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Novel taxa of Diatrypaceae from Para rubber (*Hevea brasiliensis*) in northern Thailand; introducing a novel genus *Allocryptovalsa*

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Abstract

Species of Diatrypaceae are widespread on dead wood of plants worldwide. The delineation of this family is rather problematic because the characters of ascostromata are extremely variable and the names of taxa with sequence data are often misleading. In this paper, species of Diatrypaceae were collected from Para rubber in northern Thailand for examination and illustrations. Based on morphological characteristics and phylogenetic analyses, a new genus, *Allocryptovalsa*, is introduced to accommodate a new species *A. polyspora* and two species, *A. cryptovalsoidea* and *A. rabenhorstii* are transferred to the new genus. The new species, *Diatrypella heveae* and *Peroneutypa longiasca* are also introduced in this paper. Phylogenetic analyses of combined ITS and β -tubulin sequence data show their phylogenetic affinities in Diatrypaceae. Our study also shows that phylogenetic analyses of taxa of Diatrypaceae are highly confused as some genera are shown to be polyphyletic.

Key words – Phylogeny – Sordariomycetes – taxonomy – unitunicate fungi – Xylariales

Introduction

The family Diatrypaceae (Xylariales, Sordariomycetes) has a widespread distribution on variety of plants worldwide, comprising 16 genera and more than 1500 species (Trouillas et al. 2011, Maharachchikumbura et al. 2015, 2016, Mehrabi et al. 2015, Senanayake et al. 2015, Dayarathne et al. 2016, de Almeida et al. 2016, Shang et al. 2017). Species of this family are mostly saprobes inhabiting wood and bark of various angiosperms and some species (e.g. *Eutypa leptoplaca*, *Eutypella lata*, *E. microtheca*, *Cryptosphaeria pullmanensis*, *Cryptovalsa ampelina*, and *Diatrypella vulgaris*) have been reported as pathogens and/or endophytes (Acero et al. 2004, Trouillas et al. 2004, 2010, 2011, Trouillas & Gubler 2010, 2016, Grassi et al. 2014, Paolinelli-Alfonso et al. 2015, Mehrabi et al. 2016, Shang et al. 2017).

Taxa in Diatrypaceae are characterized by perithecial ascromata, with poor or well-developed ascostromata, immersed to erumpent in the host substrates, with ostiolate, and papillate ascromata,

with short to long necks, unitunicate, cylindrical-clavate to clavate or spindle-shaped, long pedicellate asci and allantoid ascospores (Trouillas et al. 2010, Mehrabi et al. 2015, Dayarathne et al. 2016, de Almeida et al. 2016, Li et al. 2016). Species of this family are mostly found in terrestrial habitats, but they can be also found in marine habitats (e.g. *Cryptosphaeria mangrovei*, *Eutypa bathurstensis*, *Diatrypasimilis australiensis*, *Halodiatrype salinicola*, *Pedumispora rhizophorae*) (Hyde & Rappaz 1993, Chalkley et al. 2010, Abdel-Wahab et al. 2014, Jones et al. 2015, Dayarathne et al. 2016, Li et al. 2016).

Asexual morphs of Diatrypaceae have been reported as coelomycetes or hyphomycetes belonging to the genera *Cytosporina* Sacc, *Libertella* Desm. and *Phaeoisaria* von Höhn. (Glawe & Rogers 1984, Wijayawardene et al. 2012, Liu et al. 2015, Mehrabi et al. 2015, Dayarathne et al. 2016, de Almeida et al. 2016). However, the asexual morphs of many species have not yet been determined (Acero et al. 2004). Kliejunas & Kuntz (1972) reported the asexual morph of *Eutypella parasitica* as coelomycetous, occurring on the host substrate and sporulating in cultures. The genus *Libertella* has been reported as the asexual morph of *Cryptovalsa ampelina* which was not seen on host substrate, but sporulated on culture (Mostert et al. 2004). Conidia of Diatrypaceae are usually unicellular, hyaline, cylindrical or filiform or allantoid, slightly curved and group in a slim mass (Glawe & Rogers 1982, 1984, Glawe 1983, Mostert et al. 2004, Luque et al. 2006). However, the asexual morph was not considered useful in identifying species of Diatrypaceae (Glawe & Roger 1986, de Almeida et al. 2016).

Species identification based on morphological characteristics of Diatrypaceae has become difficult (Glawe & Rogers 1984, Rappaz 1987, Mehrabi et al. 2016). Most previous studies were based on morphological studies and DNA sequence comparison of the ribosomal internal transcribed spacer region (ITS) and partial sequence of the β -tubulin gene (β -tub) (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 phylogenetic relationships within Diatrypaceae are not well-resolved due to lack sequence data to clarify their taxonomic position.

Diatrypella was introduced by Cesati & De Notaris (1863), with *Diatrypella verruciformis* (Ehrh.) Nitschke as the type species. There are 140 epithets listed in Index Fungorum (2017). The genus is characterized by conical-truncate, cushion-like or discoid ascostromata, usually delimited by a black zone on host tissues, perithecial ascomata, umbilicate or sulcate ostioles, and numerous small ovoid to allantoid ascospores (Vasilyeva & Stephenson 2005, Mehrabi et al. 2015, Shang et al. 2017). *Diatrypella* has polysporous asci resembling *Cryptovalsa* in Diatrypaceae. *Diatrypella* was mentioned as the polysporous complement of *Diatrype*, while *Cryptovalsa* was a polysporous complement of *Eutypa* (Vasilyeva & Stephenson 2005). Nevertheless, it is still difficult to determine the differences between *Diatrypella* and *Cryptovalsa* based on morphological characters (Acero et al. 2004, Vasilyeva & Stephenson 2005). Therefore, phylogenetic analyses including more representative taxon sampling with multigene regions are needed to clarify the phylogenetic affinity of *Diatrypella* for the better understanding of its taxonomic relationships (Mehrabi et al. 2015).

Peroneutypa was introduced by Berlese (1902) to accommodate *P. bellula* (Desm.) Berl., *P. corniculata* (Ehrh.) Berl and *P. heteracantha* (Sacc.), which was characterized by having valsoid ascostromata, perithecial ascomata with long necks, and 8-spored asci (Saccardo 1905, Carmaran et al. 2006, de Almeida et al. 2016). Rappaz (1987) selected *P. bellula* as the genetic type and considered the genus as a synonym of *Eutypella*. Based on morphological characteristics and phylogenetic analyses, Carmaran et al. (2006) resurrected *Peroneutypa* and transferred eight *Eutypella sensu lato* species and *Echinomyces obesa* to *Peroneutypa* based on their ascal shape and phylogenetic analyses. There are presently 51 epithets for *Peroneutypa* in Index Fungorum (2017). *Peroneutypa* is characterized by valsoid, poorly developed ascostromata, perithecia with long prominent necks, 8-spored, small, clavate, sessile to subsessile asci and, allantoid ascospores (Carmaran et al. 2006, Vasilyeva & Rogers 2010, Mehrabi et al. 2016, Shang et al. 2017).

Eutypella was introduced by Saccardo (1875) with *Eutypella cerviculata* (Fr.) Sacc. as type

species. *Eutypella* is characterized by ascostromata erumpent through the host bark by clustered, sulcate perithecial beaks, 8-spored, clavate asci, with long stalks, and allantoid, hyaline or yellowish ascospores (Vasilyeva & Stephenson 2006). *Eutypella* species have been found on a wide range of hosts, especially associated with canker diseases in *Vitis vinifera* (Vasilyeva & Stephenson 2006, Trouillas et al. 2011, Luque et al. 2012). Currently, there are 248 species listed in Index Fungorum (2017), but only a few species have sequence data. Phylogenetic analyses of Diatrypaceae from previous studies show that *Eutypella* is polyphyletic (Acero et al. 2004, Chacón et al. 2013, de Almeida et al. 2016, Shang et al. 2017). Therefore, further study of the taxa in this genus is needed.

The objective of this study is to introduce a novel genus *Allocryptovalsa* to accommodate *Eutypella sensu lato* and two new Diatrypaceous species, *Diatrypella heveae* and *Peroneutypa longiasca* from Para rubber, based on morphological studies and also phylogenetic supports.

