



***Halodiatrype*, a novel diatrypaceous genus from mangroves with *H. salinicola* and *H. avicenniae* spp. nov.**

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Abstract

Collections of diatrypaceous taxa from mangroves in southern Thailand yielded two novel species from intertidal habitats. Maximum parsimony, maximum likelihood and Bayesian analyses of combined ITS and β -TUB sequence data, showed their placement in a well-separated lineage in *Diatrypaceae* (Xylariales). We therefore introduce a novel genus, *Halodiatrype* to accommodate the new species, *H. salinicola* and *H. avicenniae*. *Cryptosphaeria mangrovei* is morphologically similar to this genus, and thus it is synonymized as *Halodiatrype mangrovei*. Morphological descriptions, Fig.s. and molecular data are provided for the new taxa. Our studies show that the molecular data for *Diatrypaceae* is highly confused with species of the same genera scattered across the tree. The family therefore needs a detailed monograph with molecular data and multigene analyses with so far unpublished genes.

Key words – β -TUB – *Diatrypaceae* – ITS – marine fungi – phylogeny – taxonomy

Introduction

The family *Diatrypaceae* was introduced by Nitschke (1869) with the type genus *Diatrype* Fr. and also includes the genera *Anthostoma* Nitschke, *Cryptosphaeria* Ces. & De Not., *Cryptovalsa* Ces. & De Not. ex Fuckel, *Diatrypella* (Ces. & De Not.) De Not., *Diamantina* A.N. Mill. et al., *Diatrypasimilis* J.J. Zhou & Kohlm., *Echinomyces* Rappaz, *Eutypa* Tul. & C. Tul., *Eutypella* (Nitschke) Sacc., *Leptoperidia* Rappaz, *Libertella* Desm., *Monosporascus* Pollack & Uecker, *Pedumispora* K.D. Hyde & E.B.G. Jones, *Peroneutypa* Berl., *Phaeoisaria* Höhn. and *Quaternaria* Tul. & C. Tul. (Senanayake et al. 2015). Studies by Tiffany & Gilman (1965), Glawe & Rogers (1984), Rappaz (1987), Vasiljeva & Stephenson (2004, 2005) and Carmarán et al. (2006) have provided generic concepts and taxonomic reviews for the family. Glawe & Rogers (1984) and Tiffany & Gilman (1965) studied this family and introduced various diatrypaceous species from North America. Vasiljeva & Stephenson (2004, 2005, 2006, 2009) documented various species of *Diatrypaceae* from the Great Smoky Mountains National Park in the eastern United States. Acero

et al. (2004) established nine phylogenetic groups in *Diatrypaceae* and confirmed the polyphyletic nature of *Cryptosphaeria*, *Diatrype*, *Diatrypella*, *Eutypa* and *Eutypella* based on phylogenetic analysis of ITS rDNA sequence data. However, correlation between phylogenetic relationships and classical taxonomic schemes were discordant. New records and new species of this family were introduced by Romero & Carmarán (2003) and Carmarán et al. (2009) from Argentina. Trouillas & Gubler (2004) and Trouillas et al. (2010a, b, 2011) also reported and described many other diatrypaceous species occurring on *Vitis vinifera* and other woody plants in Australia and California based on morphological studies and phylogenetic analyses. Chacón et al. (2013) introduced a new taxon and records of diatrypaceous species from Panama. Thirteen species belonging to the genera *Diatrype* and *Cryptosphaeria* were introduced by Vasiljeva & Ma (2014) from north-eastern China and Liu et al. (2015) described a new *Diatrype* species from palms. Recent papers with sequence data providing backbone trees for the family are by Carmarán et al. (2006), Trouillas et al. (2010), Maharachchikumbura et al. (2015, 2016), Senanayake et al. (2015) and Almeida et al. (2016). Maharachchikumbura et al. (2015) included 15 genera in the *Diatrypaceae*, while Senanayake et al. (2015) put the Fig. as 17 genera.

Species of *Diatrypaceae* (Xylariales) are wide-spread on dead wood and bark of a broad variety of plants worldwide (Trouillas et al. 2011). Taxa can be recognized by their perithecial ascomata usually embedded in a black stroma, long stalked asci and allantoid ascospores (Glawe & Rogers 1984, Rappaz 1987). A few diatrypaceous taxa have been found in marine habitats (Hyde 1993, Hyde & Rappaz 1993, Hyde 1995, Jones et al. 2009, 2013, Abdel-Wahab et al. 2014, Jones et al. 2015). The delineation of diatrypaceous genera is often problematic because the characters of the stromata are extremely variable within the same species (Vasiljeva & Stephenson 2004).

The genus *Diatrype* was established by Fries (1849) with *D. disciformis* (Hoffm.) Fr. as the type species (Tilak 1964) and comprises mainly saprobes on decaying wood, while a few species are reported as pathogens forming cankers on forest trees (Senanayake et al. 2015). *Diatrype* species produce perithecia embedded in discoid or widely effuse stromata that are erumpent from the bark. The young stromata are sometimes covered with a layer of sterile tissue that eventually peels off to expose a fertile surface studded with papillate or stellate ostioles (Vasiljeva & Stephenson 2009). The concept of *Diatrype* as delimited by Rappaz (1987) is controversial, with in some instances there is no clear separation between this genus and either *Eutypa* or *Eutypella* (Vasiljeva et al. 2004). In the Dictionary of the Fungi (Kirk et al. 2001, 2008), the genus *Diatrype* was listed as comprising 56 species which is the same as given in the monograph of Rappaz (1987), and is the most comprehensive study of the genus. Our new taxa were initially identified as *Diatrype* species, but our phylogenetic analyses showed this to be incorrect.

