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Delonicicola siamense gen. & sp. nov. (*Delonicicolaceae* fam. nov., Delonicicolales ord. nov.), a saprobic species from *Delonix regia* seed pods

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Abstract – This paper introduces a new genus *Delonicicola*, to accommodate *D. siamense* sp. nov., which was found associated with *Delonix regia* seed pods, collected in Chiang Rai Province, Thailand. ITS sequence data confirmed a close relationship of *Delonicicola* with *Liberomyces* and *Asteromella* in Xylariomycetidae. Phylogenetic and molecular clock analyses of combined LSU, SSU and RPB2 sequence data provide evidence for a new family *Delonicicolaceae* and a new order Delonicicolales in Xylariomycetidae. Members of *Delonicicolaceae* are saprobes, endophytes or pathogens of angiosperms and it is characterized by pseudostromatal immersed, papillate ascomata, short pedicellate asci with a simple apex and 1-septate, hyaline ascospores. The asexual morph is coelomycetous with pycnidial conidiomata and allantoid, filiform or bacilloid, hyaline conidia.

morphology / phylogeny / seed/fruit fungi / Sordariomycetes / Xylariomycetidae

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INTRODUCTION

Liberomyces Pažoutová, M. Kolařík & Kubátová was introduced by Pažoutová *et al.* (2012) to accommodate *L. macrosporus* Pažoutová, M. Kolařík & Kubátová and *L. saliciphilus* Pažoutová, M. Kolařík & Kubátová. The genus was placed in Xylariales genera *incertae sedis* by Pažoutová *et al.* (2012), and this was followed by Maharachchikumbura *et al.* (2015, 2016). *Liberomyces* species were identified as endophytic and saprotrophic in the sapwood and phloem of broadleaf trees (*Alnus glutinosa*, *Betula pendula*, *Quercus robur*, *Salix alba*, *Ulmus laevis*). ITS sequence data from a coelomycetous leaf pathogen, *Asteromella pistaciarum*, clustered with the *Liberomyces* clade (Pažoutová *et al.* 2012). *Asteromella* was previously placed in *Mycosphaerellaceae* in Capnodiales by Sutton & Cole (1983) and Ramaley (1991). Currently *Asteromella* is referred to Dothideomycetes genera, *incertae sedis* within Ascomycota (Ruszkiewicz-Michalska 2016). Pažoutová *et al.* (2012) acknowledged the need of future studies to synonymize *A. pistaciarum* under *Liberomyces.* However ex-type sequence data for *A. pistaciarum* was not available in GenBank.

A number of fungi have been collected from *Delonix regia* seed pods (Somrithipol *et al.* 2002; Sahu *et al.* 2003). Among them, one new taxon, *Cirrenalia nigrospora*, was described by Somrithipol *et al.* (2002). We are studying seed pod fungi associated with *D. regia* in Thailand, and in this paper, we introduce a new genus and species (also family and order nov.) from *D. regia* seed pods.

MATERIALS AND METHODS

Sample collection, specimen examination and isolation

The specimen was collected from Thailand during 2015 and macroscopic and microscopic characters were observed in the laboratory. The ascomatal surface was observed using a Motic dissecting microscope (SMZ 168). Free hand sections of fruiting structures with asci, ascospores and internal tissues were mounted in water and Melzer's reagent for morphological study. Photomicrography was carried out using a Canon 450D digital camera fitted to the Nikon ECLIPSE 80i compound microscope. Measurements were made with the Tarosoft (R) Image Frame Work software. The images used for illustrating the fungus were processed with Adobe Photoshop CS5 v. 12.0 software (Adobe Systems, USA). Single spore isolates were obtained as described in Chomnunti *et al.* (2014). Colonies were sub-cultured onto Malt Extract Agar media (MEA) and incubated at 25°C.

Herbarium material was deposited in the Mae Fah Luang University herbarium, Chiang Rai, Thailand (MFLU). Living cultures were deposited in the Culture Collection at Mae Fah Luang University (MFLUCC) and Kunming Institute of Botany (KUNCC) Heilongtan, Kunming, China. *Facesoffungi* and Index Fungorum numbers were registered as explained in Jayasiri *et al.* (2015) and Index Fungorum (2017).

DNA isolation, amplification and analyses. Total genomic DNA was extracted from fungal colonies growing on MEA at 25°C using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China) according to the manufacturer's protocol. Partial gene sequences were determined for the 28S large subunit nrDNA (LSU), the internal transcribed spacer (ITS) and RNA polymerase II gene (RPB2) using the primers and PCR conditions listed in Table 1. PCR was performed in a 25 µl reaction volume containing, 12.5 µl 2 × PCR Master Mix (TIANGEN Co., China), 9.5 µl ddH_2O, 5-10 ng DNA and 1 µl of each primer

(10 μM). PCR products stained with ethidium bromide were viewed on 1% agarose gels. Purification and sequencing of PCR products were conducted by Shanghai Sangon Biological Engineering Technology & Services Co., China. Both directions of the PCR products were sequenced using the same primer pairs as used in PCR amplification, to ensure the integrity of the sequences. A consensus sequence for each gene region was assembled in ContigExpress (Vector NTI Suite 6.0). The sequence generated in this study were supplementary with the additional sequences obtained from GenBank (Table 2). The sequence data were aligned online with the MAFFT server (http://mafft.cbrc.jp/alignment/server/) and manually adjust using MEGA6 v. 6.0 where necessary (Tamura *et al.* 2011). Two different datasets were used to estimate two phylogenies. A combined dataset (LSU, SSU and RPB2) was used to infer the phylogenetic position of the new taxon *Delonicicola* in Xylariomycetidae. The ITS data set was used to infer the phylogenetic relationship of *Delonicicola* species and closely related taxa based on Blast results.

Phylogenetic analyses were based on Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP) methods. A maximum likelihood analysis was performed using RAxML GUI v. 1.3 (Silvestro & Michalak 2011). The optimal ML tree search was conducted with 1000 separate runs, 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 with the GTRGAMMA nucleotide substitution model.

MP analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) v.4.0b10 (Swofford 2002). The trees were inferred using the heuristic search option with 1000 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. Ambiguously aligned regions were excluded from all analyses and gaps were treated as missing data. Maxtrees were setup to 5 000, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1 000 replicates, each with 100 replicates of random stepwise addition of taxa (Hillis & Bull 1993). Tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC], homoplasy index [HI], and log likelihood [-ln L] (HKY model) values were calculated. The robustness of the equally most parsimonious trees was evaluated by 1 000 bootstrap replications (Felsenstein 1985) resulting from a maximum parsimony analysis, each with 10 replicates of random stepwise addition of taxa. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed to determine whether the trees inferred under different optimality criteria were significantly different.

Table 1. Information on loci and PCR protocols used in the study

Locus	*Primers (Reference)*	*PCR conditions*
ITS	ITS5, ITS4 (White *et al*., 1990)	[a]94°C: 30 s, 48–52°C: 30 s, 72°C: 1.30 min. (37 cycles) [b]
LSU	LR0R (Rehner & Samuels 1994), LR5 (Vilgalys & Hester 1990)	[a]94°C: 30 s, 48°C: 30 s, 72°C: 1.30 min. (35 cycles) [b]
SSU	NS1, NS4 (White *et al*., 1990)	[a]94°C: 30 s, 48°C: 30 s, 72°C: 1.30 min. (37 cycles) [b]
RPB2	fRPB25f fRPB27CR (Liu *et al*., 1999)	[a]95°C: 30 s, 55–56°C: 1 min., 72°C: 1.30 min. (35 cycles) [b]

[a]Initiation step of 94°C: 5 min.
[b]Final elongation step of 72°C: 7 min. and final hold at 4°C applied to all PCR thermal cycles.

