas et al. (2011)
<i>Eutypella cryptovalsoidea</i>	HVFIG02	New South Wales	F.P. Trouillas/W.M. Pitt	HQ692573	HQ692524	Trouillas et al. (2011)
<i>Eutypella cryptovalsoidea</i>	HVFIG05	New South Wales	F.P. Trouillas/W.M. Pitt	HQ692574	HQ692525	Trouillas et al. (2011)
<i>Eutypella microtheca</i>	BCMX01	Mexico	Unknown	KC405563	KC405560	Unpublished
<i>Eutypella microtheca</i>	ADEL200	Adelaide, South Australia	F.P. Trouillas	HQ692559	HQ692527	Trouillas et al. (2011)
<i>Eutypella vitis</i>	UCD2291AR	Arkansas, USA	A.K. Striegler/G.M. Leavitt	HQ288224	HQ288303	Úrbez-Torres et al. (2012)
<i>Eutypella vitis</i>	UCD2428TX	Texas, USA	Unknown	FJ790851	GU294726	Úrbez-Torres et al. (2009)
<i>Halodiatrype avicenniae</i>	MD-2016a	Thailand	M. Dayarathne	KX573916	KX573931	Dayarathne et al.(2016)

Table 1 (continued)

Taxon	Strain no.	Provenance	Collector	GenBank accession no.		Reference
				ITS	β -tub	
<i>Halodiatrype salinicola</i> Ts	MD-2016b	Thailand	M. Dayarathne	KX573915	KX573932	Dayarathne et al.(2016)
<i>Kretzschmaria deusta</i>	CBS 826.72	Mechelen, Belgium	H.A. van Kesteren	KU683767	KU684190	U'Ren et al. (2016)
<i>Monosporascus cannonballus</i> Ts	CMM3646	Brazil	Unknown	JX971617	–	Unpublished
<i>Monosporascus cannonballus</i> Ts	ATCC 26931	Unknown	Unknown	FJ430598	–	Unpublished
<i>Peroneutypa alsophila</i>	EL58C	France	F. Rappaz	AJ302467	–	Acero et al. (2004)
<i>Peroneutypa scoparia</i>	MFLUCC 11-0478	Thailand	D.Q. Dai	KU940151	–	Dai et al. (2016)
<i>Peroneutypa scoparia</i>	MFLUCC 11-0615	Thailand	D.Q. Dai	KU940152	–	Dai et al. (2016)
<i>Peroneutypa comosa</i>	BAFC:393	Argentina	C. Carmaran	KF964568	–	Grassi et al. (2014)
<i>Peroneutypa kochiana</i>	EL53M	Spain	J. Checa	AJ302462	–	Acero et al. (2004)
<i>Peroneutypa curvispora</i>	HUEFS 136877	Brazil	D.A.C. de Almeida	KM396641	–	de Almeida et al. (2016)
<i>Peroneutypa curvispora</i>	HUEFS 131248	Brazil	D.A.C. de Almeida	KM396646	–	de Almeida et al. (2016)
<i>Peroneutypa diminutispora</i>	HUEFS 192196	Brazil	D.A.C. de Almeida	KM396647	–	de Almeida et al. (2016)
<i>Peroneutypa mackenziei</i>	<u>MFLUCC 16-0072</u>	<u>Thailand</u>	<u>Q.J. Shang</u>	<u>KY283083</u>	<u>KY706363</u>	<u>This study</u>
<i>Quaternaria quaternata</i>	EL60C (CBS 278.87)	Switzerland	F. Rappaz	AJ302469	–	Acero et al. (2004)
<i>Quaternaria quaternata</i>	GNF13	Iran	M. Mehrabi	KR605645	–	Mehrabi et al. (2016)
<i>Xylaria hypoxylon</i> Ts	CBS 122620	Sweden	unknown	AM993141	–	Peršoh et al. (2009)

Ex-type strains are in **bold**; *Ts* denotes the type species of the genus; newly generated sequences are underlined