Materials & Methods

Collections, isolation and identification

Fresh materials of Para rubber (*Hevea brasiliensis* (Willd.) Muell.Arg.) were collected from Chiang Rai and Phayao Provinces during October 2016 – February 2017. Fungal structures were observed and examined by using a Motic SMZ 168 series stereomicroscope and photographed with an Axio camera on a Zeiss Discover V8 stereomicroscope. Micro-morphological structures were examined and photographed by a Nikon ECLIPSE 80i microscope with a Canon 600D digital camera. The measurement of fungal structures (e.g. ascostromata, perithecia, necks, peridium, paraphyses, asci and ascospores) were obtained by using Tarosoft® Image Framework program v.0.9.0.7. Photographic plates were made by using Adobe Photoshop CS6 version 13.0. (Adobe Systems, U.S.A.).

Single spore isolations and culture morphologies were obtained following the method described in Phookamsak et al. (2015) using malt extract agar (MEA; 33.6 g/l sterile distilled water, Difco malt extract media) and incubated overnight at room temperature (20–25 °C). Germinating ascospores were aseptically transferred to MEA plates and incubated at room temperature. Culture characteristics and growth rates were recorded after 1–4 weekly intervals.

Type specimens are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and duplicated in the herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS). Ex-type living cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) in Chiang Rai, Thailand and duplicated in the Kunming Culture Collection (KUMCC). Facesoffungi and Index Fungorum numbers are acquired as in Jayasiri et al. (2015) and Index Fungorum (2017) respectively.

DNA extraction, PCR amplification and DNA sequencing

Fungal colonies were grown on MEA at room temperature for two weeks, and the fungal mycelia were scraped off and transferred to 1.5 ml. micro centrifuge tubes. Genomic DNA was extracted by Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, China) following the manufacturer's protocol (Hangzhou, P. R. China).

The DNA amplification was performed by polymerase chain reaction (PCR) based on the relevant genes, the internal transcribed spacers (ITS1, 5.8S, ITS2) and β -tubulin (β -tub). The internal transcribed spacers (ITS) was amplified with primers pairs ITS5 and ITS4 (White et al. 1990). The β -tubulin was amplified with primers pairs T1 (O'Donnell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995). PCR reactions were performed in 25 μ l final volumes containing 8.5 μ l of sterilized water, 12.5 μ l of 2 \times Easy Taq PCR Super Mix (mixture of Easy Taq TM DNA Polymerase, dNTPs, and optimized buffer (Beijing Trans Gen Biotech Co., Chaoyang District, Beijing, PR China), 1 μ l of each forward and reverse primers (10 pM), and 2 μ l of DNA template.

The PCR thermal cycle program for ITS gene were amplified as: initially 95 °C for 3 mins, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 50 s, elongation at 72

°C for 50 s, and final extension at 72 °C for 10 mins. The PCR thermal cycle program for β -tub gene were amplified as: initially 94 °C for 2 mins, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, elongation at 72 °C for 1.50 mins, and final extension at 72 °C for 10 mins. PCR products were checked on 1% agarose gels electrophoresis stained with ethidium bromide and sent to sequence at Sangon Biotech Co., Shanghai, China.

Phylogenetic analysis

Sequences generated in this study were analysed with other related sequences of the genera in Diatrypaceae which were obtained from GenBank and also derived from recent publications (de Almeida et al. 2016, Shang et al. 2017). *Kretzschmaria deusta* (CBS 826.72) and *Xylaria hypoxylon* (CBS 121680) were selected as the out-group taxa (Table 1). The ITS and β -tub gene datasets were initially aligned by using MAFFT version 7 (Katoh & Standley 2013, <http://mafft.cbrc.jp/alignment/server/>) and improved manually where necessary in MEGA6 (Tamura et al. 2013). Ambiguous regions were excluded [exclude sites: ITS (1–631 bp) = 77–87, 128–145, 480–490, 629–631; β -tub (632–1107 bp) = 687–747, 842–916, 935–961, 1070–1107] from the analyses using Gblocks 0.91b (Talavera & Castresana 2007). The individual gene alignments were analysed separately for checking the incongruence in overall topology of the phylogenetic tree and a combined ITS and β -tub sequence data were analyzed and inferred the phylogenetic tree based on maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) analyses.

The estimated evolutionary model of Bayesian inference and maximum likelihood were performed independently for each locus using MrModeltest v. 2.3 (Nylander 2008) implemented in PAUP v. 4.0b10 (Swofford 2002). The best-fit model is resulted as GTR+I+G model for each locus under the Akaike Information Criterion (AIC).

Bayesian Inference analysis was performed by MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2003) with the best-fit model of sequences evolution under the Akaike Information Criterion (AIC). Bayesian posterior probabilities (BYPP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo Sampling (BMCMC). Six simultaneous Markov chains were run from random trees for 2000000 generations and trees were sampled every 100th generations. The Tracer v. 1.6 (Rambaut et al. 2013) program was used to examine the distribution of log-likelihood scores and determine stationary phase for each search as well as decided to acquire the extra run for achieve convergence. The first 20% of generated trees representing burn-in phase of the analysis were discarded and the remaining trees were used for calculating posterior probabilities (PP) of a majority rule consensus tree.

Maximum likelihood analysis was performed by Randomized Axelerated Maximum Likelihood (RAxML) implemented in raxmlGUI v.1.3 (Silvestro & Michalak 2011). A general time reversible model (GTR) was applied with a discrete gamma distribution which was complemented for each substitution model with four rate classes (Silvestro & Michalak 2011). One thousand rapid bootstrap analysis (Stamatakis et al. 2008) and searches for the best-scoring ML tree (RAxML option “-f a”) were applied (Silvestro & Michalak 2011). The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTR+GAMMAI substitution.

A maximum parsimony (MP) analysis was carried out with PAUP v 4.0b10 (Swofford 2002). Trees were inferred using the heuristic search function with 1000 random stepwise addition replicates and tree bisection–reconnection (TBR) as the branch–swapping algorithm. All informative characters were unordered and of equal weight. The consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were measured. Statistical supports for branches of the most parsimonious tree were estimated using maximum parsimony bootstrap (BS) analysis with 1,000 bootstrap replicates (Felsenstein 1985).

The phylogenetic tree was shown in FigTree V.1.4.3 (Rambaut 2016) and drawn in Microsoft PowerPoint 2013 and converted to jpeg file in Adobe Photoshop CS6 version 13.0. (Adobe Systems. U.S.A.). The newly sequences generated in this study were submitted in GenBank (Table

1). The final alignment and tree were deposited in TreeBASE (<http://www.treebase.org/>) under the submission ID 21525.

Results

Phylogeny

Phylogenetic analyses of a combined ITS and β -tub sequence data based on ML, MP and BI analyses were similar in overall tree topologies and not different significantly (data not shown). The dataset consists of 79 taxa from representative strains of species in Diatrypaceae. The total alignment length comprises 1107 characters including gaps.

The RAxML analysis resulted in a best scoring likelihood tree selected with a final ML optimization likelihood value of -9586.121953 which is represented in Fig. 1. The final likelihood tree was evaluated and optimized under GAMMA+P-Invar model parameters, with 521 distinct alignment patterns and 24.40% of undetermined characters or gaps. The MP analysis had 863 included remaining characters after 244 characters were excluded, with 434 characters were constant; 90 variable characters were parsimony-uninformative; and 339 were (included) parsimony-informative characters. The most parsimonious showed TL = 1769, CI = 0.418, RI = 0.734, RC = 0.307 and HI = 0.582. Bayesian posterior probabilities from MCMC were evaluated with final average standard deviation of split frequencies = 0.008511.

The phylogenetic tree based on analysis of a combined ITS and β -tub sequence data is shown the relationships of diatrypaceous taxa representing 14 clades within Diatrypaceae (Fig. 1). The new species, *Diatrypella heveae* (MFLUCC 17-0368) formed a single lineage, distinct from *D. atlantica* (HUEFS 136873 and HUEFS 194228), with no support, but its position was similar in all analyses. The comparisons of ITS sequence data show that *D. heveae* differs from *D. atlantica* in six base positions and is different in eleven base positions of β -tub pairwise comparisons. *Diatrypella* has shown to be a species complex which is difficult to distinguish based on morphological aspects. We therefore, introduced *Diatrypella heveae* as a new species, distinguishing from *D. atlantica* based on phylogenetically distinct and morphological differences (see Table 3)

Diatrypella heveae and *D. atlantica* clusters with *D. tectonae*, *D. vulgaris*, *D. frostii*, *D. major* and also the generic type, *D. verruciformis* within clade A *Diatrypella sensu stricto*. In addition, *Diatype enteroxantha*, *D. macowaniana*, *D. oregonensis* and *Eutypella caricae* are also forms a well-resolved clade within this clade.