The objective of this study is to introduce a new marine genus *Halodiatrype* comprising two novel diatrypaceous taxa, *H. salinicola* and *H. avicenniae*. Micro-morphology and maximum likelihood and Bayesian analyses, based on combined ITS and β -TUB sequence data, are used to examine their phylogenetic placements within the family *Diatrypaceae* (Xylariales). *Cryptosphaeria mangrovei* K.D. Hyde collected from a marine habitat is morphologically similar to the new genus and hence it is synonymized as *Halodiatrype mangrovei*. Comprehensive descriptions and micrographs are provided.

Materials and methods

Sample collections, specimen examination and isolation

Fresh material was collected from Tham Nam Phut, Mueang Phang-nga District, Phang-nga Province, in Southern Thailand, 8°25'52" N, 98°31'42" E, 0 m asl. and Hat Chao Samran, Phetchaburi Province, Thailand, 47° 72506' E, 40° 25038' N, 0 m asl. They were examined with a Motic SMZ 168 stereomicroscope. Micro-morphological characters of taxa were examined and images were captured under a Nikon ECLIPSE 80i compound microscope with a Canon EOS 550D digital camera. Observations and photographs were made from squash mounts of fresh fruiting bodies mounted in water and stained with Melzer's reagent and Congo red. Measurements were

taken with the Tarosoft (R) Image Frame Work and Adobe Photoshop CS3 Extended version 10.0 software was used to prepare the photo plates. Isolates were derived by single spore isolation following the method of Chomnunti et al. (2014). Germinating ascospores were transferred to potato dextrose agar (PDA) after 24 h. Cultural characteristics, such as mycelium colour, shape, texture and growth rate, were recorded after incubating at 25°C under normal light for a week. The herbarium specimens of the new species are deposited in the Mae Fah Luang University Herbarium (MFLU) and Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS), while cultures are deposited at the Mae Fah Luang University Culture Collection (MFLUCC) and International Collection of Microorganisms from Plants (ICMP), New Zealand. Faces of fungi and Index Fungorum numbers were registered according to Jayasiri et al. (2015) and Index Fungorum (2016).

DNA extractions, PCR amplifications and sequencing

Extraction of genomic DNA from mycelia (approximately 50 mg) harvested from fungal cultures grown on PDA was carried out following the manufacturer's instruction. Polymerase chain reaction (PCR) was performed for DNA amplification using the primer combination ITS4 and ITS5 (White et al. 1990) for the internal transcribed spacer (ITS), Partial sequences of the β -TUB gene was amplified using primers Bt2a and Bt2b (Glass & Donaldson 1995). Amplifications was performed in 25 μ l of PCR mixtures containing 9.5 μ l ddH₂O, 12.5 μ l 2 \times PCR Master Mix, 1 μ l of DNA template, 1 μ l of each primer (10 μ M). Amplification of ITS was accomplished by an initial step of 3 mins at 94°C, followed by 35 cycles of 30 sec at 94°C, 58 sec at 30°C, and 1 min at 72°C, with a final extension of 10 mins at 72°C. for the β -TUB amplification the 35 cycles consisted of initiation at 94°C for 3 mins, denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, elongation at 72°C for 1 min with a final extension of 10 mins at 72°C. The PCR products were observed on 1% agarose electrophoresis gels stained with Ethidium bromide. Purification and sequencing of PCR products were carried using the above mentioned PCR primers at Sun biotech company (Beijing, China).

Phylogenetic analysis

ITS and β -TUB sequence data were compared by BLAST search in the GenBank database at the National Centre for Biotechnology Information (NCBI) and sequences were analyzed with other sequences of the family *Diatrypaceae* (Table 1). Most reliable sequences for most common genera in the family *Diatrypaceae* were included (Senanayake et al. 2015). *Xylaria hypoxylon* (CBS 121680) and *Kretzschmaria deusta* (CBS 826.72) were selected as the out group taxa. Sequence data were aligned by MAFFT v. 6.864b (<http://mafft.cbrc.jp/alignment/server/index.html>), and combined using Bioedit 7 (Hall 1999) and MEGA 5.0 (Tamura et al. 2011) and refined visually. Phylogenetic analysis consisted of three methods: Maximum parsimony analysis was performed to obtain the most parsimonious tree. Trees were inferred using the heuristic search option with 1000 random sequence additions. Maxtrees were setup to 1000 and branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI]) were calculated for trees generated under different optimality criteria. Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989) were performed in order to determine whether trees were significantly different. Maximum likelihood analysis was performed using RAxML GUI v. 1.3 (Silvestro & Michalak 2011). General time reversible model (GTR) using proportion of invariable sites was applied with a discrete gamma distribution and four rate classes. The best scoring tree was selected with a final likelihood value of -4587.577780. Bayesian analysis was performed using MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2003). Nucleotide substitution models were determined with MrModeltest v. 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala et al. 1998, Zhaxybayeva & Gogarten 2002) were defined by Bayesian Markov Chain Monte Carlo (BMCMC) sampling method in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov

chains were run for 1000000 generations and trees were sampled every 100th generation resulting in 10000 total trees. 8000 trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree, after discarding the first 2000 trees representing the burn-in phase (20 %) of the analysis. Resulting trees were visualized with TreeView v. 1.6.6 (Page 1996). Maximum likelihood bootstrap values (ML) equal or greater than 60%, Bayesian posterior probabilities (PP equal or greater than 0.90%) and Maximum parsimony bootstrap values (MP) equal or greater than 60% are given above the nodes (Fig. 1). Sequences generated in this study were deposited in GenBank.