ATCC American Type Culture Collection, Manassas, USA; CBS Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; CMM Culture Collection of Phytopathogenic Fungi “Prof. Maria Menezes”; HUEFS Herbarium of the State University of Feira de Santana; HVFIG Hoosic Valley Family Interest Group; IPV Istituto di Patologia Vegetale, Milan, Italy, and E. and J. Gallo, Modesto, CA; MFLUCC Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; UCD University of California, Davis; UFMGCB Culture Collection of Microorganisms and Cells of the Universidade Federal of Minas Gerais

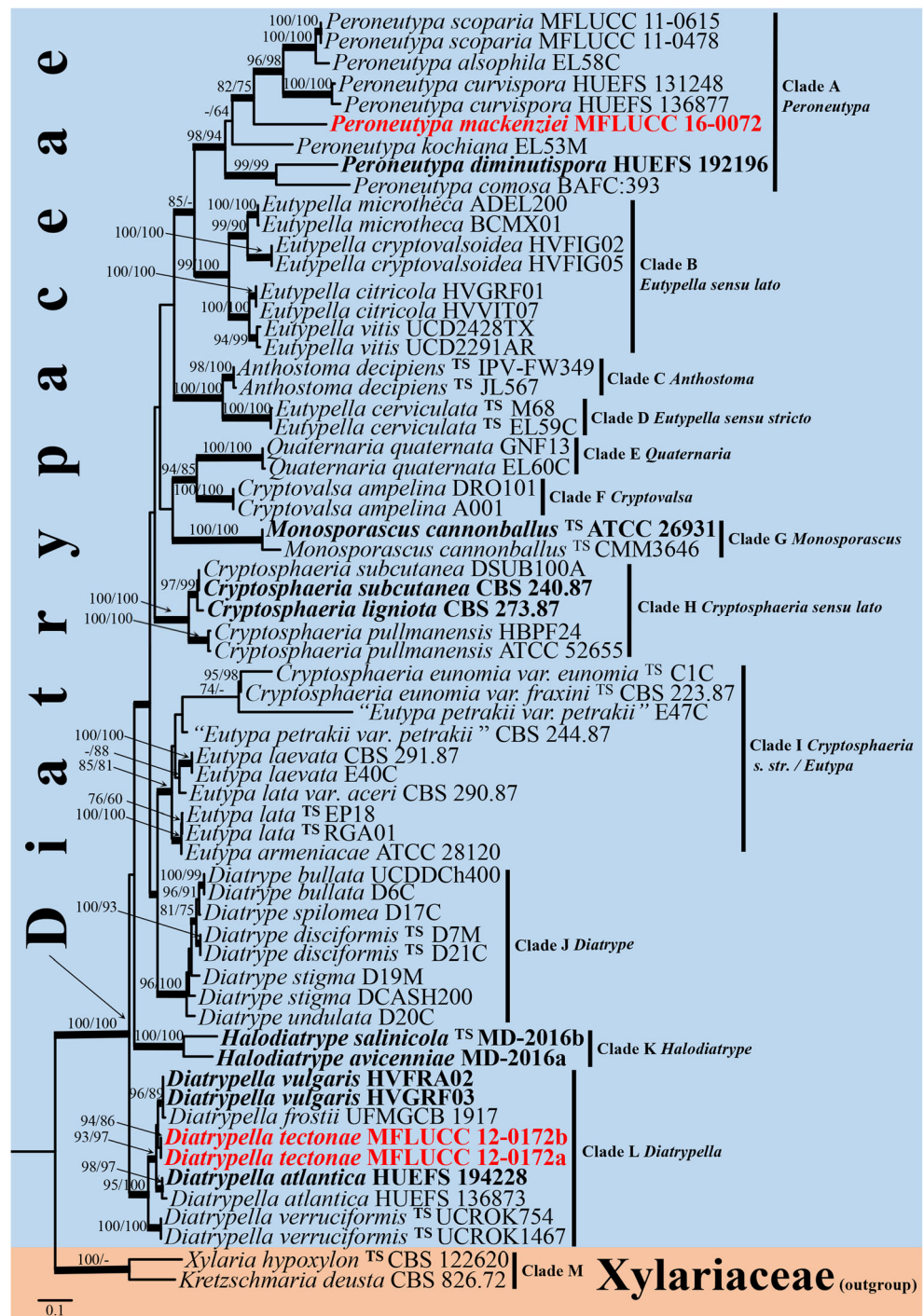
spore-bearing part length [p. sp.] (80–)100–128(–146) μm (\bar{x} = 114 μm , n = 97), polysporous, unitunicate, clavate, with moderately short stalks, apically rounded, with J-apical ring. Ascospores (5–)7–9(–12) \times (1.5–)2–2.5(–3) μm (\bar{x} = 8 \times 2.3 μm , n = 170), overlapping, yellowish to brown, ellipsoidal to cylindrical or elongate-allantoid, aseptate, smooth-walled. **Asexual morph:** Undetermined.