Allocryptovalsa polyspora (MFLUCC 17-0371) forms a sister clade with *Eutypella cryptovalsoidea* (HVFIG02 and HVFIG05) with strong support (100% ML, 99% MP and 1.00 PP) (Fig. 1; clade H) and nests with *Cryptovalsa rabenhorstii* (WA07CO and WA08CB) and other *Eutypella sensu lato* viz. *E. microtheca*, *E. citricola*, *E. vitis* and *E. leprosa*.

Peroneutypa longiasca (MFLUCC 17-0371) forms a distinct lineage sister to *P. mackenziei* (MFLUCC 16-0072) (Fig. 1; clade I) with moderate support (82% ML, 81% MP and 1.00 PP). *Peroneutypa* forms a well-resolved clade (99% ML, 93% MP and 1.00 PP) in Diatrypaceae comprising *P. alsophila*, *P. comosa*, *P. curvispora*, *P. diminutispora*, *P. kochiana*, *P. longiasca*, *P. mackenziei* and *P. scoparia*. *Eutypa microasca* grouped in *Peroneutypa* as well. The species formed a strongly supported clade (96% ML, 85% MP and 1.00 PP) with *P. comosa* and *P. diminutispora*

Taxonomy

Based on morphological characters and phylogenetic analyses, a new genus *Allocryptovalsa* is introduced to accommodate the new species *A. polyspora* and cryptovalsa-like taxa. Furthermore, two new species, *Diatrypella heveae* and *Peroneutypa longiasca* are also introduced in this study. Detailed descriptions and illustrations are provided.

Allocryptovalsa Senwana, Phookamsak & K.D. Hyde gen. nov.

Index Fungorum number: IF553857; Facesoffungi number: FoF03773

Etymology – In reference to the morphological resemblance to *Cryptovalsa*

Saprobic on wood. Sexual morph: *Ascstromata* present or absent. If present; embedded in host tissue. *Ascomata* perithecial, solitary to scattered, immersed to semi-immersed in host substrate, globose to subglobose, dark brown to black. *Ostiolar canal* central filled with periphyses. *Peridium* thick, composed of several cell layers of brown to black cells of *textura angularis*, outer cell fused with host cells. *Hamathecium* comprising dense, arising from the base of perithecia, hyaline, unbranched, septate, slightly constricted at septa, filamentous paraphyses, narrowing and tapering toward at apex. *Asci* polysporous, unitunicate, thin-walled, clavate to cylindrical-clavate, long pedicellate, apical round to truncate, with a refractive J- subapical ring. *Ascospores* crowded, hyaline to pale yellowish, or pale brown at maturity, oblong to allantoid, aseptate, smooth-walled, with small guttules. Asexual morph: Undetermined.

Type species – *Allocryptovalsa polyspora* C. Senwana, Phookamsak & K.D. Hyde

Notes – *Cryptovalsa* is typified by *C. protracta* (Pers.) De Not. (1863) that is characterized by immersed ascostromata but sometimes invading bark tissues, polysporous, cylindrical or clavate asci, and allantoid ascospores (Vasilyeva & Stephenson 2005). The phylogenetic study from de Almeida et al. (2016) showed that *Cryptovalsa rabenhorstii* clustered with *Eutypella cryptovalsoidea* and other *Eutypella sensu lato* species. In their analyses, de Almeida et al. (2016) suggested that taxa included in this clade could represent a new genus and also appropriated to accommodate the type species of *Cryptovalsa* according to the presence of *C. rabenhorstii* and *Eutypella cryptovalsoidea*. However, *Cryptovalsa rabenhorstii* formed a distinct lineage separate from *Cryptovalsa ampelina* (Nitschke) Fuckel (clade L *Cryptovalsa sensu lato*) and the generic type of *Cryptovalsa* has not yet been sequenced. Therefore, phylogenetic affinity of this genus is uncertain.

In this study, our species is characterized by perithecia immersed in host tissue, polysporous asci, and allantoid ascospores. Phylogenetic analyses of a combined ITS and β -tub sequence data showed that our species clustered with the polysporous species, *Cryptovalsa rabenhorstii* and *Eutypella cryptovalsoidea* and also resembles morphological characters as described in Trouillas et al. (2011). Therefore, we introduce *Allocryptovalsa* as a new genus in Diatrypaceae to accommodate a new species *Allocryptovalsa polyspora* and two new combination species *Allocryptovalsa cryptovalsoidea* (\equiv *Eutypella cryptovalsoidea*) and *Allocryptovalsa rabenhorstii* (\equiv *Cryptovalsa rabenhorstii*) which are also designated and accommodated in this genus.

Allocryptovalsa cryptovalsoidea (Trouillas, W.M. Pitt & Gubler) Senwana, Phookamsak & K.D. Hyde, comb. nov.

\equiv *Eutypella cryptovalsoidea* Trouillas, W.M. Pitt & Gubler, in Trouillas, Pitt, Sosnowski, Huang, Peduto, Loschiavo, Savocchia, Scott & Gubler, Fungal Diversity 49: 215 (2011)

Index Fungorum number: IF553863

Notes – *Eutypella cryptovalsoidea* was introduced by Trouillas et al. (2011) which was characterized by ascostromata mostly in the bark, asci clavate to spindle-shape, long pedicellate, polysporous asci, and allantoid to sub-allantoid ascospores. This species is similar to *Allocryptovalsa polyspora* in its asci, ascospore shape and colour. *Eutypella cryptovalsoidea* differs from other *Eutypella* species in having polysporous asci (Vasilyeva & Stephenson 2005, de Almeida et al. 2016). Phylogenetic analyses showed *E. cryptovalsoidea* grouped with *Cryptovalsa rabenhorstii* and *Allocryptovalsa polyspora*, which also have polysporous asci (Fig. 1). Therefore, we transfer the species to the genus *Allocryptovalsa* as *A. cryptovalsoidea*.

Allocryptovalsa polyspora Senwana, Phookamsak & K.D. Hyde, sp. nov.,

Fig. 2

Index Fungorum number: IF553858; Facesoffungi number: FoF03774

Etymology – The epithet “polyspora” refers to the polysporous asci

Holotype – MFLU 17-1218

Saprobic on dead twig of *Hevea brasiliensis* (Willd.) Muell. Arg. Sexual morph: *Ascstromata* solitary to gregarious, 1–3-loculate, immersed to semi-immersed, becoming raised to erumpent through the host tissue. *Ascromata* 100–400 µm diam., 80–425 µm high, perithecial, dark brown to black, gregarious or solitary, immersed to semi-immersed in substrate, globose to subglobose, dark brown to black, ostiolate, papillate. *Ostiolar canal* 20–40 µm wide, central, not prominent, cylindrical, straight, dark brown to black, with periphysate. *Peridium* 15–33 µm wide, composed of two type 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* 3–10 µm wide, comprising dense, hyaline, septate, unbranched, slightly constricted at the septa, filamentous paraphyses, tapering towards the apex. *Asci* (33–)70–120(–177) × (4–)13–19(–21) µm (\bar{x} = 104 × 16.3 µm, n = 20), spore-bearing part length [p. sp.] (29–) 40–75(–90) µm (\bar{x} = 59.73 µm, n = 45), polysporous, unitunicate, thin-walled, clavate, long pedicellate, apically rounded, with a refractive, J- subapical ring. Ascospores 5–14 × 2–4 µm (= 10.8 × 2.8 µm, n = 105), crowded, pale yellowish to pale brown at maturity, oblong to allantoid, aseptate, slightly curved, smooth-walled, with small guttules. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinated on MEA within 24 hours and germ tube produced from one or both sides of the ascospore. Colonies on MEA reaching 5.5 cm diam. after 14 days at 25 °C, colonies medium dense, irregular, flat or effuse, slightly raised, fluffy, white, not produced pigmentation on MEA media.

Material examined – THAILAND, Phayao Province, Muang District, on dead twig of *Hevea brasiliensis* (Euphorbiaceae), 29 January 2017, C. Senwanna, RBPY046 (MFLU 17-1218, holotype, HKAS100697 isotype), ex-type living culture, MFLUCC 17-0364 = KUMCC17-0261.

Addition GenBank number – LSU = MF959503.

Notes – *Allocryptovalsa polyspora* shares a size range of the asci and ascospores with *A. cryptovalsoidea*, but is typical smaller than *A. cryptovalsoidea* and *A. rabenhorstii*. However, *A. polyspora* differs from *A. cryptovalsoidea* and *A. rabenhorstii* in a having shorter of spore-bearing part (see Table 2).