Results

Phylogenetic analysis

Fifty-nine taxa were included in the combined ITS and β -TUB data set with *Xylaria hypoxylon* (CBS 121680) and *Kretzschmaria deusta* (CBS 826.72) as the out group taxa. Parsimony analysis indicated that alignment comprised 1461 characters (including gaps) and 558 characters were constant; 76 variable characters were parsimony-uninformative; and 441 characters were parsimony informative. The most parsimonious tree out of 14 trees showed TL = 1739, CI = 0.505, RI = 0.764, RC = 0.386, HI = 0.495 values. Tree topology of the maximum parsimony, Bayesian analysis (not shown) was almost compatible with the ML tree and the best scoring RAxML tree, with a final likelihood value of -4587.577780 is presented in Fig. 1. The novel taxa *Halodiatrype avicenniae* and *H. salinicola* grouped as a separate lineage with high bootstrap support and posterior probability (100% ML/ 100% MP/ 1.00 PP).

The phylogram (Fig. 1) represents 13 clades (A–M) of genera of *Diatrypaceae*. Clade A represents *Eutypella sensu lato* with *Eutypella cryptovalsoidea* Trouillas, W.M. Pitt & Gubler, *E. microtheca* Trouillas, W.M. Pitt & Gubler, *E. citricola* Speg. and *E. vitis* (Schwein.) Ellis & Everh. *Eutypella sensu stricto* (Clade D) contains the type species, *E. cerviculata* (Fr.) Sacc., which forms a robust clade with the genus *Anthostoma* and is distinct from *Eutypella sensu lato*. Clade A probably represents a novel genus.

Clade B represents the genus *Peroneutypa* comprising *Peroneutypa scoparia* (Schwein.) Carmarán & A.I. Romero, *P. alsophila* (Durieu & Mont.) Carmarán & A.I. Romero, *P. curvispora* (Starbäck) Carmarán & A.I. Romero, *P. kochiana* Rehm, *P. comosa* (Speg.) Carmarán & A.I. Romero. The generic type, *P. bellula* (Desm.) Berl., has yet to be sequenced.

Clade C represents the genus *Anthostoma* with the putative species, *A. decipiens* (DC.) Nitschke, the generic type of *Anthostoma*. This genus needs epitypifying (*sensu* Ariyawansa et al. 2014). Clade E represents *Cryptosphaeria sensu lato*, comprising *C. subcutanea* (Wahlenb.) Rappaz, *C. ligniota* (Fr.) Auersw. and *C. pullmanensis* Glawe. These species form a separate clade from *Cryptosphaeria sensu stricto* and hence a novel genus will probably be needed for these species. *Cryptosphaeria eunomia* var. *fraxini* (Richon) Rappaz and the generic type, *C. eunomia* var. *eunomia* (Fr.) Fuckel (Clade I), form a distinct clade away from *Cryptosphaeria sensu lato* and cluster with *Eutypa*.

Clade F comprises *Cryptovalsa ampelina* (Nitschke) Fuckel which represents the genus *Cryptovalsa*, and is sister to the genus *Quaternaria* (Clade G) which comprises a putative species *Q. quaternata*. The generic types, *Cryptovalsa protracta* (Pers.) De Not. and *Quaternaria persoonii* Tul. & C. Tul. lack sequence data, and thus these genera need further confirmation. Clade H represents the genus *Eutypa* comprising *E. armeniacae* Hansf. & M.V. Carter, *E. lata* (Pers.) Tul. & C. Tul. (generic type), *E. lata* var. *aceri* Rappaz, *E. laevata* (Nitschke) Sacc. and *E. petrakii* Rappaz. This genus forms a well-resolved clade within *Diatrypaceae*. Clade J comprises *Diatrype disciformis* (Hoffm.) Fr. (generic type), *D. stigma* (Hoffm.) Fr., *D. spilomea* Syd., *D. bullata* (Hoffm.) Fr. and *D. undulata* (Pers.) Fr. *Diatrype brunneospora* Trouillas, Sosnowski & Gubler forms a single clade distinct from other *Diatrype* species. This species needs to be re-visited to clarify its phylogenetic affinity. Clade K is represented by two strains of the type species of *Monosporascus*, *M. cannonballus* Pollack & Uecker.

Table 1 GenBank and culture collection accession numbers of species treated in the phylogenetic analyses. Bold accession numbers from ex-type strains and blue, bold accession numbers were generated in this study.