Culture characteristics Ascospores germinating on PDA within 12 h. Germ tubes produced around ascospore.

Colonies on PDA reaching 2.2–3.8 cm diam. after 7 days in darkness at 25 °C, colonies irregular in shape, medium dense, flat or effuse, slightly raised, with edge fimbriate, fluffy to fairly fluffy, white from above, light yellow from below, becoming white to yellow or pale brown from above and pale brown from below after 30 days; not producing pigments in agar.

Material examined THAILAND, Chiang Rai Province, Mae Lao District, on dead branch of *Tectona grandis* Linn.

Fig. 1 Maximum likelihood majority rule consensus tree of analysis of species in Diatripaceae generated from a combined dataset of ITS and β -tub sequence data. Bootstrap support values for maximum likelihood (ML, left) and maximum parsimony (MP, right) equal to or greater than 60% (ML/MP) are shown above the nodes. The Bayesian posterior probabilities (PPs) greater than 0.95 are indicated by thickened lines. The new isolates are in *red*, ex-type strains are in **bold** and type species are denoted with the superscript ^{TS} after the species name. The tree is rooted to *Xylaria hypoxylon* and *Kretzschmaria deusta*



(Lamiaceae), 5 February 2012, M. Doilom, MKT 034 (MFLU 15-3430, **holotype**; KUN-HKAS 95024, **isotype**), ex-type culture, MFLUCC 12-0172 = KUMCC 16-0023.

Notes: *Diatrypella tectonae* fits well with the classical description of *Diatrypella*. *Diatrypella tectonae* resembles to *D. atlantica* D.A.C. de Almeida, Gusmão & A.N. Mill., *D. vulgaris* and *D. frostii* Peck. However, *D. tectonae* can be distinguished from *D. atlantica* by its longer spore-bearing part of asci (see Table 2), as well as the black outer layer of

the entostroma (de Almeida et al. 2016). *Diatrypella tectonae* differs from *D. vulgaris* and *D. frostii* by its yellowish inner entostroma and longer asci (see Table 2). Phylogenetic analyses based on ITS and β -tub sequence data (Fig. 1) show that *D. tectonae* (MFLUCC 12-0172) forms a separate lineage, but clusters with *D. atlantica*, *D. frostii* and *D. vulgaris*. *Diatrypella* species form a well-resolved clade in this study coupled with morphological differences, we introduce *D. tectonae* as a new species.