Table 2. GenBank accession numbers of the isolates used in this study

Species	*Isolate no./Culture Collection no.*	*GenBank Accession No.*			
		LSU	*SSU*	*RPB2*	*ITS*
Adisciso yakushimense	KT1907	AB593721	AB593700	–	–
Ambrosiella xylebori	AFTOL-ID 1285	DQ470979	DQ471031	DQ470931	–
Amphibambusa bambusicola	MFLUCC 11-0617	KP744474	–	–	–
Amphiporthe castanea	CBS 392.93	AF277128	AF277117	DQ368644	–
Amphisphaeria sorbi	MFLUCC 13-072	KP744475	–	–	–
Amphisphaeria umbrina	HKUCC 994	AF452029	AY083811	–	–
A. umbrina	AFTOL-ID 1229	FJ176863	FJ176809	FJ238348	–
Annulohypoxylon cohaerens	CBS 114744	–	–	–	AY616688
Apiospora bambusae	ICMP 6889	DQ368630	DQ368662	DQ368649	–
Ap. montagnei	AFTOL-ID 951	DQ471018	–	DQ470921	–
Ap. setosa	ICMP 4207	DQ368631	DQ368661	–	–
Arecophila bambusae	HKUCC 4794	AF452038	AY083802	–	–
Arthrinium hydei	CBS 114990	KF144936	–	–	–
Asteromella pistaciarum	ISPaVe2106	–	–	–	FR681905
Bartalinia robillardoides	CBS 122705	KJ710438	–	–	–
Beltrania pseudorhombica	CPC 23656 = CBS 138003	KJ869215	–	–	–
Beltraniella endiandrae	CPC 22193	KJ869185	–	–	–
Beltraniopsis neolitseae	CPC 22168 = CBS 137974	KJ869183	–	–	–
Biscogniauxia nummularia	MUCL 51395	KT281894	–	–	–
Bombardia bombarda	SMH3391 = AFTOL-ID 967	DQ470970	DQ471021	DQ470923	–
Botryotinia fuckeliana	AFTOL-ID 59	AY544651	AY544732	DQ247786	–
Broomella vitalbae	MFLUCC 15-0023	KP757751	KP757759	–	–
Cainia graminis	CBS 136.62	AF431949	AF431948	–	–
Camarops microspora	CBS 649.92	AY083821	AY083800	DQ470937	–
Camarops ustulinoides	AFTOL-ID 72	AY346267	FJ190588	DQ470882	–
Ceratocystis fimbriata	TCH-C89 = CBS 374.83	AF221009	U32418	DQ368641	–
Cercophora caudata	CBS 606.72	AY999113	DQ368659	DQ368646	–
Chaetomium elatum	IFO 6554	DQ368628	M83257	AF107791	–
Ciferriascosea fluctamurum	MFLUCC 15-0541	KR092778	–	–	–
C. rectamurum	MFLUCC 15-0542	KR092776	–	–	–
Ciliochorella mangiferae	MFLUCC12-0310 = TCL067	KF827445	KF827446	KF827479	–
Coniochaeta discoidea	SANK12878	AY346297		AY780191	–
Co. velutina	IFO 9439 = 93.262 = MA3370	AF353594	AJ496244	DQ631959	–
Cordyceps militaris	NRRL 28021 = OSU 93623	AF327374	AF049146	AY545732	–
Cordyceps cardinalis	OSC 93609	AY184962	NG013131	–	–
Cordyceps irangiensis	OSC 128578	DQ518770	DQ522556	DQ522445	–

Species	*Isolate no./Culture Collection no.*	*GenBank Accession No.*			
		LSU	*SSU*	*RPB2*	*ITS*
Corollospora maritima	CBS 264.59	AF491260	U46871	DQ368632	–
Cordyceps militaris	OSC 93623	AY184966	AY184977	–	–
Creosphaeria sassafras	CM AT 018	DQ840056	–	–	–
Cr. sassafras	ANM 1978	JN673042	–	–	–
Cryphonectria parasitica	SA713 = CP155 = CMW13749	AF277132	AF277116	AY485619	–
Cryptodiaporthe corni	ATCC 66834 = CBS 245.90	AF408343	AF277119	AF277149	–
Delonicicola siamense	MFLUCC 15-0670	MF158345	MF158347	MF158346	MF167586
Diaporthe eres	AR 3519	AF362565	–	–	–
Diaporthe phaseolorum	FAU458 = NRRL13736 = CBS 435.87	U47830	L36985	AY641036	–
Diatrype disciformis	AFTOL ID-927	DQ470964	DQ471012	DQ470915	–
Diatrype palmicola	MFLUCC 11-0020	KP744482	KP753950	–	–
D. whitmanensis	ATCC MYA-4417	FJ430587	–	–	–
Discosia aff. Artocreas	MAFF 242776 = KT 2010	AB593715	AB593699	–	–
Discula destructiva	MD233 = ATCC 76230	AF362568	AF429718	AF277147	–
Endophyte	XL-A23	–	–	–	EF488447
Endophyte	E8520c	–	–	–	HQ117861
Endophyte	VegaE4-79	–	–	–	EU009996
Endophyte	VegaE4-79	–	–	–	FJ025239
Eutypa lata	AFTOL-ID 929	DQ836903	DQ836896	DQ836889	–
Faurelina elongata	CBS 126.78	DQ368625	DQ368657	DQ368639	–
Gnomonia ribicola	CBS 115443	DQ368626	DQ368658	DQ368642	–
Gnomonia virginianae	CBS 121913 = AR 4077	EU255105	–	–	EU219309
Graphostroma platystoma	AFTOL-ID 1249	DQ836906	–	–	–
Halosarpheia fibrosa	AFTOL-ID 786 = JK 5132C = JK 5166A	U46886	AF352078	KT225543	–
Hyalotiella spartii	MFLUCC 13-0397	KP757752	KP757760	–	–
Hydropisphaera erubescens	ATCC 36093	AY545726	AY545722	AY545731	–
Hypocrea pallida	GJS89-83	U00740	AF281672	AY015636	–
H. schweinitzii	CBS 243.63	U47833	L36986	DQ368635	–
Hypomyces polyporinus	CBS 168.89	AF543793	U32410	DQ368636	–
Hypoxylon macrocarpum	agtS139	–	–	–	AY616705
Idriella lunata	CBS 204.56	KP858981	–	–	–
Iodosphaeria tongrenensis	QL-2015 = MFLU 15-0393	KR095283	KR095284	–	–
Kretzschmaria deusta	CBS 163.93	KT281896	–	–	–
Lasiosphaeria ovina	CBS 958.72	AF064643	AY083799	AY600292	–
Leotia lubrica	AFTOL-ID 1	AY544644	AY544746	DQ470876	–
Lepteutypa cupressi	IMI 052255	AF382379	AY083813	–	–
Liberomyces macrosporus	CCF4028 = AK242/05	FR715522	FR715503	FR715509	FR715522
L. saliciphilus	H041	FR715510	FR715500	FR715507	FR715510

Table 2. GenBank accession numbers of the isolates used in this study *(continued)*