Species	Strain no.	GenBank accession no.	
		ITS	β -TUB
<i>Anthostoma decipiens</i>	IPVFW349	AM399021	AM920693
<i>A. decipiens</i>	JL567	JN975370	JN975407
<i>Cryptosphaeria eunomia</i> var. <i>fraxini</i>	CBS 223.87	KT425231	KT425166
<i>C. eunomia</i> var. <i>eunomia</i>	C1C	AJ302417	–
<i>C. subcutanea</i>	DSUB100A	KT425189	KT425124
<i>C. subcutanea</i>	CBS 240.87	KT425232	KT425167
<i>C. ligniota</i>	CBS 273.87	KT425233	KT425168
<i>C. pullmanensis</i>	HBPF24	KT425202	GQ294014
<i>C. pullmanensis</i>	ATCC 52655	KT425235	KT425170
<i>Cryptovalsa ampelina</i>	A001	GQ293901	GQ293972
<i>C. ampelina</i>	DRO101	GQ293902	GQ293982
<i>Diatrypella vulgaris</i>	HVFRA02	HQ692591	HQ692503
<i>D. vulgaris</i>	HVGRF03/CBS 128327	HQ692590	HQ692502
<i>D. verruciformis</i>	UCROK1467	JX144793	JX174093
<i>D. verruciformis</i>	UCROK754	JX144783	JX174083
<i>D. frostii</i>	UFMGCB 1917	HQ377280	–
<i>D. atlantica</i>	HUEFS 194228	KM396615	KR363998
<i>D. atlantica</i>	HUEFS 136873	KM396614	KR259647
<i>Diatrype brunneospora</i>	DAR 80711	HM581946	HQ692478
<i>D. bullata</i>	D6C	AJ302422	–
<i>D. bullata</i>	UCDDCh400	DQ006946	DQ007002
<i>D. disciformis</i>	D21C	AJ302437	–
<i>D. disciformis</i>	D7M	AJ302423	–
<i>D. undulata</i>	D20C	AJ302436	–
<i>D. stigma</i>	DCASH200	GQ293947	GQ294003
<i>Diatrype spilomea</i>	D17C	AJ302433	–
<i>Eutypa armeniaca</i>	ATCC 28120	DQ006948	DQ006975
<i>E. lata</i>	EP18	HQ692611	HQ692501
<i>E. lata</i>	RGA01	HQ692614	HQ692497
<i>E. petrakii</i> var. <i>petrakii</i>	CBS 244.87	HM164735	HM164769
<i>E. petrakii</i> var. <i>petrakii</i>	E46C	AJ302455	–
<i>E. lata</i> var. <i>aceri</i>	CBS 290.87	HM164736	HM164770
<i>E. laevata</i>	E40C	AJ302449	–
<i>E. laevata</i>	CBS 291.87	HM164737	HM164771
<i>Eutypella citricolca</i>	HVVIT07	HQ692579	HQ692512
<i>E. citricolca</i>	HVGRF01	HQ692589	HQ692521
<i>E. cryptovalsoidea</i>	HVFIG02	HQ692573	HQ692524
<i>E. cryptovalsoidea</i>	HVFIG05	HQ692574	HQ692525
<i>E. microtheca</i>	ADEL200	HQ692559	HQ692527
<i>E. microtheca</i>	BCMx01	KC405563	KC405560
<i>E. cerviculata</i>	M68	JF340269	–
<i>E. cerviculata</i>	EL59C	AJ302468	–
<i>E. vitis</i>	UCD2428TX	FJ790851	GU294726
<i>E. vitis</i>	UCD2291AR	HQ288224	HQ288303
<i>Monosporascus cannonballus</i>	ATCC 26931	NR111370	–
<i>M. cannonballus</i>	CMM3646	JX971617	–
<i>Peroneutypa alsophila</i>	EL58C	AJ302467	–
<i>P. scoparia</i>	MFLUCC 11-0478	KU940151	–
<i>P. scoparia</i>	MFLUCC 11-0615	KU940152	–
<i>P. comosa</i>	BAFC:393	KF964568	–
<i>P. kochiana</i>	EL53M	AJ302462	–
<i>P. curvispora</i>	HUEFS136877	KM396641	–
<i>P. curvispora</i>	HUEFS131248	KM396646	–
<i>Halodiatrype avcenniae</i>	MFLUCC15–0953	KX573916	KX573931
<i>H. salinicola</i>	MFLUCC15–1277	KX573915	KX573932

<i>Quaternaria quaternata</i>	EL60C/CBS 278.87	AJ302469	–
<i>Q. quaternata</i>	GNF13	KR605645	–
<i>Xylaria hypoxylon</i>	CBS 122620	AM993141	–
<i>Kretzschmaria deusta</i>	CBS 826.72	KU683767	KU684190

Abbreviations: ATCC: American Type Culture Collection, Virginia, USA; BAFC: Herbarium, Department of Biological Sciences, Faculty of Natural Sciences, Buenos Aires' University, Argentina; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; UCD: University of California, Davis; HUEFS: Herbarium of Universidade Estadual de Feira de Santana.

Seven strains represent the genus *Diatrypella* (clade L) including *D. verruciformis* Ehrh., *D. vulgaris* Trouillas, W.M. Pitt & Gubler (generic type), *D. frostii* Peck and *D. atlantica* D.A.C. Almeida, Gusmão & A.N. Mill. Clade M represents the novel genus proposed in this study and comprises *Halodiatrype salinicola* Dayarathne & K.D. Hyde and *H. avicenniae* Dayarathne & K.D. Hyde

Taxonomy

Halodiatrype Dayarathne & K.D. Hyde, **gen. nov.**

Index Fungorum number: IF552254

Facesoffungi number: FoF02535

Etymology – Name reflects the saline environment from where the taxa were found and the similarity to *Diatrype*

Saprobic on marine wood. **Sexual morph:** *Ascostromata* or *pseudostromata* present or absent. If present; effuse, embedded in decorticated wood and incorporating host tissues, rather eutypoid, blackening the host surface. *Ascomata* perithecial, solitary to scattered, immersed, dark brown to black, globose to subglobose, ostiolate, papillate. *Ostioles* central, sometimes apex of the ostiole composed of brown outer amorphous layer and inner yellow cells of *textura porrecta* and ostiolar canal filled with periphyses. *Peridium* thick, composed of several cell layers of brown to dark cells of *textura angularis*, outer cell layer fuse with the host cells. *Paraphyses* arising from the base of perithecia, composed of long, wide, thin-walled cells, unbranched, septate, slightly constricted at the septum, narrowing and tapering towards the apex, with apex blunt, smooth to rough-walled with small guttules. *Asci* 8-spored, unitunicate, cylindrical to clavate, pedicellate, apically rounded or flat, lacking an apical ring, initially deliquescing. *Ascospores* overlapping uni- to bi-seriate, tri- to tetra-seriate at maturity, oblong to allantoid or sub-inaequilateral, aseptate to septate, not constricted at the septum, light brown, with one to few small guttules, slightly curved, smooth-walled. **Asexual morph:** Undetermined.

Type species – *Halodiatrype salinicola* Dayarathne & K.D. Hyde, **sp. nov.**

Notes – *Halodiatrype* generally appears to have many morphological characters in common with members of *Diatrypaceae*. It mainly differs from other genera in this family by having ascomata lacking stromatal tissues and comparatively large ascospores with septa. Generally, *Diatrypaceae* species have well-developed ascostromata with aseptate ascospores (Maharachchikumbura et al. 2015). Species belong to this novel genus formed a well-separated clade apart from other genera within *Diatrypaceae* with high bootstrap support (100% ML/ 1.00 PP/ 100% MP), basal to the genus *Diatrypella*.