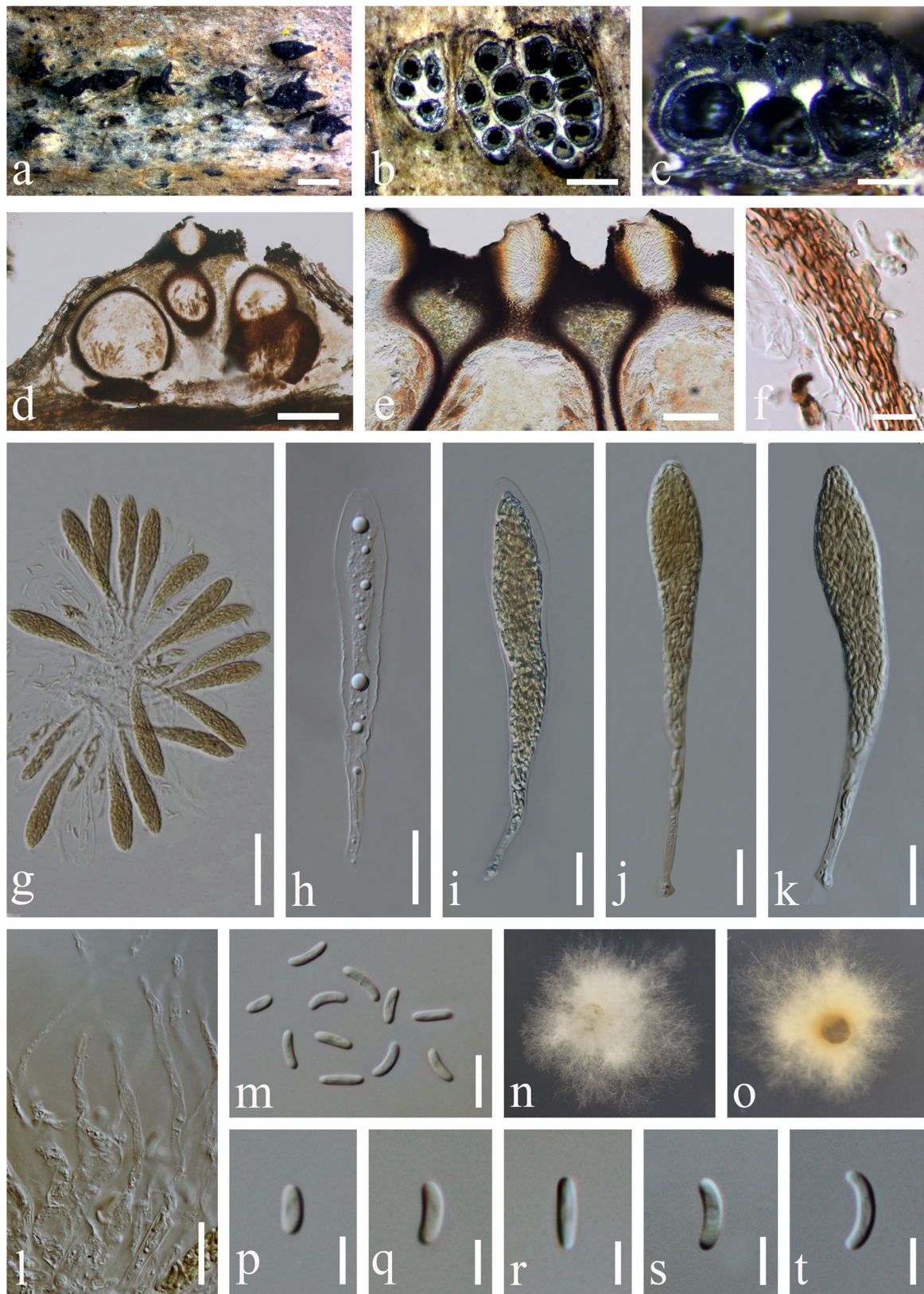


Fig. 2 *DiatryPELLA tectonae* (MFLU 15-3430, holotype). **a** Stromata on substrate. **b** Cross-section through stroma. **c, d** Vertical section through stroma showing ostioles and perithecia. **e** Ostiolar canals. **f** Peridium. **g–k** Asci. **l** Paraphyses. **m, p–t** Ascospores. **n, o** Culture characteristic on

PDA after 7 days (**n** = colony from above, **o** = colony from below). Scale bars: **a** = 1000 μm , **b** = 500 μm , **c, d** = 250 μm , **e, g** = 50 μm , **f, m** = 10 μm , **h–k, l** = 20 μm , **p–t** = 5 μm

Table 2 Synopsis of *Diatrypella tectonae* and related species discussed in this study