Species	*Isolate no./Culture Collection no.*	*GenBank Accession No.*			
		LSU	*SSU*	*RPB2*	*ITS*
L. saliciphilus	AK8/09	–	–	–	FR715513
L. saliciphilus	CCF4020	–	–	–	FR715515
L. saliciphilus	CCF4021	–	–	–	FR715519
L. saliciphilus	CCF4022	–	–	–	FR715516
L. saliciphilus	CCF4023	–	–	–	FR715521
L. saliciphilus	CCF4025	–	–	–	FR715514
L. saliciphilus	CCF4026	–	–	–	FR715518
L. saliciphilus	CCF4027	–	–	–	FR715517
L. saliciphilus	H077	–	FR715501	FR715508	FR715511
L. saliciphilus	H133	–	–	–	FR715512
Lindra thalassiae	JK 4322	AF195633	AF195632	DQ470897	–
Lopadostoma turgidum	LT2	KC774618	–	KC774563	–
Lulworthia grandispora	JK 4686	DQ522856	DQ522855	DQ518181	–
Melanospora zamiae	ATCC 12340 = CBS 421.87	U17405	AY046578	DQ368634	–
Melogramma campylosporum	MBU	JF440978	–	–	–
Menispora tortuosa	AFTOL-ID 278	AY544682	AY544747	DQ836884	–
Microascus trigonosporus	RSA1942 = IFO 3222	U47835	L36987	AF107792	–
Microdochium phragmitis	CBS 423.78	KP858948	–	KP859121	–
M. trichocladiopsis	CBS 623.77	KP858934	–	KP859107	–
Nectria cinnabarina	CBS 125165 = AR 4477 = CLL 7152	HM484562	–	KM232402	–
Nimbospora effusa	JK 5104A = AFTOL-ID 761	U46892	U46877	DQ836887	–
Obolarina dryophila	8401 = H86	GQ428313	Z49784	FR715505	–
Ophiocordyceps halabalaensis	BCC 21491 = MY1308	–	KM655825	–	–
Ophiocordyceps gracilioides	TSJ935	KJ130992	–	–	–
Ophiocordyceps melolonthae	TSJ679	KJ130990	–	–	–
Ophiocordyceps sinensis	YN09-6	JX968033	JX968028	JX968013	–
Ophiostoma piliferum	CBS 129.32 = CBS 158.74	AY281094	AJ243295	–	–
O. stenoceras	AFTOL-ID 1038	DQ836904	DQ836897	DQ836891	–
O. ulmi	ATCC 32437 = CBS 298.87	DQ368627	M83261	DQ368645	–
Oxydothis garethjonesii	MFLUCC 15-0287	KY206762	KY206768	–	–
O. metroxylonicola	MFLUCC 15-0281	KY206763	KY206769	KY206781	–
O. metroxylonis	MFLUCC 15-0283	KY206764	KY206770	–	–
O. palmicola	MFLUCC 15-0806	KY206765	KY206771	KY206782	–
Parapleurotheciopsis inaequiseptata	MUCL 41089 = INIFAT C98 = 30-1	EU040235	–	–	–
Pestalotiopsis adusta	CGMCC 3.9103	JN940828	JN940796	–	–
Pseudopestalotiopsis theae	SAJ 0021	JN940838	JN940785	–	–

Species	*Isolate no./Culture Collection no.*	*GenBank Accession No.*			
		LSU	*SSU*	*RPB2*	*ITS*
Petriella setifera	ATCC 26490 = CBS 110344	U48421	U43908	DQ368640	–
Phlogicylindrium eucalyptorum	CBS 111680	KF251707	–	KF252209	–
Ph. eucalyptorum	CBS 111689	KF251708	–	KF252210	–
Ph. uniforme	CBS 131312	JQ044445	–	–	–
Plagiostoma euphorbiae	CBS 340.78	AF277131	AF277114	DQ368643	–
Pleospora herbarum	ATCC11681 = CBS 191.86 = EGS04-188C	AF382386	AF229513	AF107804	–
Podosordaria tulasnei	CBS 128.80	KT281897	–	–	–
Robillarda africana	CBS 122.75	KR873281	–	–	–
R. roystoneae	CBS 115445	KR873282	–	–	–
R. sessilis	CBS 101440	KR873283	–	–	–
R. sessilis	CBS 114312	KR873284	–	–	–
R. terrae	CBS 587.71	KJ710459	–	–	–
Sarcostroma restionis	CBS 118154	DQ278924	–	–	–
Seimatosporium cornii	MFLUCC 14-0467	KR559739	KR559741	–	–
S. tosta	HKUCC 1004	AF382380	AY083814	–	–
Seiridium phylicae	CPC 19962	KC005807	–	–	–
Selenodriella cubensis	CBS 683.96	KP858990	–	–	–
Se. fertilis	CBS 772.83	KP858992	–	–	–
Seynesia erumpensnew	AFTOL-ID 392 = SMH 1291	AF279410	AF279409	AY641073	–
Sordaria fimicola	CBS 723.96	AF132330	X69851	DQ368647	–
So. macrospora	ATCC 36709 = Buck s.n.	AY346301	AY641007	AY780195	–
Subramaniomyces fusisaprophyticus	CBS 418.95 = INIFAT C94 = 134	EU040241	–	–	–
Trichoderma viride	GJS89-127	AY489726	–	–	–
Truncatella spartii	MFLUCC 15-0573	KR092782	KR092783	–	–
Tolypocladium capitatum	OSC 71233	AY489721	AY489689	–	–
Tolypocladium japonicum	OSC 110991	DQ518761	DQ522547	DQ522428	–
Torrubiella wallacei	CBS 101237	AY184967	AY184978	EF469119	–
Uncultured	Singleton_50-2942_0537	–	–	–	FJ762875
Uncultured	OTU#644-28-3825_3534	–	–	–	GQ508897
Varicosporina ramulosa	AFTOL-ID 794 = RVG-113	VRU44092	VRU43846	DQ836888	–
Vialaea minutella	BRIP 56959	KC181924	–	–	–
V. mangiferae	MFLUCC 12-0808 = ICS-2013	KF724975	–	–	–
Xylaria hypoxylon	ATCC 42768	AY327480	–	–	–
Zetiasplozna acaciae	CBS 137994 = CPC 23421	KJ869206	–	–	–

* Ex-type strains are bold.

Bayesian analysis was performed using MrBayes v. 3.2.0 (Huelsenbeck & Ronquist 2001). The best-fit evolutionary models for phylogenetic analyses were selected independently for each gene region using MrModeltest v. 2.2 (Nylander 2004). GTR + I + G model was selected for LSU and RPB2 genes and SYM + I + G was selected for SSU gene, and incorporated into the analysis. Two parallel analyses of each consisting of six Markov Chain Monte Carlo (MCMC) chains, run from random trees for 5 000 000 generations were sampled every 100 generations resulting in 20 000 total trees. The first 8 000 trees, representing the burn in phase of the analyses were discarded from each run. For the single gene analysis of ITS gene, SYM + G was selected as the best fit evolutionary model. Two parallel analyses of each consisting of six Markov Chain Monte Carlo (MCMC) chains, run from random trees for 3 000 000 generations were sampled every 100 generations resulting in 20 000 total trees. The first 10 000 trees, representing the burn in phase of the analyses were discarded from each run. The remaining trees were used to calculate posterior probabilities (PP) in the majority rule consensus tree.

Molecular clock analysis

Molecular clock analysis was carried out using two calibrating points consisting of fossil and secondary calibrating points. *Paleoophiocordyceps coccophagus* Sung *et al.* a fossil from the class Sordariomycetes (Hypocreomycetidae, Hypocreales) was selected for calibrating the tree (Sung *et al.* 2008; Samarakoon *et al.* 2016; Hongsanan *et al.* 2017, Hyde *et al.* 2017). This fossil data was used for the calibration of the node of the *Ophiocordyceps* crown (Exponential distribution, offset 100, mean 27.5, with 95% credibility interval of 182.4 Mya). The mean divergence age of the Sordariomycetes crown was used as the secondary calibrating point following previous studies (Prieto & Wedin 2013; Beimforde *et al.* 2014; Pérez-Ortega *et al.* 2016) (Normal distribution, mean 250 Mya, 45 Mya SD, with 95% credibility interval of 338 Mya).

Molecular dating analysis was performed using the BEAST v1.8.0 (Drummond *et al.* 2012). BEAUti v1.8.0 (BEAST package) was used to obtain the XML file including the partitioned alignment of LSU, SSU and RPB2 using the GTR (LSU, RPB2: GTR + I + G) and SYM (SSU: SYM + I + G) were selected as substitution models. BEAST analyses were performed for 100 000 000 generations, logging parameters and trees were obtained for every 5,000 generations. Effective sample sizes (ESS) of parameters were checked using Tracer v.1.6 (ESS > 200). A burn-in of first 10% trees was removed from each analysis based on the ESS values using Tracer v1.6 (Rambaut *et al.* 2013). The remaining trees were used to generate a maximum clade credibility tree by using Logcombiner v1.8.0 and TreeAnnotator v1.8.0. The resulting trees were viewed with FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/) and edited with Microsoft PowerPoint 2016. The most recent International Chronostratigraphic Chart (v2017/02) from the International Commission on Stratigraphy (IUGS), available from www.stratgraphy.org, was referred to indicate the ages for geological time periods at the base of the evolution tree (Mapook *et al.* 2016).