Allocryptovalsa rabenhorstii (Nitschke) C. Senwanna, Phookamsak & K.D. Hyde, comb. nov.

≡ *Valsa rabenhorstii* Nitschke, Pyrenomyc. Germ. 1: 158 (1867)

= *Cryptovalsa rabenhorstii* (Nitschke) Sacc., Syll. fung. (Abellini) 1: 190 (1882)

Index Fungorum number: IF553864

Notes – *Cryptovalsa rabenhorstii* is similar to *Allocryptovalsa cryptovalsoidea* and *A. polyspora* in having polysporous asci (Trouillas et al. 2011). Based on phylogenetic analyses this species clustered with *A. cryptovalsoidea* and *A. polyspora* and formed separate clade with *Cryptovalsa ampelina* (Fig. 1; Clade H). However, the type of *Cryptovalsa*, *C. protracta* (Pers.) De Not., lacks sequence data in GenBank. Until more species are collected and sequenced, understanding of the *Cryptovalsa* will remain incomplete. Therefore, we transfer the species to the genus *Allocryptovalsa* as *A. rabenhorstii*.

Diatrypella (Ces. & De Not.) De Not., Sfer. Ital.: 29 (1863)

Type species – *Diatrypella verruciformis* (Ehrh.) Nitschke 1867

Note – *Diatrypella* is feature characterized by “ascostromata conical or truncate, cushion-like or discoid, usually delimited by a black zone on host tissues, perithecial ascromata, umbilicate or sulcate ostioles; asci polysporous, cylindrical, with long stalks and ascospores are ovoid to allantoid, hyaline or yellowish” (Vasilyeva & Stephenson 2005, Mehrabi et al. 2015, Shang et al. 2017). Acero et al. (2004) confirmed the polyphyletic groups of *Diatrypella* in Diatrypaceae based on phylogenetic analysis of ITS rDNA data and this was concurred from previous studies (de Almeida et al. 2016, Mehrabi et al. 2016, Shang et al. 2017).

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

Taxon	Strain no.	Provenance	Collector	GenBank accession		Reference
				ITS	β -tubulin	
<i>Allocryptovalsa cryptovalsoidea</i> “ <i>Eutypella</i> HVFIG02 <i>cryptovalsoidea</i> ”		New South Wales	F.P. Trouillas/W.M. Pitt	HQ692573	HQ692524	Trouillas et al. (2011)
<i>Allocryptovalsa cryptovalsoidea</i> “ <i>Eutypella</i> HVFIG05 <i>cryptovalsoidea</i> ”		New South Wales	F.P. Trouillas/W.M. Pitt	HQ692574	HQ692525	Trouillas et al. (2011)
<i>Allocryptovalsa polyspora</i>	<u>MFLUCC 17–0364</u>	<u>Thailand</u>	<u>C. Senwana</u>	<u>MF959500</u>	<u>MG334556</u>	<u>This study</u>
<i>Allocryptovalsa rabenhorstii</i> “ <i>Cryptovalsa</i> WA07CO <i>rabenhorstii</i> ”		Western Australia	F.P. Trouillas	HQ692620	HQ692522	Trouillas et al. (2011)
<i>Allocryptovalsa rabenhorstii</i> “ <i>Cryptovalsa</i> WA08CB <i>rabenhorstii</i> ”		Western Australia	F.P. Trouillas	HQ692619	HQ692523	Trouillas et al. (2011)
<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>	C1C (CBS 216.87)	Switzerland	F. Rappaz	AJ302417	–	Acero et al. (2004)
<i>Cryptosphaeria eunomia</i> var. <i>fraxini</i>	CBS223.87	Switzerland	F. Rappaz	AJ302421	–	Acero et al. (2004)
<i>Cryptosphaeria ligniota</i>	CBS273.87	Switzerland	F. Rappaz	KT425233	KT425168	Acero et al. (2004)
<i>Cryptosphaeria moravica</i>	CBS244.87	Switzerland	F. Rappaz	HM164735	HM164769	Trouillas & Gubler 2010
<i>Cryptosphaeria pullmanensis</i>	HBPF24	USA	F.P. Trouillas	KT425202	GQ294014	Trouillas et al. (2010)
<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>	CBS240.87	Norway	F. Rappaz	KT425232	KT425167	Trouillas et al. (2015)
<i>Cryptovalsa ampelina</i>	A001	Australia	M.L. Creaser	GQ293901	GQ293972	Trouillas et al. (2010)
<i>Cryptovalsa ampelina</i>	DRO101	California, USA	F.P. Trouillas	GQ293902	GQ293982	Trouillas et al. (2010)
<i>Diatrype bullata</i>	UCDDCh400	USA	Unknown	DQ006946	DQ007002	Rolshausen et al. (2006)
<i>Diatrype disciformis</i> Ts	D21C (CBS 205.87)	Switzerland	F. Rappaz	AJ302437	–	Acero et al. (2004)
<i>Diatrype disciformis</i> Ts	D7M (GB5815)	New Jersey, USA	G.F. Bills	AJ302423	–	Acero et al. (2004)
<i>Diatrype exteroxantha</i>	HUEFS155114	Brazil	D.A.C. Almeida	KM396617	KT003700	de Almeida et al. (2016)
<i>Diatrype exteroxantha</i>	HUEFS155116	Brazil	D.A.C. Almeida	KM396618	KT022236	de Almeida et al. (2016)
<i>Diatrype macowaniana</i>	CBS214.87	Australia	F. Rappaz	AJ302413	–	Acero et al. (2004)
<i>Diatrype oregonensis</i>	DPL200	USA	F.P. Trouillas	GQ293940	GQ293999	Trouillas et al. (2010)
<i>Diatrype stigma</i>	DCASH200	USA	F.P. Trouillas	GQ293947	GQ294003	Trouillas et al. (2010)
<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. Almeida	KM396615	KR363998	de Almeida et al. (2016)
<i>Diatrypella atlantica</i>	HUEFS 136873	Brazil	D.A.C. Almeida	KM396614	KR259647	de Almeida et al. (2016)

Table 1 Continued.

Taxon	Strain no.	Provenance	Collector	GenBank accession		Reference
				ITS	β -tubulin	
<i>Diatrypella favaceae</i>	Isolate 380	USA	L. Vasilyeva	KU320616	_	de Almeida et al. (2016)
<i>Diatrypella frostii</i>	UFMGCB 1917	Brazil	M. L.A. Vieira	HQ377280	_	Vieira et al. (2011)
<i>Diatrypella heveae</i>	<u>MFLUCC 17-0368</u>	<u>Thailand</u>	<u>C. Senwanna</u>	<u>MF959501</u>	<u>MG334557</u>	<u>This study</u>
<i>Diatrypella major</i>	Isolate 1058	USA	A.N. Miller	KU320613	_	de Almeida et al. (2016)
<i>Diatrypella pulvinata</i>	H048	Czech Republic	P. Srutka and J. Holusa	FR715523	FR715495	de Almeida et al. (2016)
<i>Diatrypella tectonae</i>	MFLUCC 12-0172a	Thailand	M. Doilom	KY283084	_	Shang et al. (2017)
<i>Diatrypella tectonae</i>	MFLUCC 12-0172b	Thailand	M. Doilom	KY283085	KY421043	Shang et al. (2017)
<i>Diatrypella verruciformis</i> Ts	UCROK1467	USA	S.C. Lynch	JX144793	JX174093	Lynch et al. (2013)
<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 astroidea</i>	CBS 292.87	Switzerland	Unknown	AJ302458	DQ006966	Rolshausen et al. (2006)
<i>Eutypa flavovirens</i>	CBS 272.87	France	Unknown	AJ302457	DQ006959	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>	CBS290.87	Switzerland	F. Rappaz	HM164736	HM164770	Trouillas & Gubler 2010
<i>Eutypa laevata</i>	E40C (CBS 291.87)	Switzerland	F. Rappaz	AJ302449	_	Acero et al. (2004)
<i>Eutypa lejoplaca</i>	CBS 248.87	Switzerland	Unknown	DQ006922	DQ226974	Rolshausen et al. (2006)
<i>Eutypa leptoplaca</i>	CBS 287.87	Switzerland	Unknown	DQ006924	DQ006961	Rolshausen et al. (2006)
<i>Eutypa maura</i>	CBS 219.87	Switzerland	Unknown	DQ006926	DQ006967	Rolshausen et al. (2006)
<i>Eutypa microasca</i>	BAFC 51550	Argentina	C. Carmaran	KF964566	KF964572	Grassi et al. (2014)
<i>Eutypa sparsa</i>	3802 3b	Switzerland	F. Rappaz	AY684220	AY684201	Trouillas & Gubler (2004)
<i>Eutypella caricae</i>	EL51C	France	F. Rappaz	AJ302460	_	Acero (2000)
<i>Eutypella cerviculata</i> Ts	M68	Latvia	Unknown	JF340269	_	Arhipova et al. (2012)
<i>Eutypella cerviculata</i>	EL59C	Switzerland	F. Rappaz	AJ302468	_	Acero et al. (2004)
<i>Eutypella citricolca</i>	HVVIT07	New South Wales	F.P. Trouillas/W.M. Pitt	HQ692579	HQ692512	Trouillas et al. (2011)
<i>Eutypella citricolca</i>	HVGRF01	New South Wales	F.P. Trouillas/W.M. Pitt	HQ692589	HQ692521	Trouillas et al. (2011)
<i>Eutypella leprosa</i>	EL54C	_	_	AJ302463	_	Acero et al. (2004)
<i>Eutypella leprosa</i>	Isolate 60	USA	L. Vasilyeva	KU320622	_	de Almeida et al. (2016)
<i>Eutypella microtheca</i>	BCMX01	Mexico	Unknow	KC405563	KC405560	Paolinelli-Alfonso et al. (2015)