Species name	Colour of entostroma (inner/outer layer)	Ascus length/([p. sp.] μm)	Ascus width (μm)	Ascospore length (μm)	Ascospore width (μm)	Reference
<i>Diatrypella atlantica</i> (holotype)	White/yellowish	112–193/(30–92)	12–26	4–10.5	1.5–2.5	de Almeida et al. (2016)
<i>Diatrypella frostii</i>	Yellow-green, olive-green/black	40/27	8–12	6–8	–	Saccardo (1882); Glawe (1983); Boehm (2002)
<i>Diatrypella tectonae</i> (holotype)	White to yellow/black	(107–)123–153(–173)/(80–)100–128(–146)	(13.5–)15.5–21.5(–30.6)	(4.9–)7.1–9.0(–11.7)	(1.5–)2–2.6(–3.3)	This study
<i>Diatrypella vulgaris</i> (holotype)	White/–	(82–)104–121(–150)/(65–)80–130(–155)	(12–)18–20	(7–)8–10(–12)	2–2.5	Trouillas et al. (2011)

Peroneutypa mackenziei Q.J. Shang, Phookamsak & K.D. Hyde, sp. nov.

Mycobank number: MB 820324 *Facesoffungi number*: FoF 02404; Fig. 3

Etymology: The specific epithet “*mackenziei*” refers to the name in honour of the mycologist Dr. Eric H.C. McKenzie for his immense contribution to mycology

Holotype: MFLU 16-1441

Saprobic on bark. **Sexual morph**: *Stromata* 1.2–2.2 mm wide, with poorly developed interior, solitary to gregarious, immersed, becoming raised to erumpent by a long ostiolar canal, dark brown to black, glabrous, circular to irregular in shape, arranged in longitudinal or valsoid configuration, multi-ascoma, with conspicuous, clustered, roundish to cylindrical prominent ostioles in the centre. *Ascomata* (excluding necks) 375–555 μm high, 260–450 μm diam. (\bar{x} = 466 \times 356 μm , n = 45), perithecial, immersed in an stroma, tightly aggregated in a horizontal perspective in 1–3 rows, dark brown to black, globose to subglobose, glabrous, individual ostiole with long neck. *Ostiolar canal* 105–420 μm high, 80–120 μm diam. (\bar{x} = 265 \times 100 μm , n = 25), cylindrical, sulcate, at the apex curved, periphysate. *Peridium* 45–65 μm wide, composed of two section layers, outer section comprising 3–4 layers, of relatively small, brown to dark brown, thick-walled cells, arranged in *textura globulosa* to *textura angularis*, inner part comprising 8–10 layers of hyaline cells of *textura angularis*. *Hamathecium* composed of 2.5–4.5 μm wide, dense, filamentous, aseptate, hyaline, paraphyses, embedded in a mucilaginous matrix. *Asci* (14.5–)15.5–20(–21.5) \times (3–)3.5–5 μm (\bar{x} = 17.7 \times 4.2 μm , n = 40), 8-spored, unitunicate, cylindrical or clavate, sessile, apically rounded to truncate, with a J-apical ring. *Ascospores* (3.4–)4.5–6.5(–8) \times (0.8–)1–2 μm (\bar{x} = 5.6 \times 1.6 μm , n = 150), overlapping 2–3-seriate, subhyaline to pale yellowish, elongate-allantoid, aseptate, smooth-walled, with small guttules. **Asexual morph**: Undetermined.

Culture characteristics Ascospores germinating on malt extract agar (MEA) within 48 h. Germ tubes produced from all sides. Colonies on MEA reaching 1.5–2.5 cm diam. after 7 days in darkness at 25 °C, colonies circular to irregular, medium dense, flat or effuse, slightly raised, with edge fimbriate, fluffy to fairly fluffy, white from above, black from below; not producing pigments in agar.

Material examined THAILAND, Chiang Rai Province, Khun Korn waterfall, on undetermined decaying wood, 22 January 2015, Q. J. Shang, KK-14 (MFLU 16-1441, **holotype**; KUN-HKAS 95023, **isotype**), ex-type culture, MFLUCC 16-0072 = KUMCC 16-0022.