Halodiatrype salinicola Dayarathne & K.D. Hyde, **sp. nov.**

Fig. 2

Index Fungorum number: IF552253

Facesoffungi number: FoF02021

Etymology – Name reflects the saline environment where the taxon was found and “-cola” means loving.

Holotype – MFLU 15-0179

Saprobic on decayed wood at a mangrove stand. **Sexual morph:** *Ascomata* 125–140 µm high, 105–115 µm diam., solitary to scattered, lacking stromatic tissues, deeply immersed, dark brown to black, globose to subglobose, uni-loculate, ostiolate, papillate. *Ostioles* central, apex composed of brown outer amorphous layer and inner yellow cells of *textura porrecta* and ostiolar canal filled with periphyses. *Peridium* up to 2.5 µm wide, thick, a single layer of amorphous fungal tissue fused with the host cells, incorporating occasionally in the host tissues. *Paraphyses* 2–3.5 µm wide, arising from the base of perithecia, composed of long, wide, filamentous, thin-walled cells, guttulate, unbranched, septate, slightly constricted at septum, narrow towards the apex, with apex blunt. *Asci* 40–50 × 7.5–10.5 µm (\bar{x} = 45 × 2.8 µm, n = 20), 8-spored, unitunicate, clavate, long pedicellate, apically rounded, with J- subapical ring, initially deliquescing. *Ascospores* 7.5–8.5 × 2.5–3 µm (\bar{x} = 8 × 2.8 µm, n = 30), overlapping bi- to tetra-seriate, allantoid, light brown, aseptate, 1-septate at maturity, not constricted at the septum, smooth-walled with one to few small guttules, slightly curved. **Asexual morph:** Undetermined.

Culture characteristics – Colonies on PDA, reaching 10 cm diam. after 2 weeks at 20–25°C, medium dense, irregular, slightly raised, surface slightly rough, with uneven edge, undulate margin, cottony, colony from above: white to pale grey at the margin, greenish-grey at the centre; from below: yellowish white at the margin, yellow to brown at the centre; mycelium greenish-grey, not produced pigmentation on PDA media.

Material examined – THAILAND, Phang-nga Province, Mueang Phang-nga District, Tham Nam Phut, 8°25'52" N, 98°31'42" E, 0 m asl., on submerged marine wood, 7 December 2014, M. Dayarathne, KRB010 (MFLU 15-0179, **holotype**), ex-type living culture, MFLUCC 15–1277, ICMP.

Notes – The new species *H. salinicola* is clearly different from other taxa in the family *Diatrypaceae* based on molecular data and morphology. It is characterized by immersed ascomata with a cylindrical or subconical, periphysate ostiole, apically rounded, clavate asci and allantoid, 0–1-septate, and light brown ascospores. *Halodiatrype salinicola* can be clearly distinguish from *H. avicenniae* by having unique ascomatal characters and apex composed of brown outer amorphous layer and inner yellow cells of *textura porrecta* and aseptate ascospores becoming 1-septate at maturity. *Halodiatrype salinicola* and *H. avicenniae* grouped as a separate lineage with high bootstrap support (100% ML/ 1.00 PP/ 100% MP). The two novel species share some common features including ascomata lacking stromatic tissues and allantoid, light brown and ascospores and resemble *Cryptosphaeria* species. The marine species, *Cryptosphaeria mangrovei* is morphologically similar, but differences are discussed under that species.

Halodiatrype avicenniae Dayarathne & K.D. Hyde, **sp. nov.**

Fig. 3

Index Fungorum number: IF552255

Facesoffungi number: FOF02020

Etymology – Name referring the host, of which the species was collected.

Holotype – MFLU 16-1185

Saprobic on intertidal decaying wood of *Avicennia* sp. **Sexual morph:** *Ascomata* 130–240 µm diam., dark brown to black, solitary to scattered, deeply immersed in the darken pseudostroma, globose to subglobose, uni-loculate, papillate, ostiolate. *Ostioles* central, ostiolar canal filled with periphyses. *Peridium* 25–35 µm wide, composed of several cell layers of dark brown to black cells of *textura angularis*, outer cell layer fusing with the host cells. *Paraphyses* 2–4 µm wide, arising from base of perithecia, composed of long, narrow, unbranched, septate, guttulate, narrowing and tapering towards the apex, with apex blunt. *Asci* 138–180 × 15–28.5 µm (\bar{x} = 159 × 21.5 µm, n = 20), 8-spored, unitunicate, clavate, short pedicellate, apically rounded, with J- subapical ring, early deliquescing. *Ascospores* 45–52 × 10–14 µm (\bar{x} = 48.5 × 12 µm, n = 30), overlapping bi-seriate, allantoid, aseptate, hyaline to brown, with one to few small guttules, slightly to moderately curved and smooth-walled. **Asexual morph:** Undetermined.

Culture characteristics – Colonies on PDA reaching 8 cm diam. after 2 weeks at 20–25°C, medium dense, irregular, slightly raised, surface smooth with undulate edge, cottony, colony from

above: white at the margin, yellowish white at the centre; from below: yellowish white at the margin, yellow to pale brown at the centre; mycelium greenish-grey, not produced pigmentation on PDA media.

Material examined – THAILAND, Phetchaburi Province, Hat Chao Samran, 47° 72506' E, 40° 25038' N, 0 m asl., on intertidal decayed wood of *Avicennia* sp. at a mangrove stand, 28 August 2015, M. Dayarathne, CHAM020 (MFLU 16-1185, **holotype**), ex-type living culture, MFLUCC15-0953, ICMP.

Notes – *Halodiatrype avicenniae* can be distinguished from *H. salinicola* in having larger ascospores (45–52 × 10–14 µm vs 7.5–8.5 × 2.5–3 µm), which are hyaline to light brown. Morphologically, *H. avicenniae* is similar to *Cryptosphaeria mangrovei* in its ascomata, asci, ascospore shape and colour (Hyde 1993), but *C. mangrovei* differs in having 1–4 septate ascospores, while they are aseptate in *H. avicenniae*. However, there is no molecular data to compare the phylogenetic affinities of *H. mangrovei* with *H. avicenniae*.