Table 1 Continued.

Taxon	Strain no.	Provenance	Collector	GenBank accession		Reference
				ITS	β -tubulin	
<i>Eutypella microtheca</i>	ADEL200	Adelaide, South Australia	F. P. Trouillas	HQ692559	HQ692527	Trouillas et al. (2011)
<i>Eutypella semicircularis</i>	MP4669	Panama	Unknown	JQ517314	_	Mehrabi et al. (2016)
<i>Eutypella vitis</i>	UCD2291AR	Arkansas, USA	AK. 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 avcenniae</i>	MFLUCC 15-0953	Thailand	M. Dayarathne	KX573916	KX573931	Dayarathne et al.(2016)
<i>Halodiatrype salinicola</i> Ts	MFLUCC 15-1277	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 comosa</i>	BAFC 393	Argentina	C. Carmaran	KF964568	_	Grassi et al. (2014)
<i>Peroneutypa curvispora</i>	HUEFS 136877	Brazil	D.A.C. Almeida	KM396641	_	de Almeida et al. (2016)
<i>Peroneutypa diminutispora</i>	HUEFS 192196	Brazil	D.A.C. Almeida	KM396647	_	de Almeida et al. (2016)
<i>Peroneutypa kochiana</i>	EL53M	Spain	J. Checa	AJ302462	_	Acero et al. (2004)
<i>Peroneutypa longiasca</i>	<u>MFLUCC 17-0371</u>	<u>Thailand</u>	<u>C. Senwana</u>	<u>MF959502</u>	<u>MG334558</u>	<u>This study</u>
<i>Peroneutypa mackenziei</i>	MFLUCC 16-0072	Thailand	Q.J. Shang	KY283083	KY706363	Shang et al. (2017)
<i>Peroneutypa scoparia</i>	MFLUCC 11-0478	Thailand	Dong-Qin Dai	KU940151	_	Dai et al. (2017)
<i>Quaternaria quaternata</i>	EL60C (CBS 278.87)	Switzerland	F. Rappaz	AJ302469	_	Acero et al. (2004)
<i>Quaternaria quaternata</i>	GNF13	Iran	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

Abbreviations: **ATCC**: American Type Culture Collection, Manassas, America; **BAFC**: Herbarium, Department of Biological Sciences, Faculty of Natural Sciences, Buenos Aires' University, Argentina; **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, 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**: Instituto 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.

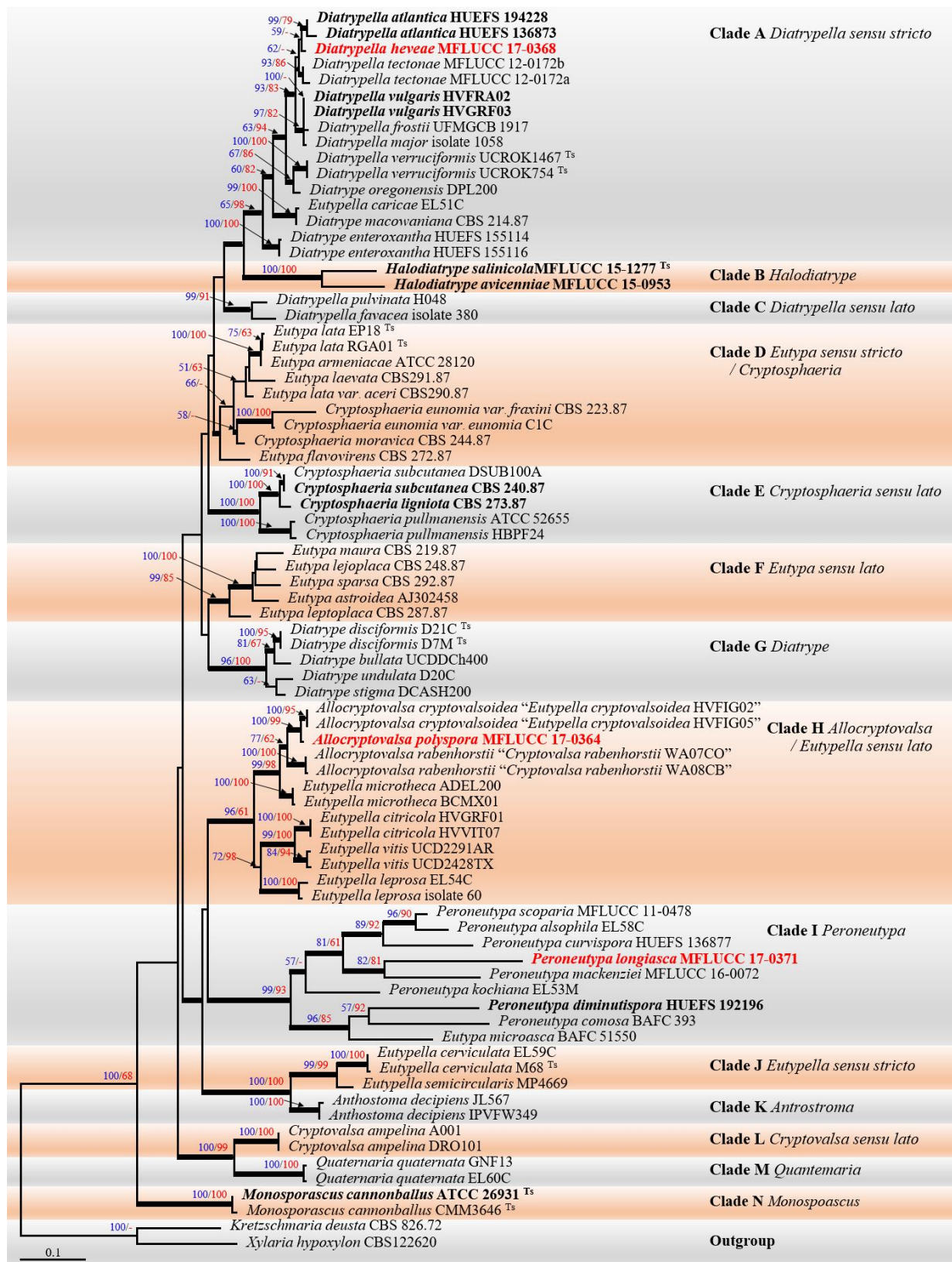


Figure 1 – Phylogram generated from the best scoring of the RAxML tree based on a combined ITS and β -tubulin sequence data. Bootstrap support values for maximum likelihood (ML, blue) and maximum parsimony (MP, red) equal or greater than 50% are defined above the node. Bayesian posterior probabilities (BYPP) equal or greater than 0.95 are shown as bold branch. The tree is rooted to *Xylaria hypoxylon* (CBS 122620) and *Kretzschmaria deusta* (CBS 826.72). All sequences from ex-type strains are in bold and type species are denoted with the superscript “Ts” after the species name. Newly generated strains are indicated in red