RESULTS

Phylogenetic analyses

Analysis of combined LSU, SSU and RPB2 sequence data was carried out to clarify the phylogenetic placement of our strain within *Sordariomycetes* (Table 2).

The combined LSU, SSU and RPB2 dataset consisted of 124 isolates including three outgroup taxa (*Botryotinia fuckeliana*, *Leotia lubrica* and *Pleospora herbarum*). The aligned dataset comprised 2974 characters including gaps (LSU: 1-853, SSU: 854-1859 and RPB2: 1860-2974), with 1329 parsimony-informative characters. The parsimony analysis resulted in 108 equally most parsimonious trees (tree length = 11733 steps, CI = 0.254, RI = 0.541, RC = 0.137, HI = 0.746). Bayesian inference and maximum parsimony analyses yielded trees with similar topologies to support the same terminal clades as obtained from maximum likelihood (ML) analysis. The ML tree generated from RAxML is illustrated in Fig 1. In the phylogenetic analysis, our isolate from *Delonicicola* clustered with *Liberomyces saliciphilus*, *L. macrosporus*, the coelomycetous pathogen *Asteromella pistaciarum*, endophytes from various angiosperms and an uncultured taxon from *Quercus*. The whole clade is basal to other known families or orders of Xylariomycetidae with good support (89% ML/1.00 PP), and introduced here as Delonicicolaes ord. nov. and *Delonicicolaceae* fam. nov. Two different datasets were used to estimate two phylogenies. A combined dataset (LSU, SSU and RPB2) was used to infer the position of *Delonicicola* in Xylariomycetidae. An ITS data set was used to infer the phylogenetic relationship among *Delonicicola* species and related taxa within *Delonicicolaceae*.

Another phylogenetic analysis was performed with ITS sequences of *Delonicicola* and closely related taxa with two outgroup taxa (*Annulohypoxylon cohaeren* and *Hypoxylon macrocarpum*) (Fig. 2). The ITS dataset consisted of 22 taxa, 581 characters (including gaps) with 162 parsimony informative sites. The parsimony analysis resulted in 108 equally most parsimonious trees (tree length = 443 steps, CI = 0.797, RI = 0.816, RC = 0.650, HI = 0.203). The Bayesian inference and maximum parsimony analyses yielded trees similar to the topology of the tree obtained from maximum likelihood analysis. The ML tree generated from RAxML is illustrated in Fig 2.

Divergence time estimation

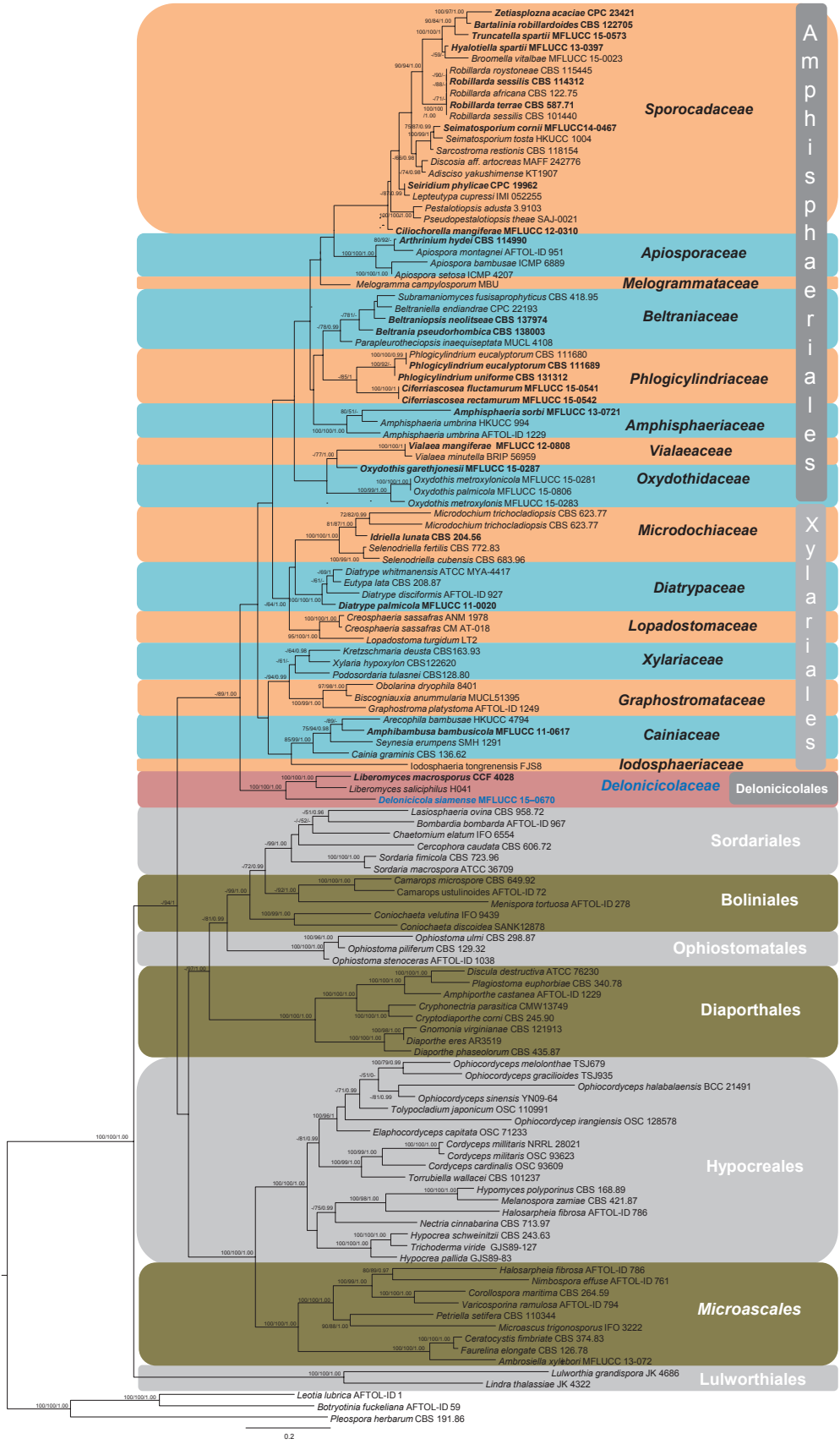
The class Sordariomycetes diverged around 343 Mya (257-436), while the crown age is around 306 Mya (247-371) during the Paleozoic (Fig. 3). The subclass Lulworthiomycetidae diverged approximately at 284 Mya (247-371). The subclasses Hypocreomycetidae and Savoryellomycetidae diverged within 177-279 Mya during the late Mesozoic. The split between Diaporthomycetidae and Sordariomycetidae occurred approximately 228 Mya (172-288) during late Mesozoic. According to our analysis subclasses Xylariomycetidae diverged approximately at 262 Mya (205-315) during early Paleozoic. Amphisphaeriales, Xylariales and newly introduced order Delonicicolales diverged at 181 Mya (133-234) from the common ancestor. The estimated stem ages of order Delonicicolales (181 MYA (133-234) is within the adjusted stem ages (150-250 MYA) of an order, proposed by Hyde *et al.* (2017). The divergence time estimations of this study are overlapping estimations of previous studies (Sung *et al.* 2008; Samarakoon *et al.* 2016; Hongsanan *et al.* 2017; Hyde *et al.* 2017).