Notes: Based on phylogenetic analyses and morphological comparison, our isolate belongs to the genus *Peroneutypa* in Diatrypaceae. Phylogenetic analyses of combined ITS and β -

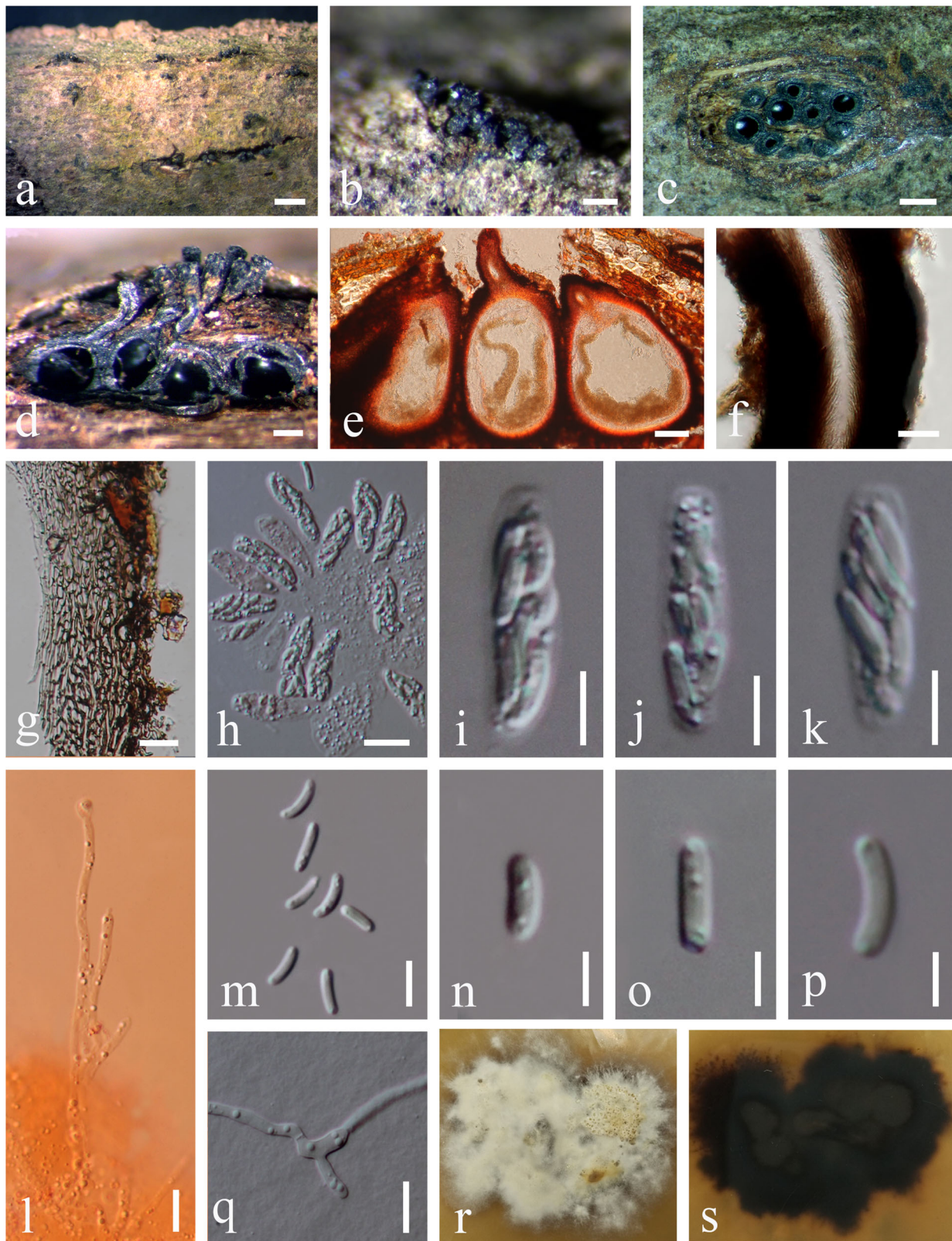


Fig. 3 *Peroneutypa mackenziei* (MFLU 16-1441, holotype). **a, b** Appearance of stromata on substrate. **c** Cross-section through stroma. **d, e** Vertical section through stroma (**e** = stained with Congo red). **f** Ostiolar canal. **g** Peridium. **h–k** Asci. **l** Paraphyses stained with Congo red. **m–p**