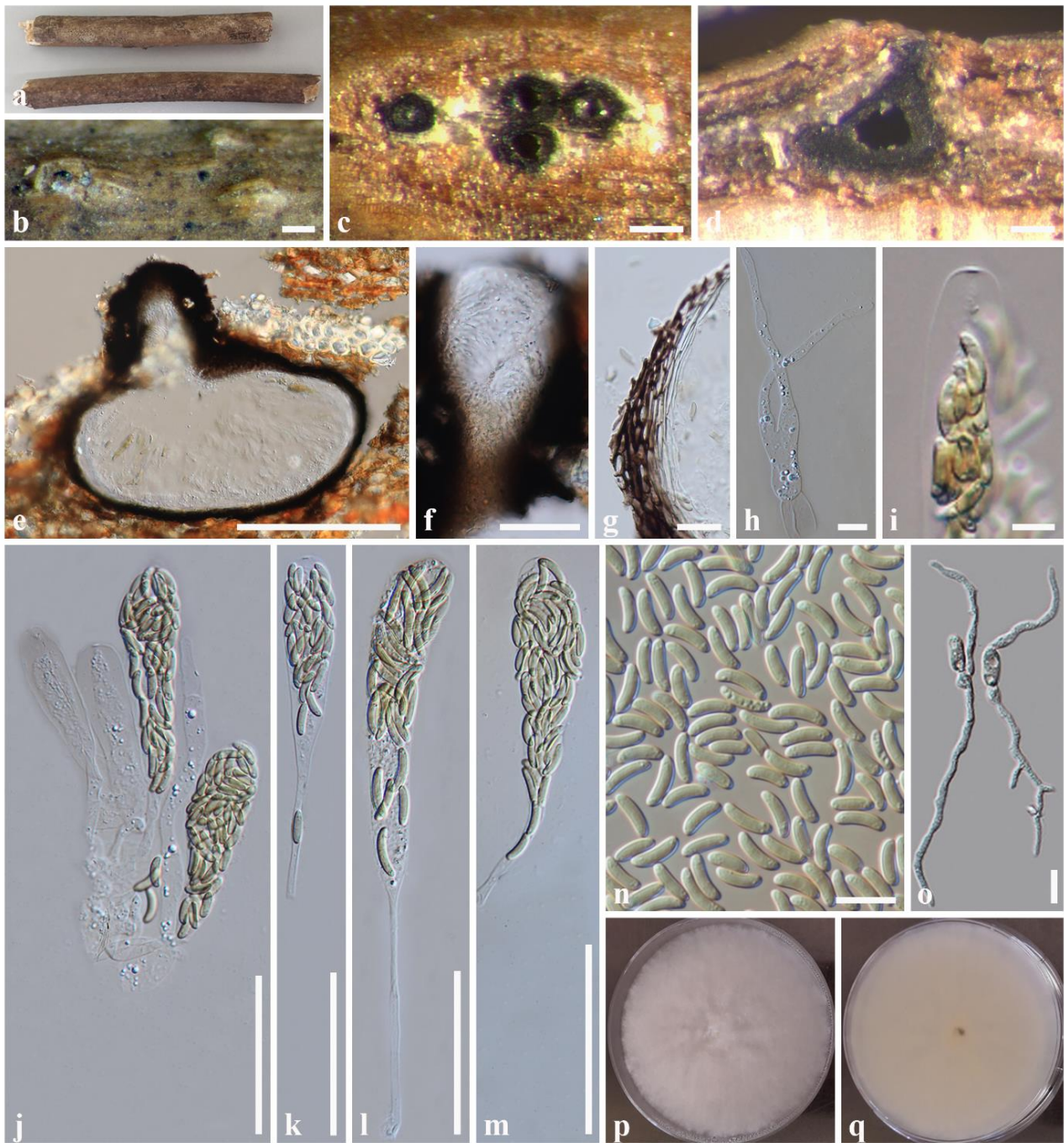


Figure 2 – *Allocryptovalsa polyspora* (MFLU 17-1218, holotype). a–b Habit of ascostromata on substrate. c Transverse sections through ascostroma. d Vertical section through ascostroma. e Section through the ascoma. f Ostiole with short periphyses. g Peridium. h Paraphyses. i Refractive J- subapical ring. j–m Asci. n Ascospores. o Germinated ascospores. p–q Culture characteristic on PDA after 14 days (p = colony from above, q = colony from below). Scale bar: b = 200 μ m, c, d, e = 100 μ m, f, j–m = 50 μ m, g–i = 20 μ m, n–o = 10 μ m.

Diatrypella heveae Senwanna, Phookamsak & K.D. Hyde, sp. nov.,

Fig. 3

Index Fungorum number: IF553859; Facesoffungi number: FoF03775

Etymology – The epithet “*heveae*” refers to the host genus *Hevea* on which the species was collected.

Holotype – MFLU 17-1216

Saprobic on dead twig of *Hevea brasiliensis*. Sexual morph: *Ascostromata* 410–700 μ m high, 980–1910 μ m diam., visible as black, rounded to irregular in shape on the host surface, erumpent

through host epidermis, with bark adhering to ascostroma, solitary to gregarious, entostromatic region, occupying by white pseudoparenchymatous cells, with a powdery, thin black pseudoparenchymatous tissue around the white entostroma, carbonaceous. *Ascomata* 200–600 μm

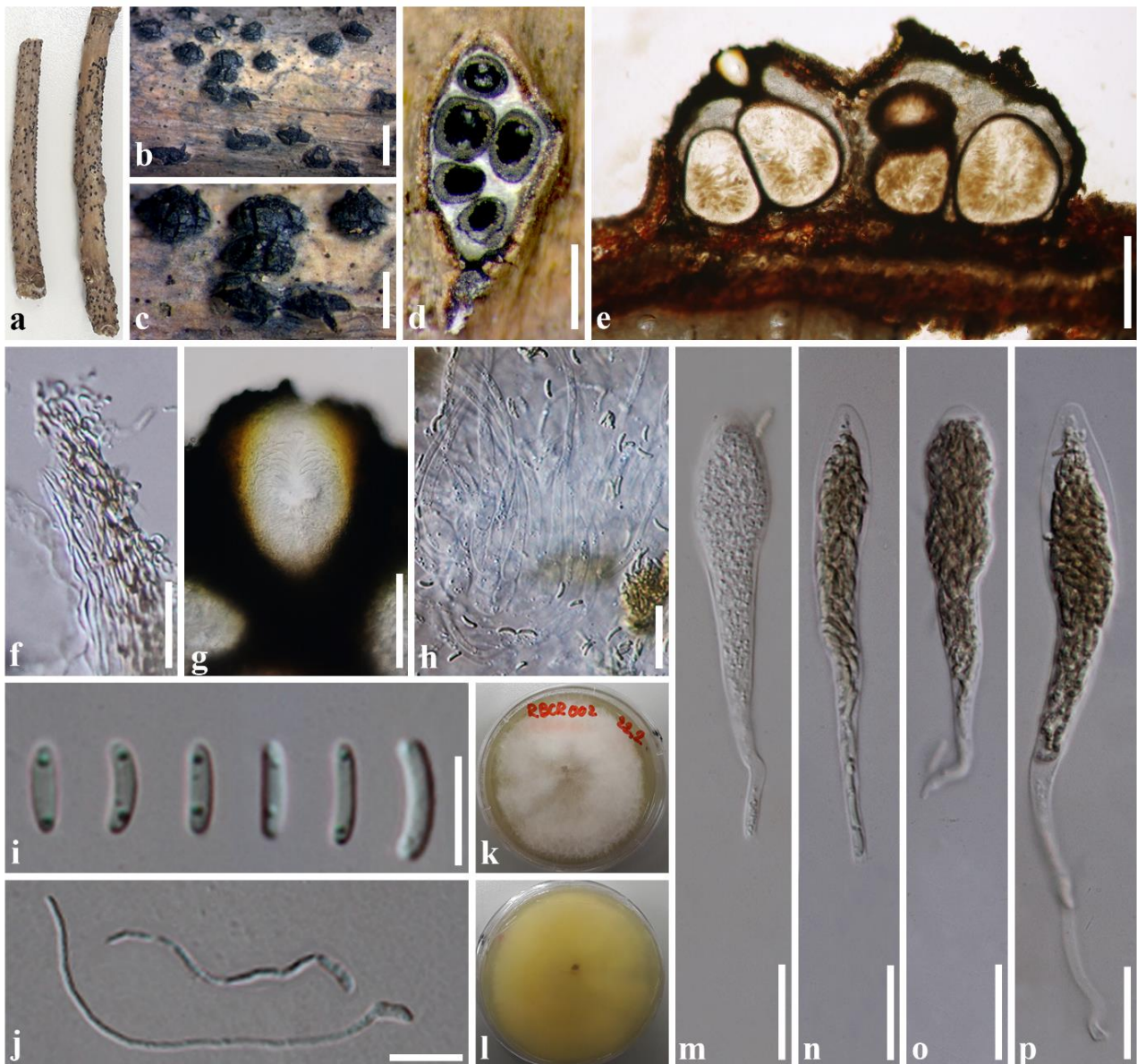


Figure 3 – *Diatrypella heveae* (MFLU 17-1216, holotype). a–c Appearance of ascostromata on the host substrate. d Transverse sections through ascostroma. e Section through the ascostroma. f Peridium. g Ostiole in section with periphyses. h Paraphyses. i Ascospores. j Germinated spores. k–l Culture characteristic on MEA after 14 days (k = colony from above, l = colony from below). m–p Asci. Scale bar: b = 1000 μm , c = 500 μm , e = 300 μm , f–h, j, m–p = 20 μm , i = 10 μm .