Delonicicolales R.H. Perera, Maharachch. & K.D. Hyde, ***ord. nov.***

Index fungorum number: IF553775

Facesoffungi number: FoF 03603

Saprobic, *pathogenic* and/or *endophytic* in angiosperms hosts. **Sexual morph**: *Pseudostromata* aggregated, immersed, dark brown to black. *Ascomata*, perithecial, aggregated, immersed in host tissues, ostiolate, papillate. *Peridium*,


Amphisphaeriales
Xylariales
Sporocadaceae
Zetiasplozna acaciae CPC 23421
Bartalinia robillardoides CBS 122705
Truncatella spartii MFLUCC 15-0573
Hyalotiella spartii MFLUCC 13-0397
Broomella vitalbae MFLUCC 15-0023
Robillarda roystoneae CBS 115445
Robillarda sessilis CBS 114312
Robillarda africana CBS 122.75
Robillarda terrae CBS 587.71
Robillarda sessilis CBS 101440
Seimatosporium cornii MFLUCC14-0467
Seimatosporium tosta HKUCC 1004
Sarcostroma restionis CBS 118154
Discosia aff. artocreas MAFF 242776
Adisciso yakushimense KT1907
Seiridium phylicae CPC 19962
Lepteutypa cupressi IMI 052255
Pestalotiopsis adusta 3.9103
Pseudopestalotiopsis theae SAJ-0021
Ciliochorella mangiferae MFLUCC 12-0310
Apiosporaceae
Arthrinium hydei CBS 114990
Apiospora montagnei AFTOL-ID 951
Apiospora bambusae ICMP 6889
Apiospora setosa ICMP 4207
Melogrammataceae
Melogramma campylosporum MBU
Beltraniaceae
Subramaniomyces fusisaprophyticus CBS 418.95
Beltraniella endiandrae CPC 22193
Beltraniopsis neolitseae CBS 137974
Beltrania pseudorhombica CBS 138003
Parapleurotheciopsis inaequiseptata MUCL 4108
Phlogicylindriaceae
Phlogicylindrium eucalyptorum CBS 111680
Phlogicylindrium eucalyptorum CBS 111689
Phlogicylindrium uniforme CBS 131312
Ciferriascosea fluctamurum MFLUCC 15-0541
Ciferriascosea rectamurum MFLUCC 15-0542
Amphisphaeriaceae
Amphisphaeria sorbi MFLUCC 13-0721
Amphisphaeria umbrina HKUCC 994
Amphisphaeria umbrina AFTOL-ID 1229
Vialaeaceae
Vialaea mangiferae MFLUCC 12-0808
Vialaea minutella BRIP 56959
Oxydothidaceae
Oxydothis garethjonesii MFLUCC 15-0287
Oxydothis metroxylonicola MFLUCC 15-0281
Oxydothis palmicola MFLUCC 15-0806
Oxydothis metroxylonis MFLUCC 15-0283
Microdochiaceae
Microdochium trichocladiopsis CBS 623.77
Microdochium trichocladiopsis CBS 623.77
Idriella lunata CBS 204.56
Selenodriella fertilis CBS 772.83
Selenodriella cubensis CBS 683.96
Diatrypaceae
Diatrype whitmanensis ATCC MYA-4417
Eutypa lata CBS 208.87
Diatrype disciformis AFTOL-ID 927
Diatrype palmicola MFLUCC 11-0020
Lopadostomaceae
Creosphaeria sassafras ANM 1978
Creosphaeria sassafras CM AT-018
Lopadostoma turgidum LT2
Xylariaceae
Kretzschmaria deusta CBS163.93
Xylaria hypoxylon CBS122620
Podosordaria tulasnei CBS128.80
Graphostromataceae
Obolarina dryophila 8401
Biscogniauxia anummularia MUCL51395
Graphostroma platystoma AFTOL-ID 1249
Cainiaceae
Arecophila bambusae HKUCC 4794
Amphibambusa bambusicola MFLUCC 11-0617
Seynesia erumpens SMH 1291
Cainia graminis CBS 136.62
Iodosphaeriaceae
Iodosphaeria tongrenensis FJS8
Delonicicolales
Delonicicolaceae
Liberomyces macrosporus CCF 4028
Liberomyces saliciphilus H041
Delonicicola siamense MFLUCC 15–0670
Sordariales
Lasiosphaeria ovina CBS 958.72
Bombardia bombarda AFTOL-ID 967
Chaetomium elatum IFO 6554
Cercophora caudata CBS 606.72
Sordaria fimicola CBS 723.96
Sordaria macrospora ATCC 36709
Boliniales
Camarops microspore CBS 649.92
Camarops ustulinoides AFTOL-ID 72
Menispora tortuosa AFTOL-ID 278
Coniochaeta velutina IFO 9439
Coniochaeta discoidea SANK12878
Ophiostomatales
Ophiostoma ulmi CBS 298.87
Ophiostoma piliferum CBS 129.32
Ophiostoma stenoceras AFTOL-ID 1038
Diaporthales
Discula destructiva ATCC 76230
Plagiostoma euphorbiae CBS 340.78
Amphiporthe castanea AFTOL-ID 1229
Cryphonectria parasitica CMW13749
Cryptodiaporthe corni CBS 245.90
Gnomonia virginianae CBS 121913
Diaporthe eres AR3519
Diaporthe phaseolorum CBS 435.87
Hypocreales
Ophiocordyceps melolonthae TSJ679
Ophiocordyceps gracilioides TSJ935
Ophiocordyceps halabalaensis BCC 21491
Ophiocordyceps sinensis YN09-64
Tolypocladium japonicum OSC 110991
Ophiocordycep irangiensis OSC 128578
Elaphocordyceps capitata OSC 71233
Cordyceps militaris NRRL 28021
Cordyceps militaris OSC 93623
Cordyceps cardinalis OSC 93609
Torrubiella wallacei CBS 101237
Hypomyces polyporinus CBS 168.89
Melanospora zamiae CBS 421.87
Halosarpheia fibrosa AFTOL-ID 786
Nectria cinnabarina CBS 713.97
Hypocrea schweinitzii CBS 243.63
Trichoderma viride GJS89-127
Hypocrea pallida GJS89-83
Microascales
Halosarpheia fibrosa AFTOL-ID 786
Nimbospora effusa AFTOL-ID 761
Corollospora maritima CBS 264.59
Varicosporina ramulosa AFTOL-ID 794
Petriella setifera CBS 110344
Microascus trigonosporus IFO 3222
Ceratocystis fimbriata CBS 374.83
Faurelina elongate CBS 126.78
Ambrosiella xylebori MFLUCC 13-072
Lulworthiales
Lulworthia grandispora JK 4686
Lindra thalassiae JK 4322
Leotia lubrica AFTOL-ID 1
Botryotinia fuckeliana AFTOL-ID 59
Pleospora herbarum CBS 191.86
0.2

composed of layers of hyaline cells of *textura angularis*. *Hamathecium* comprising dense filamentous, aseptate, unbranched paraphyses. *Asci* 8-spored, unitunicate, clavate, with short pedicel, apex simple. *Ascospores* uniseriate to biseriate, hyaline, trans-septate. **Asexual morph:** *Conidiomata* pycnidial, single or aggregated, uni- or irregularly multi-locular, ostiolate. *Peridium* composed of several layers, dark brown cells at the outside. *Conidiophores* irregularly branched, simple or more complex, hyaline. *Conidiogenous cells* holoblastic with sympodial proliferation. *Conidia* allantoid or bacilloid, 1-celled, hyaline.

Type family: *Delonicicolaceae* R. H. Perera, Maharachch. & K.D. Hyde.