Ascospores. **q** Germinating ascospore. **r, s** Culture characteristic on MEA after 10 days (**r** = colony from above, **s** = colony from below). Scale bars: **a** = 1000 μm , **b** = 250 μm , **c** = 500 μm , **d** = 200 μm , **e** = 100 μm , **f** = 25 μm , **g**, **h** = 20 μm , **i–k**, **m** = 5 μm , **l**, **q** = 10 μm , **n–p** = 3 μm

tub sequence data (Fig. 1) show that *Peroneutypa mackenziei* (MFLUCC 16-0072) clusters with *Peroneutypa* species, with moderate support (ML 82%, MP 75% and 0.99 PP). *Peroneutypa mackenziei* forms a separate clade, away from *P. curvispora* (Starbäck) Carmarán & A.I. Romero (HUEFS 136877 and HUEFS 131248), *P. scoparia* (Schwein.) Carmarán & A.I. Romero (MFLUCC 11-0615 and MFLUCC 11-0478) and *P. alsophila* (Durieu & Mont.) Carmarán & A.I. Romero (EL58C). Moreover, the morphology of stromata, ascumata, asci and ascospore of our isolate fits well with the description of the genus *Peroneutypa* (Carmarán et al. 2006; de Almeida et al. 2016). *Peroneutypa mackenziei* is morphologically distinct from other species of *Peroneutypa* in having cylindrical or clavate, sessile asci (Fig. 3).

Discussion

From the results of this study, we introduce two new species in the family Diatrypaceae. An updated tree for the genera of Diatrypaceae is also provided. Our results concur with the previous molecular analyses of Trouillas et al. (2011), Mehrabi et al. (2015, 2016) and de Almeida et al. (2016), which support Diatrypaceae as a monophyletic family, comprising some polyphyletic genera.

The identification of *Diatrypella* and *Peroneutypa* species is based on molecular phylogenetic analyses of combined ITS and β -tub sequence data as well as morphology, the latter still being important (Hyde et al. 2010). Following Maharachchikumbura et al. (2015, 2016) and de Almeida et al. (2016), 11 representative genera in Diatrypaceae were selected to investigate the phylogenetic placement of our taxa. However, some genera in Diatrypaceae, such as the genera *Echinomyces* and *Leptoperidia*, lack sequence data (Dai et al. 2016; de Almeida et al. 2016; Maharachchikumbura et al. 2016; Mehrabi et al. 2016). Species of Diatrypaceae are predominantly saprotrophic on the bark of angiosperms, while a few have been reported as pathogens (Trouillas and Gubler 2004; Trouillas et al. 2011) or endophytes in petioles (Carroll et al. 1977) and woody tissue (de Errasti et al. 2010). They are also present in various ecological niches as decomposers on dead wood worldwide (Chacón et al. 2013).

Peroneutypa (clade A; Fig. 1) appears to be a monophyletic group in Diatrypaceae and this was concurred from previous phylogenetic studies (Acero et al. 2004; Carmarán et al. 2006, 2009; Trouillas et al. 2011; Dai et al. 2016). *Peroneutypa* has recently been studied by Carmarán et al. (2014), de Almeida et al. (2016) and Dai et al. (2016). Molecular data are available for *P. alsophila*, *P. comosa* (Speg.) Carmarán & A.I. Romero, *P. curvispora*, *P. diminutispora* D.A.C. de Almeida, Gusmão & A.N. Mill., *P. kochiana* (Rehm) Carmarán & A.I. Romero and *P. scoparia* (Grassi et al. 2014; de Almeida et al. 2016; GenBank 2016). *Peroneutypa mackenziei* forms a well-

supported branch in the genus. *Peroneutypa* has a unique character due to its conspicuous sessile asci (Saccas 1954; Carmarán et al. 2006; Vasilyeva and Rogers 2010; Radu 2013; Carmarán et al. 2014; Raymundo et al. 2014). Other taxa in Diatrypaceae typically have long pedicellate asci (Acero et al. 2004; Carmarán et al. 2009; Trouillas et al. 2010a, b, 2011; Mehrabi et al. 2015; de Almeida et al. 2016). Thus, this work opens up another interesting area of research concerning the pedicellate reduction.