high, 160–460 μm diam., perithecial, black, clustered, immersed to semi-immersed in ascostroma, ovoid to subglobose, 4–5 locules in ascostroma, glabrous, ostioles non prominent, with or without papillate, carbonaceous. *Ostiole canal* 150–245 μm high, 45–140 μm diam., cylindrical, sulcate, periphysate, with yellowish pigment around ostioles. *Peridium* 12–33 μm wide, composed of two type 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* to *textura prismatica*. *Hamathecium* 3.5–11 μm wide. (\bar{x} = 7.02, n = 20), comprising dense, hyaline, septate, unbranched, filamentous paraphyses, tapering towards the apex, embedded

in hyaline gelatinous matrix. *Asci* 70–136 × 10–21 μm (\bar{x} = 101.9 × 14.6 μm, n = 45), spore-bearing part length [p. sp.] (65–)80–113(–126) μm (\bar{x} = 92.95 μm, n = 20), polysporous, unitunicate, thin-walled, clavate to cylindrical-clavate, long pedicellate, apically rounded to truncate with indistinct J- subapical ring. *Ascospores* 5–9 × 1–3 μm (\bar{x} = 6.9 × 1.8 μm, n = 45), crowded, initially hyaline, becoming pale yellowish to pale brown at maturity, oblong to allantoid, aseptate, slightly curved, smooth-walled, mostly with small guttules. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinated on MEA within 24 hours and germ tube produced from one end of the ascospore. Colonies on MEA reaching 4.5–5 cm diam. after 14 days at 25 °C, colonies medium dense, irregular, flat or effuse, slightly raised, cottony, white, margin rough, not produced pigmentation on MEA media.

Material examined – THAILAND, Chiang Rai Province, Wiang Chiang Rung District, on dead twig of *Hevea brasiliensis* (Euphorbiaceae), 1 November 2016, C. Senwanna, RBCR002 (MFLU 17-1216, holotype; HKAS100695, isotype), ex-type living culture MFLUCC 17-0368 = KUMCC17-0260.

Addition GenBank number – LSU = MF959504.

Notes – The new species, *Diatrypella heveae* is similar to *D. atlantica*, but differs by black outer layer of the entostroma, shorter asci and longer spore-bearing part length (Table 3). *Diatrypella heveae* differs from *D. tectonae* by its shorter asci and ascospore (Table 3). In the phylogenetic analyses based on ITS and β-tub sequence data (Fig. 1) indicated that *D. heveae* forms a separate lineage, but clusters with *D. atlantica* and *D. tectonae*.

Peroneutypa Berl., Icon. fung. (Abellini) 3(3-4): 80 (1902)

Type species – *Peroneutypa bellula* (Desm.) Berl.

Note – This genus is characterized by valsoid ascostromata, perithecia with long necks octosporous, clavate, sessile to subsessile asci, allantoid, hyaline or yellowish ascospores (Carmarán et al. 2006, Vasilyeva and Rogers 2010, Mehrabi et al. 2016, Shang et al. 2017). The molecular phylogenetic analysis of *Peroneutypa* species appears to be a monophyletic group in Diatrypaceae (Carmarán et al. 2006, Dai et al. 2016, Shang et al. 2017).

Peroneutypa longiasca Senwanna, Phookamsak & K.D. Hyde, sp. nov.,

Fig. 4

Index Fungorum number: IF553860; Facesoffungi number: FoF03776

Etymology – In reference to the long pedicellate of the asci

Holotype – MFLU 17-1217

Saprobic on dead twig of *Hevea brasiliensis*. Sexual morph: *Ascostromata* solitary to gregarious, immersed, becoming raised to erumpent through host tissue by a long ostiolar canal, dark brown to black, irregular in shape, arranged in longitudinal configuration. *Ascomata* 180–450 μm high, 170–390 μm diam., perithecial, with long neck, single to aggregated, immersed in substrate, globose to subglobose, dark brown to black. *Ostiolar canal* 20–50 μm wide, with long necks, 190–440 μm length, cylindrical, straight, dark-brown to black. *Peridium* 14–47 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* to hyaline, thin-walled, clavate to cylindrical, short to long pedicellate, apically rounded to truncate with indistinct J- subapical ring. *Ascospores* 3–7 × 1–3 μm (\bar{x} = 5.8 × 2 μm, n = 30), overlapping 2–3-seriate, hyaline, oblong to allantoid, aseptate, slightly curved, smooth-walled, with small guttules. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinated on MEA within 24 hours and germ tube produced from one or both sides of the ascospore. Colonies on MEA reaching 2–4.5 cm diam. after 14 days at 25 °C, colonies irregular, flat or effuse, slightly raised, margin rough, white, with aerial mycelium, reverse side white at the margin, yellow to pale brown at the center, not produced pigmentation on MEA media.