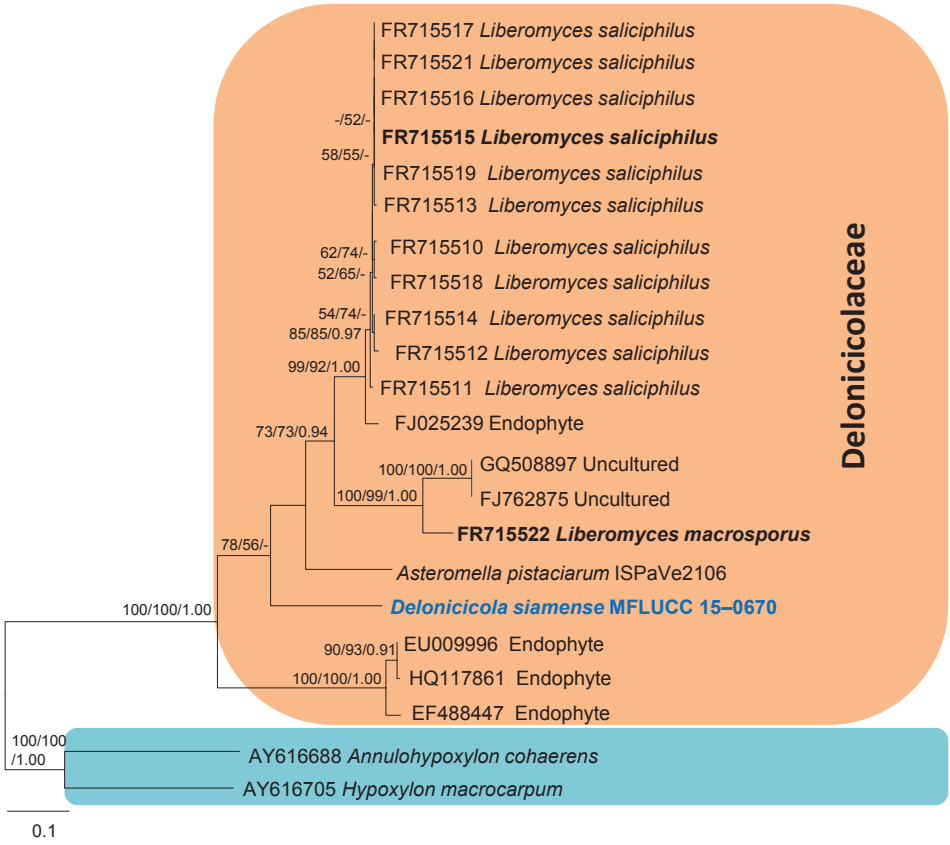
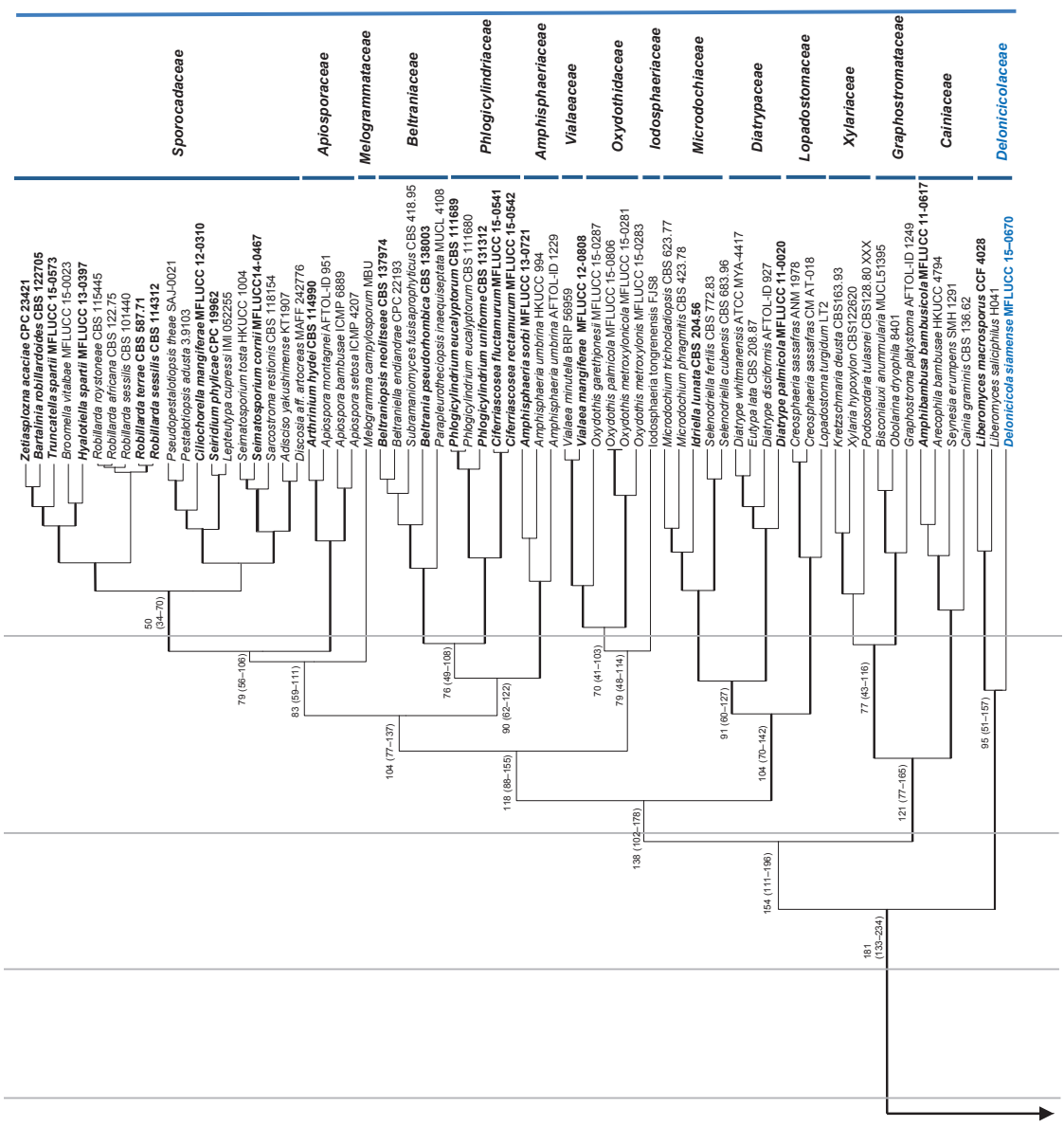


Fig. 2. Phylogram generated from RAxML based on ITS sequence data of analyzed *Delonicicolaceae* taxa. Values above the branches indicate maximum parsimony and maximum likelihood bootstrap ≥ 50%, (MP/ML). Values at the third positions represent Bayesian inference posterior probabilities (PP ≥ 0.90). The ex-type strains are in bold and the new isolate is in blue. The tree is rooted with *Annulohypoxylon cohaerens* and *Hypoxylon macrocarpum*.

◀ Fig. 1. Phylogram generated from maximum likelihood analysis of combined LSU, SSU and RPB2 sequence data of selected taxa of Sordariomycetes. Values above the branches indicate maximum parsimony and maximum likelihood bootstrap ≥ 70%, (MP/ML). Values at the third positions, respectively, above or below the branches represent posterior probabilities (PP ≥ 0.95) from Bayesian inference analysis. The ex-types strains are in bold, the new isolates are in blue. The tree is rooted with *Botryotinia fuckeliana*, *Leotia lubrica* and *Pleospora herbarum*.


Xylariomycetidae
Sporocadaceae
Apiosporaceae
Melogrammataceae
Beltraniaceae
Phlogicylindriaceae
Amphisphaeriaceae
Vialaeaceae
Oxydothidaceae
Iodosphaeriaceae
Microdochiaceae
Diatrypaceae
Lopadostomaceae
Xylariaceae
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Delonicicolaceae
Zetaspiozna acaciae CPC 23421
Bartalinia robillardoides CBS 122705
Truncatella spartii MFLUCC 15-0573
Broomella vitalbae MFLUCC 15-0023
Hyalotiella spartii MFLUCC 13-0397
Robillarda roystoneae CBS 115445
Robillarda africana CBS 122.75
Robillarda sessilis CBS 101440
Robillarda terrae CBS 587.71
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Phlogicylindrium eucalyptorum CBS 111680
Phlogicylindrium uniforme CBS 131312
Ciferriascosea fluctamurum MFLUCC 15-0541
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Vialaea mangiferae MFLUCC 12-0808
Oxydothis garethjonesi MFLUCC 15-0287
Oxydothis palmicola MFLUCC 15-0806
Oxydothis metroxylonicola MFLUCC 15-0281
Oxydothis metroxylonis MFLUCC 15-0283
Iodosphaeria tongrenensis FJS8
Microdochium trichocladiopsis CBS 823.77
Microdochium phragmitis CBS 423.78
Idriella lunata CBS 204.56
Selenodriella fertilis CBS 772.83
Selenodriella cubensis CBS 683.96
Diatrype whitmanensis ATCC MYA-4417
Eutypa lata CBS 208.87
Diatrype disciformis AFTOL-ID 927
Diatrype palmicola MFLUCC 11-0020
Creosphaeria sassafras ANM 1978
Creosphaeria sassafras CM AT-018
Lopadostoma turgidum LT2
Kretzschmaria deusta CBS163.93
Xylaria hypoxylon CBS122620
Podosordaria tulasnei CBS128.80 XXX
Bisconiauxi anummularia MUCL51395
Obolarina dryophila 8401
Graphostroma platystoma AFTOL-ID 1249
Amphibambusa bambusicola MFLUCC 11-0617
Arecophila bambusae HKUCC 4794
Seynesia erumpens SMH 1291
Cainia graminis CBS 136.62
Liberomyces macrosporus CCF 4028
Liberomyces saliciphilus H041
Delonicicola siamense MFLUCC 15–0670
50 (34–70)
79 (56–106)
83 (59–111)
104 (77–137)
76 (49–108)
90 (62–122)
118 (88–155)
70 (41–103)
79 (48–114)
138 (102–178)
91 (60–127)
104 (70–142)
154 (111–196)
77 (43–116)
121 (77–166)
181 (133–234)
95 (51–157)