Clade B represents *Eutypella sensu lato*, which separates from the type species *Eutypella cerviculata* (clade D). *Eutypella cerviculata* (clade D) forms a well-resolved clade (100% ML, 100% MP, 1.00 PP), clustering with *Anthostoma* (clade C; Fig. 1). *Eutypella* can be considered as a species complex with a polyphyletic status. However, the strains of *E. cerviculata* were not represented by the type strain; therefore, further study of the taxa in this genus is needed.

Cryptovalsa (clade F) and *Quaternaria* (clade E) clustered together with high support value (94% ML, 85% MP, 0.99 PP), as observed in previous studies of Maharachchikumbura et al. (2015, 2016) and de Almeida et al. (2016). However, the type species of *Cryptovalsa*, *Cryptovalsa protracta* (Pers.) De Not., and *Quaternaria*, *Quaternaria persoonii* Tul. & C. Tul., lack molecular data and phylogenetic analysis to resolve their natural placements. Therefore, more taxa and sequence data of the species in these genera is required.

Clade G represents the genus *Monosporascus*, comprising two strains of a putative species *M. cannonballus*. The genus forms a well-resolved clade, closely related to *Cryptovalsa* and *Quaternaria*. *Cryptosphaeria sensu lato* (clade H) forms a robust clade distinct from *Cryptosphaeria sensu stricto* (clade I), which is represented by the type species *C. eunomia* var. *eunomia* and *C. eunomia* var. *fraxini* (synonym of *Cryptosphaeria eunomia*). *Cryptosphaeria sensu stricto* clusters with the genus *Eutypa* and this result is similar to de Almeida et al. (2016). However, these are not type strains and, thus, the representative strains of the generic type needs to be recollected, epitypified to obtain molecular data for clarifying the placement of these genera.

Diatrype (clade J), *Diatrypella* (clade L), *Eutypa* (clade J) and *Halodiatrype* (clade K) form well-resolved clades in Diatrypaceae. However, previous phylogenetic studies of *Diatrype*, *Diatrypella* and *Eutypa* in Diatrypaceae showed that they are polyphyletic (Acero et al. 2004; Trouillas et al. 2010b, 2011; Grassi et al. 2014; de Almeida et al. 2016). In the present study, the phylogenetic analyses show better concordance with morphology, except for the genera *Cryptosphaeria* and *Eutypella*, possibly caused by the low amount of representative species in each clade. Thus, it is necessary to include more taxa and specific genes to obtain a better understanding of the family Diatrypaceae.

A salient characteristic of the genus *Diatrypella* is the occurrence of polysporous asci (Vasilyeva and Stephenson

2005) and the unique features of the genus were highlighted by Saccardo (1882), Ellis and Everhart (1892), Berlese (1902), Chacón (2003), Vasilyeva and Stephenson (2005), Trouillas et al. (2011) and Mehrabi et al. (2016). Molecular data are available for *D. atlantica*, *D. favacea*, *D. frostii*, *D. iranensis* Mehrabi and R. Hemmati, *D. major* (Berl.) Lar. N. Vassiljeva, *D. prominens* (Howe) Ellis & Everh., *D. pulvinata* Nitschke, *D. quercina* (Pers.) Cooke and *D. verruciformis* (Ehrh.) Nitschke (GenBank 2016; EBI 2016). In this study, the strains of *Diatrypella* form a well-resolved clade (95% ML, 100% MP, 1.00 PP). The novel species *D. tectonae* was introduced by evidence of morphological and phylogenetic study. *Diatrypella tectonae* forms a sister clade to *D. atlantica*, *D. frostii* and *D. vulgaris* (Fig. 1). However, in the previous study by de Almeida et al. (2016), some strains of *Diatrypella* often form distinct lineages in Diatrypaceae; these strains might be misidentified, as they are not the type strain nor are they isolated from endophytes. Therefore, the epitypification of the *Diatrypella* species is required to resolve their phylogenetic placement.

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