Halodiatrype mangrovei (K.D. Hyde) Dayarathne & K.D. Hyde, **comb. nov.**

≡ *Cryptosphaeria mangrovei* K.D. Hyde, Trans. Mycol. Soc. Japan 34(3): 311 (1993)

Description – see Hyde (1993)

Notes – *Cryptosphaeria mangrovei* was introduced by Hyde (1993) and is characterized by clavate, extremely thin-walled, slightly truncate asci, with an indistinct apical thickening, lacks an apical ring, and allantoid or subinequilateral, one-cell with guttules ascospores. This species is similar to species of *Halodiatrype* by having allantoid or subinequilateral, light brown ascospores and was collected from intertidal mangrove on dead roots of *Rhizophora apiculata* (*Rhizophoraceae*). Therefore, we transfer the species to the genus *Halodiatrype* as *H. mangrovei*.

Key to *Halodiatrype* species

- 1. Ascospores aseptate*H. avicenniae*
- 1. Ascospores septate2
- 2. Ascomata 125–140 × 105–115 µm*H. salinicola*
- 2. Ascomata 480–920 × 630–970 µm *H. Mangrovei*

Discussion

Species of *Diatrypaceae* (Xylariales) are present in various ecosystems growing on woody plants (Carter 1957, 1991, Bolay & Moller 1977, Moller & Kasimatis 1978, Munkvold & Marois 1994). They are decomposers of dead wood worldwide (Kirk et al. 2008). The diatrypaceous species occurring in marine habitats has remained largely unexplored (Jones et al. 2015). Diatrypaceous taxa occurring in marine habitats included *Cryptosphaeria mangrovei* K.D. Hyde (Hyde 1993), *C. eunoma* (Fr.) Fuckel, Jb. Nassau. Ver. Naturk. (Jones et al. 2015), *Eutypa bathurstensis* K.D. Hyde & Rappaz (Hyde & Rappaz 1993), *Eutypella naqsii* K.D. Hyde (Hyde 1995), *Pedumispora rhizophorae* K.D. Hyde & E.B.G. Jones (Jones et al. 2015) and *Diatrypasimilis australiensis* J.J. Zhou & Kohlm. (Chalkley et al. 2010, Abdel-Wahab et al. 2014, Jones et al. 2015) (Table 3). These species share features with taxa in *Diatrypaceae*, especially the formation of crustose stroma-like structures on the host surface, asci with a long pedicel and an apical ring and allantoid ascospores (Klaysuban et al. 2014). However, none of these species (with the exception of *Diatrypasimilis australiensis* J.J. Zhou et Kohlm and *Pedumispora rhizophorae* K.D. Hyde & E.B.G. Jones) have been analyzed phylogenetically to confirm their taxonomic placement within *Diatrypaceae* (Chalkley et al. 2010, Abdel-Wahab et al. 2014, Klaysuban et al. 2014).

The genus *Diatrype* is far from being morphologically conclusively defined, according to the molecular data of Trouillas et al. (2011). Phylogenetic hypotheses based on molecular sequence data of species of *Diatrypaceae* are provided mainly by Acero et al. (2004), Trouillas et al. (2010, 2011) and Dorge et al. (2013). It will be interesting to establish if clades within the family comprising distinct sets of characters or are host specific that will require their own generic status,