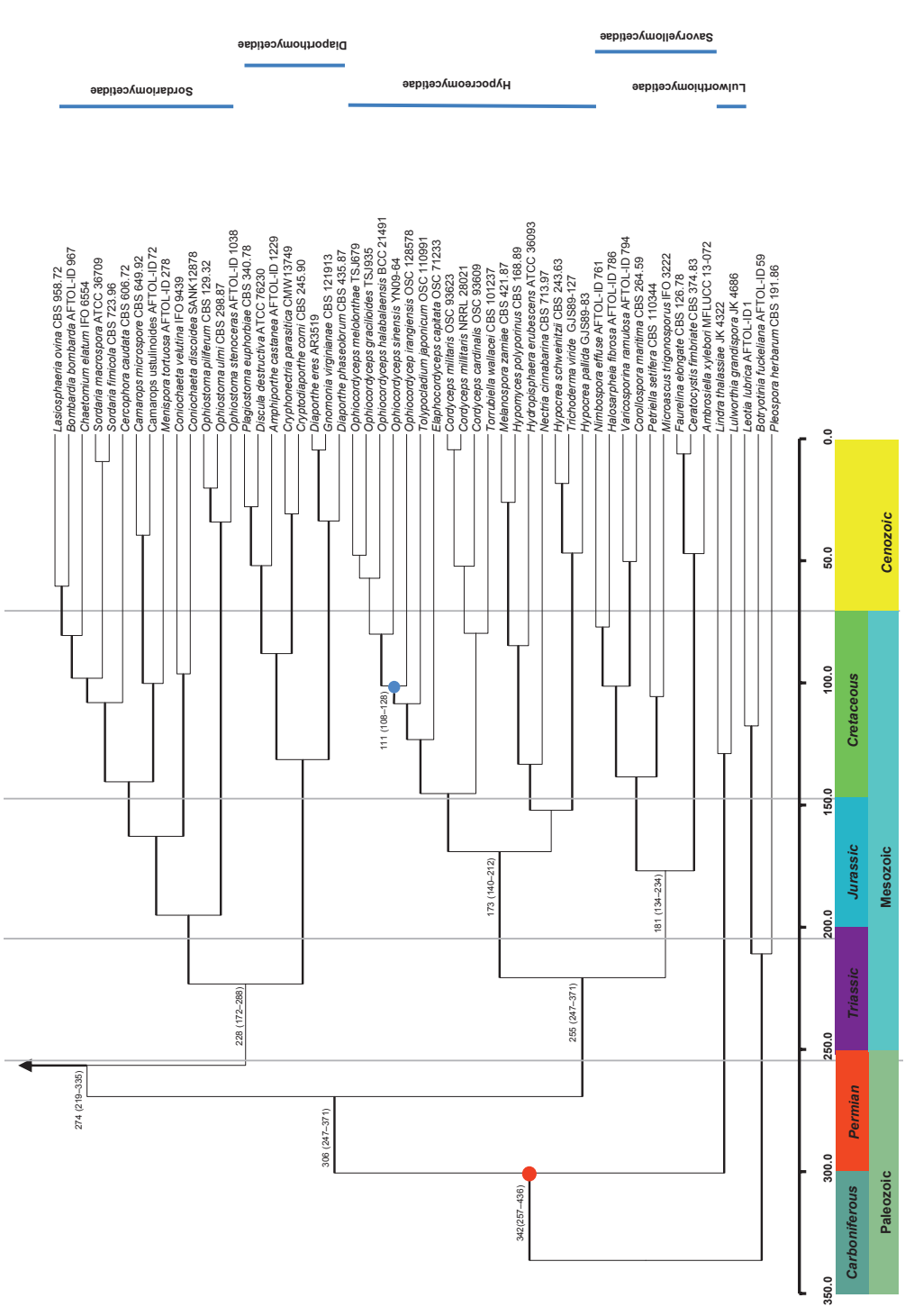


Fig. 3. Sordariomycetes divergence time tree estimated using lognormal relaxed clock model (uncorrelated) in BEAST, with representative families. Median age and 95% highest posterior density (HPD) are shown near the nodes in Mya. The blue and red circles indicate the fossil and secondary points respectively. Branches > 0.9PP are in bold.

Delonicicolaceae R. H. Perera, Maharachch. & K.D. Hyde, ***fam. nov.***

Index fungorum number: IF553776
Facesoffungi number: FoF 03604
Saprobic, *pathogenic* and/or *endophytic* in angiosperms hosts. **Sexual morph**: *Pseudostromata* aggregated, immersed, dark brown to black, visible as raised, dark spots on the host surface. *Ascomata*, perithecial, aggregated, immersed in host tissues, globose, subglobose to conical or irregular, hyaline, ostiolate, papillate. *Peridium*, composed of 5-8 layers of hyaline cells of *textura angularis*. *Hamathecium* comprising dense filamentous, aseptate, unbranched paraphyses. *Asci* 8-spored, unitunicate, clavate, with short pedicel, apex simple. *Ascospores* uniseriate to biseriate, 1-septate, ellipsoid, equilateral or inequilateral, hyaline, without a mucilaginous sheath. **Asexual morph:** *Conidiomata* pycnidial, rounded to oval, single or aggregated, uni- or irregularly multi-locular, ostiolate. *Peridium* composed of several layers, inner layers consisting of paler cells, outer layers with thick-walled, dark brown cells, basal part sometimes consisting of thin-walled non-melanized cells. *Conidiophores* irregularly branched, simple or more complex, hyaline, arising all around the cavity of the conidioma. *Conidiogenous cells* holoblastic with sympodial proliferation. *Conidia* allantoid or bacilloid, 1-celled, hyaline, sometimes producing synanamorph with filiform, one-celled, hyaline, conidia.
Type genus: *Delonicicola* R.H. Perera, Maharachch. & K.D. Hyde.
Notes: In the phylogenetic analyses (Fig. 1), *Delonicicola* groups with *Liberomyces, Asteromella pistaciarum* and endophytes from various plants of tropical origin forming a distinct clade in Xylariomycetidae. *Liberomyces* species are endophytic or pathogenic coelomycetes, with sympodial proliferation of conidiogenous cells, allantoid conidia and sometimes produce synanamorph with filiform conidia (Pažoutová *et al.* 2012). *Asteromella pistaciarum* is a leaf pathogenic coelomycete, with rounded to oval conidiomata (45-90 µm diam) and bacilloid conidia (3-5 × 1 mm), straight or rarely curved, the type of conidial developmental pattern was not mentioned in the original description (Bremer & Petrak 1947). In the multi-gene analysis, this clade is distinct from the other orders and families in Xylariomycetidae and thus we introduce a new order and family to accommodate these taxa. *Delonicicolaceae* resembles Xylariomycetidae in its life mode, as they are saprobic, pathogenic and/or endophytic (Bremer & Petrak 1947; Pažoutová *et al.* 2012; Senanayake *et al.* 2015).