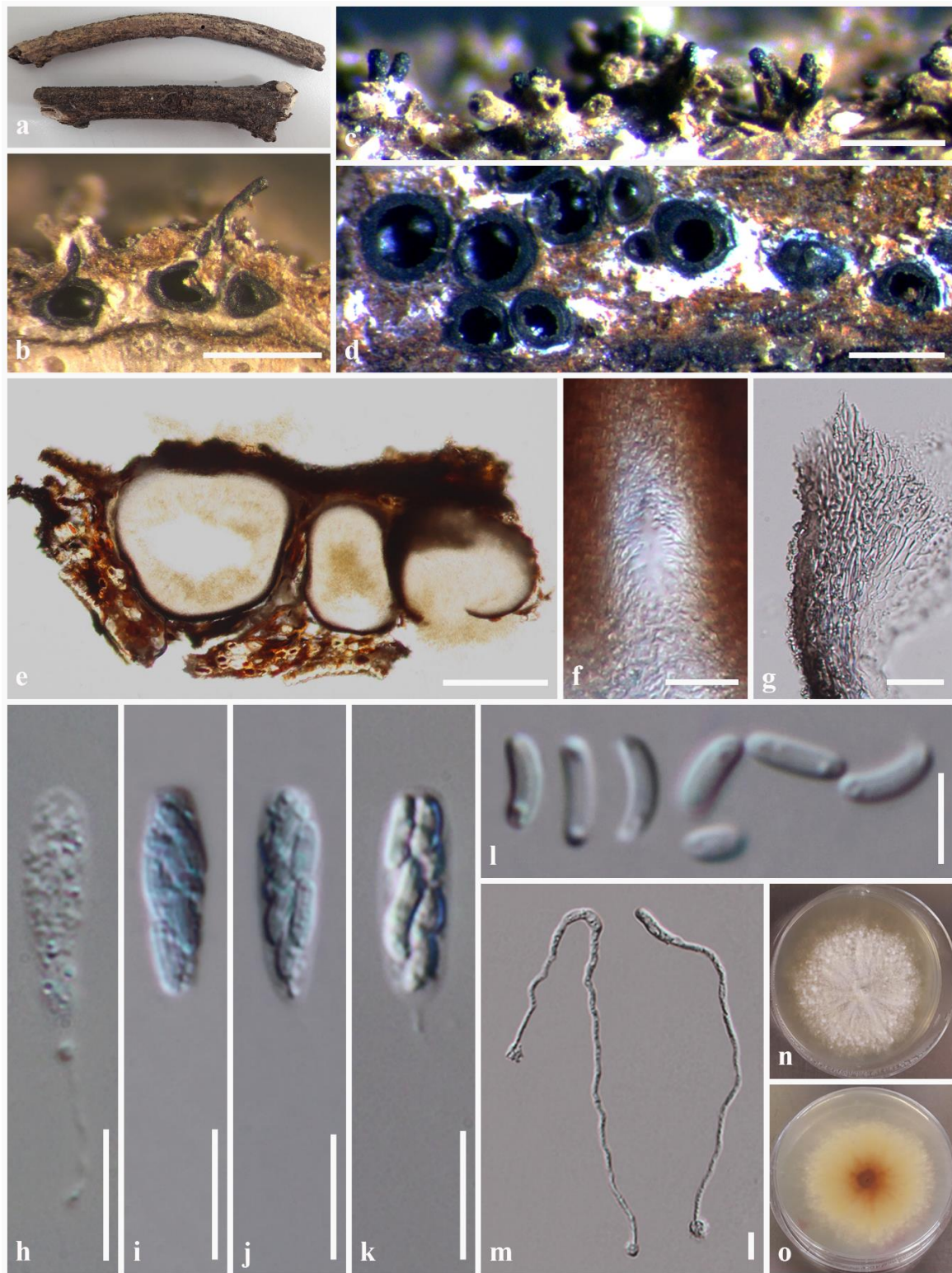


Figure 4 – *Peroneutypa longiasca* (MFLU 17-1217, holotype). a, c Appearance of ascostromata on substrate. b Vertical section through ascostroma. d Transverse sections through ascostromata. e Section through the ascostroma. f Ostiolar canal. g Peridium. h–k Asci. i–j Asci in lactophenol cotton blue. l Ascospores in lactophenol cotton blue. m Germinated spores. n–o Culture characteristic on MEA after 14 days (n = colony from above, o = colony from below). Scale bar: b–d = 500 μ m, e = 200 μ m, f, h–k = 10 μ m, g = 20 μ m, l–m = 5 μ m.

Material examined – THAILAND, Chiang Rai Province, Wiang Chiang Rung District, on dead twig of *Hevea brasiliensis* (Euphorbiaceae), 1 November 2016, C. Senwanna, RBCR005 (MFLU 17-1217, holotype; HKAS100696, isotype), ex-type living culture, MFLUCC 17-0371.

Addition GenBank number – LSU = MF959505

Notes – *Peroneutypa longiasca* is introduced as a new species based on morphology characteristics and phylogenetic analyses. This species resembles to *P. mackenziei*. However asci dimensions of *P. longiasca* are longer and wider than *P. mackenziei* (Table 4) and *P. longiasca* do not appear paraphyses. In this study phylogenetic analyses of a combined ITS and β -tub sequence data (Fig. 1) showed that *P. longiasca* (MFLUCC17-0371) forms a distinct lineage sister to *P. mackenziei*.

Discussion

Taxa in Diatrypaceae are difficult to distinguish based on the morphological characters (Acero et al. 2004, Vasilyeva & Stephenson 2005). Therefore, multigene phylogenetic investigations are needed to clarify phylogenetic affinities of the genera in this family. Some genera in Diatrypaceae lack molecular data to clarify their placement as well as the generic types have not yet been sequenced. Therefore, more taxon sampling of representative species from genera in Diatrypaceae are acquired in further work and the taxonomic revision of this family is needed to be revisited.

In this study, phylogenetic analyses based on a combined ITS and β -tubulin sequence data showed that *Cryptosphaeria*, *Diatrype*, *Diatrypella*, *Eutypa* and *Eutypella* are polyphyletic within the family and this is concurred the results from previous studies (Acero et al. 2004, Trouillas et al. 2011, Chacón et al. 2013, de Almeida et al. 2016, Shang et al. 2017).

The new genus *Allocryptovalsa* is introduced to accommodate cryptovalsa-like taxa forming polysporous asci viz. *A. cryptovasoidea*, *A. polyspora* and *A. rabenhorstii*. *Allocryptovalsa* clusters with *Eutypella sensu lato* which is characterized by 8-spored asci and this is concurs with the results of de Almeida et al. (2016). *Eutypella microtheca* (clade H; *sensu lato*) forms a robust clade (99% ML, 98% MP and 1.00 PP) with *Allocryptovalsa*. However, the species differs from *Allocryptovalsa* in having 8-spored asci. Furthermore, other *Eutypella sensu lato* in Clade H forms a separated clade with *Eutypella sensu stricto* (Clade J) which is represented by the type species *Eutypella cerviculata* (Fr.) Sacc., Therefore, the new genera may need to designate for accommodating taxa in this clade when more taxon sampling and informative genes can be resolved their generic boundaries.

Phylogenetic analyses showed a polyphyletic in *Diatrypella* and *Diatrype* (Acero et al. 2004, Trouillas et al. 2011, Grassi et al. 2014, de Almeida et al. 2016, Shang et al. 2017). *Diatrypella sensu lato* (Clade C) comprises *D. favacea* and *D. pulvinata*, form a distinct lineages with *Diatrypella sensu stricto* (clade A) and this concur the phylogenetic results of de Almeida et al. (2016). Taxa in this clade need to be revisited.

Diatrype enteroxantha, *D. macowaniana*, *D. oregonensis* and *Eutypella caricae* form a well-resolved clade together with *Diatrypella sensu stricto* (clade A). However, these taxa are morphologically different from *Diatrypella* due to their octosporous asci. Therefore, these taxa are needed to be revisited based on morphological study and multigene phylogenetic analyses to determine their taxonomic placement. Furthermore, the new genus is required to accommodate these taxa in further study.

In clade J, *Eutypa microasca* clusters with *Peroneutypa* species, which is concurred with de Almeida et al. (2016). *Eutypa microasca* should be transferred to the genus *Peroneutypa* in further studies. *Peroneutypa* has recently been studied by Carmarán et al. (2006), de Almeida et al. (2016), Dai et al. (2017) and Shang et al. (2017). The phylogenetic analyses of ITS and β -tub showed that *Peroneutypa* formed a well-resolved clade in Diatrypaceae. The new species, *P. longiasca* sister to *P. mackenziei*, but they are different in morphological characters as *P. longiasca* lacks paraphyses and long pedicellate, which is different from previous studies that reported this genus has conspicuous sessile asci (Carmarán et al. 2006, Vasilyeva & Rogers 2010, Shang et al. 2017).

Table 2 Synopsis of *Allocryptovalsa polyspora* and related species discussed in this study

Species name	Asci length/ ([p. sp.] (μm))	Asci width (μm)	Ascospores length (μm)	Ascospore width (μm)	Reference
<i>Allocryptovalsa polyspora</i> (polysporous)	(33.5–)70–120–177)/(41–)54–74(–101)	(4–)13–19(–21)	(4–)7–12(–14)	2–4	This study
<i>Allocryptovalsa cryptovalsoidea</i> (holotype) (polysporous)	–/65–120	15–20	8–12(–13.5)	2–3	Trouillas et al. 2011
<i>Allocryptovalsa rabenhorstii</i> (polysporous)	–/ (55–)70–90 (–95)	(15–)18–22(–27)	(10–)13.5–15 (–17.5)	(3–)4–5(–6)	Trouillas et al. 2011

Table 3 Synopsis of *Diatrypella heveae* and related species discussed in this study.

Species name	Colour of entostroma (inner/outer layer)	Asci length/ ([p. sp.] (μm))	Asci width (μm)	Ascospores 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 heveae</i> (holotype)	White/black	(70–)89–122(–136)/ (65–)80–13(–127)	(10–)12–17(–21)	(5.4–)5.8–7.8(–8.9)	1.3–2.2(–2.7)	This study
<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)	Shang et al. (2017)

Table 4 Synopsis of *Peroeutypa longiasca* and related species discussed in this study.

Species name	Asci length (μm)	Asci width (μm)	Ascospores length (μm)	Ascospores width (μm)	Reference
<i>Peroeutypa longiasca</i> (holotype)	(10.5–)13.5–20(–32.5)	(4.5–)5–6	(3.5–)5–7	1.5–2	This study
<i>Peroneutypa mackenziei</i> (holotype)	(14.5–)15.5–20(–21.5)	3–3.5(–3.5)	(3–)4.5–6.5(–8)	1–2	Shang et al. (2017)

Shang et al. (2017) mentioned that the pedicellate character might be significant to determine the species in this genus. In this study, the absence of paraphyses and conspicuous sessile asci may not be significant characters to distinguish the genus from other genera in Diatrypaceae.

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