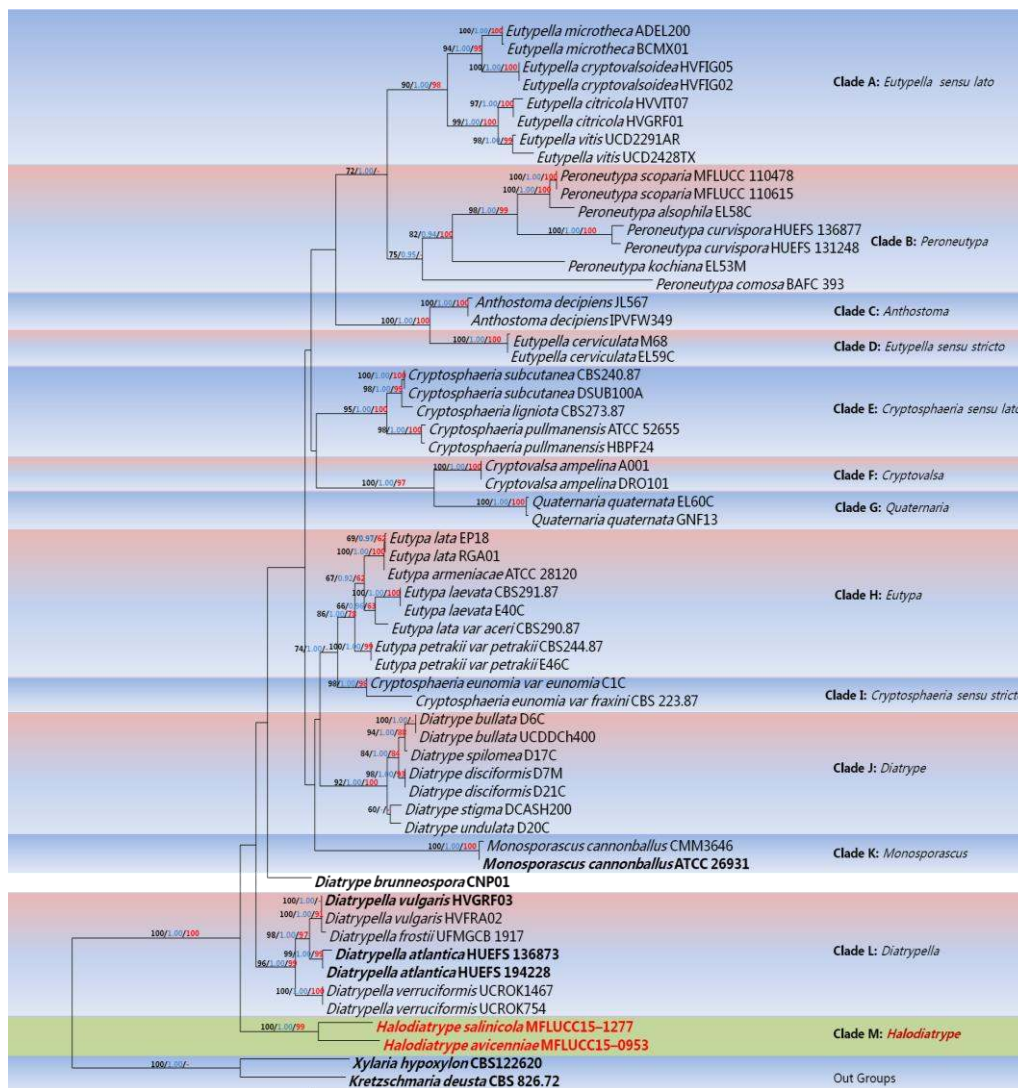


Fig. 1 – Best scoring RAxML tree based on a combined dataset of ITS and β -TUB sequence data. Bootstrap support values for maximum likelihood (ML, black) and maximum parsimony (MP, red) equal or greater than 60% and Bayesian posterior probabilities (PP, blue) equal to or greater than 0.90 are shown above the nodes. The tree is rooted to *Xylaria hypoxylon* (CBS 121680) and *Kretzschmaria deusta* (CBS 826.72). All sequences from ex-type strains are in bold.

as more collections are sequenced. According to our studies the genera *Cryptosphaeria*, *Eutypella* and *Diatrype* are polyphyletic within the family. Most of *Cryptosphaeria* and *Eutypella* species clustered distantly from their type species, hence there is a need to revise that genera further with more fresh collections. We defined *Cryptosphaeria sensu stricto* and *Eutypella sensu stricto* based on the sequence data published from non-type strains of the type species since no molecular data available for any ex-type strains of *C. millepunctata* (Syn. *C. eunomia*) and *E. cerviculata*. However, fresh collections and epitypes need to be designated (*sensu* Ariyawansa et al. 2014) to further confirm *Cryptosphaeria sensu stricto* and *Eutypella sensu stricto*. There is also a possibility of renaming *Cryptosphaeria sensu lato* and *Eutypella sensu lato* as separate novel genera. The type species of the genus *Diatrype*, *D. disciformis* did not cluster with our new taxa even if though they share some morphological features, such as clavate asci and allantoid ascospores. The well-developed, multi-perithecial, discoid stromata found in *D. disciformis* (Maharachchikumbura et al. 2015), versus the lack of stromata, place them as distantly related genera. *Diatrype brunneospora* shows some morphological resemblance to our novel taxa in ascospore shape and colour, but it differs in ascospore size and septation. However, this species clusters away from the other *Diatrype* species and it is necessary to reconsider its placement with multiple genes studies.



Fig. 2 – *Halodiatrype salinicola* (MFLU 15-0179, **holotype**). **a.** Appearance of ascomata opening on host. **b.** Transverse section of ascoma on the host. **c.** Vertical section through ascoma. **d.** Peridium. **e.** Section through neck with periphyses. Note the yellow colouration. **f, g** Asci. **h.** Asci stained with congo red. **i.** Paraphyses. **j–m.** Ascospores. **n.** Germinating spore. **o, p.** Culture on MEA (**o** = from above, **p** = from below). Scale bars: **c** = 100 μm , **d** = 50 μm , **e** = 20 μm , **f–i** = 10 μm , **j–n** = 5 μm .

Depending on the stromatal morphology, and the nature of asci and ascospores described by Vasilyeva & Stephenson (2006), our new strains mostly resembled the type of *Cryptosphaeria*; *Cryptosphaeria eunomia*. The novel taxa morphologically best fit *Cryptosphaeria*. However, phylogenetic analyses of combined ITS and β -TUB sequence data, indicate that the novel taxa has no affinity with *Cryptosphaeria* species including; *C. eunomia*, *C. ligniota*, *C. pullmanensis* and *C. subcutanea*.

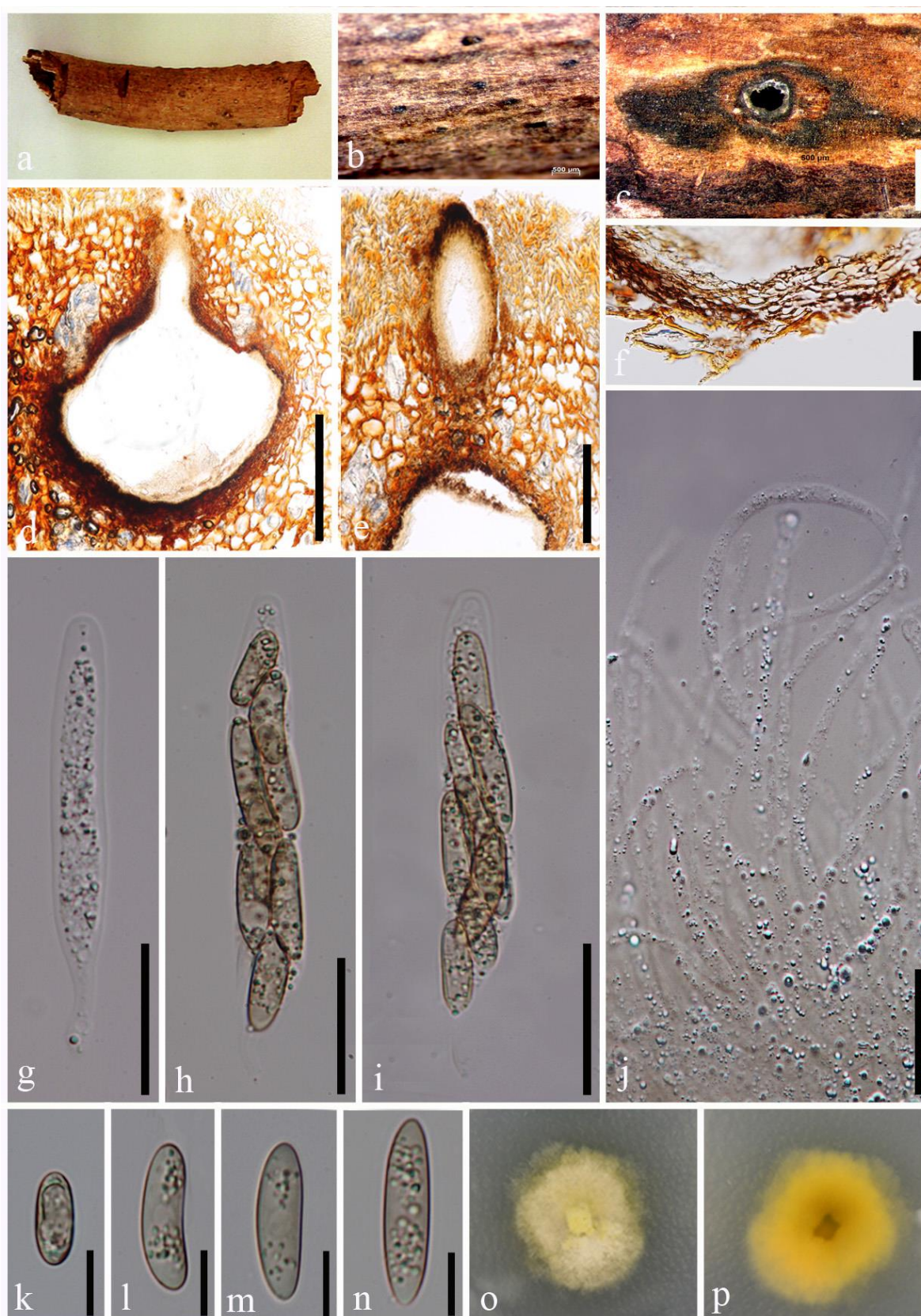


Fig. 3 – *Halodiatrype avicenniae* (MFLU 16-1185, **holotype**) **a.** Host specimens. **b.** Appearance of ascomata on host surface. **c.** Transverse section of ascoma in the darkened pseudostroma. **d.** Vertical section through ascoma. **e.** Section through neck. **f.** Peridium. **g–i.** Asci. **j.** Paraphyses. **k–m.** Ascospores. **n, o.** Culture on PDA (**n** = from above, **o** = from below) Scale bars: c, d, f–i = 50 μ m, e, j–m = 10 μ m.

Table 2 Synopsis of the characteristics of species belongs to the new genus *Halodiatrype*.

Species	Ascomata size (µm)	Asci (µm)	Ascospores			Host	Locality	Literature
			Size (µm)	Colour	No. of septa			
<i>Halodiatrype avicenniae</i>	130–240	138–180 × 15–28.5	45–52 × 10–14	hyaline to brown	0	<i>Avicennia</i> sp.	Thailand	In this paper
<i>Halodiatrype salinicola</i>	125–140 × 105–115	40–50 × 7.5–10.5	7.5–8.5 × 2.5–3	dark brown	1	Mangrove sp.	Thailand	In this paper
<i>Halodiatrype mangrovei</i>	480–920 × 630–970	76–95 × 10–15	7.5–15.5 × 1.8–4.0	yellowish brown	1–4	<i>Rhizophora apiculata</i>	Thailand	Hyde (1993)

Table 3 Synopsis of the characteristics of other marine species in *Diatrypaceae*

Species	Ascostromata size (µm)	Asci (µm)	Ascospores			Host	Locality	Literature
			Size (µm)	Colour	Shape & No. of septa			
<i>Eutypella naqsii</i>	650–840 × 520–650	28–50 × 3.6–6.4	6.4–8 × 1.2–1.6	Hyaline to pale-yellow	Allantoid, aseptate	<i>Avicennia</i> sp.	Queensland	Hyde (1995)
<i>Eutypa bathurstensis</i>	300–600 × 800	30–50 × 8–10.1	6–12 × 2–2.8	Olive-brown	Allantoid, aseptate	<i>Avicennia</i> sp.	Queensland	Hyde & Rappaz (1993)
<i>Pedumispora rhizophorae</i>	100–125 × 488–500	202–255 × 17–33	153–213 × 5	Slightly brown	Filiform, (7–)12–13	<i>Rhizophora mucronata</i>	Central Thailand	Klaysuban et al. (2014)
<i>Diatrypasimilis australiensis</i>	1500 diam.	-	11–15 × 3.5–6	Dark brown	Ellipsoidal, aseptate	<i>Rhizophora</i> sp.	Queensland	Chalkley et al. (2010)

In our phylogenetic analyses two novel taxa grouped together as separate lineages. *Cryptosphaeria mangrovei* introduced by Hyde (1993) is most similar to *H. avicenniae* in ascomata, asci, ascospore shape and colour and therefore we transfer this species to *Halodiatrype mangrovei*. However, future studies should confirm the placement of this species within our new genus with molecular data from several collections, as currently we depend only on the morphologies. Further studies on *Diatrypaceae* are needed because of the taxonomic confusion resulting from the difficulty in separating the various genera by morphological characteristics and the limited availability of sequence data of types or authenticated specimens and lack of reference cultures in collections (Trouillas et al. 2011, Mehrabi et al. 2015).

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