Delonicicola R.H. Perera, Maharachch. & K.D. Hyde, ***gen. nov.***

Index fungorum number: IF553772
Facesoffungi number: FoF 03605
Etymology: Referring to the host genus.
Saprobic on seed pods of *Delonix*. **Sexual morph**: *Pseudostromata* aggregated, immersed, dark brown to black, visible as raised, dark spots on the host surface. *Ascomata* perithecial, aggregated, immersed in host tissues, globose, subglobose to conical or irregular, hyaline, ostiolate, papillate. *Papilla* short, immersed or protruding through the host tissue, conical, periphysate. *Peridium*, composed of 5-8 layers of hyaline cells of *textura angularis*. *Hamathecium* comprising dense filamentous, aseptate, unbranched paraphyses. *Asci* 8-spored, unitunicate, clavate, with short pedicel, apex simple. *Ascospores* uniseriate to biseriate, 1-septate, not constricted at the septum, ellipsoid, equilateral or inequilateral, with narrowly rounded ends, straight, hyaline, smooth-walled, without a mucilaginous sheath. **Asexual morph:** Undetermined.

Type species: *Delonicicola siamense* R. H. Perera, Maharachch. & K.D. Hyde

Notes: *Delonicicola* is introduced as a monotypic genus to accommodate *D. siamense*, saprobic on *Delonix* seed pods based on sequence data and morphology. The ITS phylogeny showed that *Delonicicola* forms a distinct clade sister to *Liberomyces*, *Asteromella pistaciarum* and related sequences of endophytes of angiosperms within the family *Delonicicolaceae* (Fig. 2).

Delonicicola siamense R.H. Perera, Maharachch. & K.D. Hyde, ***sp. nov.*** **Fig. 4**

Index fungorum number: IF553771

Facesoffungi number: FoF 03606

Etymology: Referring to the country where the fungus was first collected.

HOLOTYPUS: MFLU 17-0739

Saprobic on seed pods of *Delonix regia*. **Sexual morph**: *Pseudostromata* aggregated, immersed, dark brown to black, visible as raised, dark spots on the host surface. *Ascomata* 112-267 *μm* high, 75-264 *μm* diam. ($\bar{x}$ = 162 × 125 *μm*, n = 20), perithecial, aggregated, immersed in host tissues, globose, subglobose to conical or irregular, hyaline, ostiolate, papillate. *Papilla* short, immersed or protruding through the host tissue, conical, periphysate. *Peridium* 10-16 *μm* wide ($\bar{x}$ = 12 *μm*, n = 20), composed of 5-8 layers of hyaline cells of *textura angularis*. *Hamathecium* comprising dense filamentous, aseptate, unbranched paraphyses, 0.8-1.1 *μm* wide ($\bar{x}$ = 0.9 *μm*, n = 20). *Asci* 29-42 × 3.4-4.9 *μm* ($\bar{x}$ = 35 × 4.2 *μm*, n = 25), 8-spored, unitunicate, clavate, with short pedicel, apex simple. *Ascospores* 3.8-6.9 × 1-1.4 *μm* ($\bar{x}$ = 5.4 × 1.2 *μm*, n = 20), uniseriate to biseriate, 1-septate, not constricted at the septum, ellipsoid, equilateral or inequilateral, with narrowly rounded ends, straight, hyaline, smooth-walled, without a mucilaginous sheath. **Asexual morph:** Undetermined

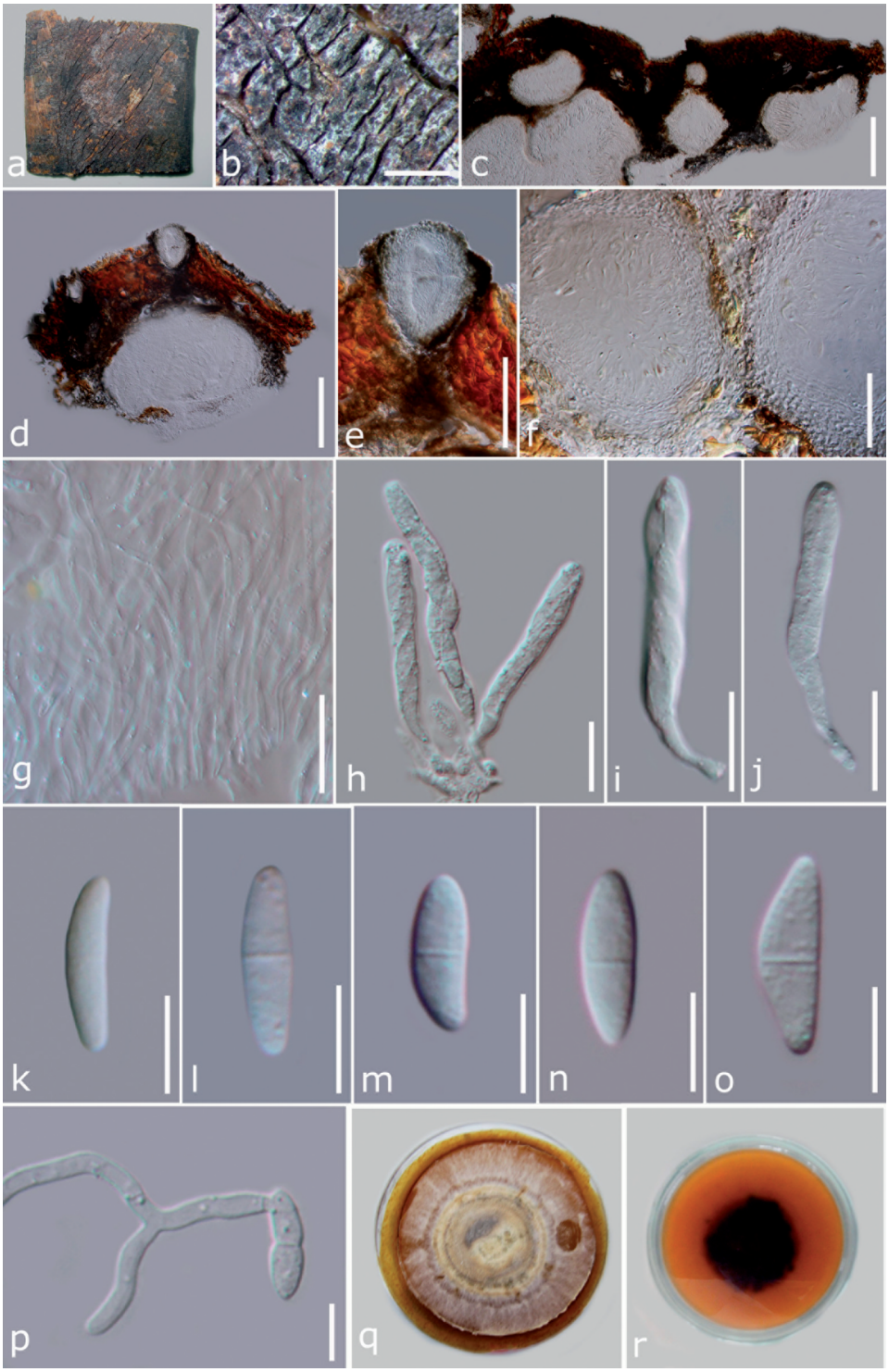
Culture characters: Colonies growing on MEA, reaching 7 cm within 18 days, at 25°C, circular, white, even margin with zonation, becoming pale yellow with time, flat, lacking aerial mycelium, underneath brown.

Material examined: THAILAND, Chiang Rai, Mae Fah Luang University premises, on dried seed pods of *Delonix regia* (Boj. ex Hook.) Raf. (*Fabaceae*), 2 June 2015, R.H. Perera, RHP 92 (MFLU 17-0739, **holotype**) *ibid.* (HKAS, **isotype**), ex-type living culture = MFLUCC 15-0670 = KUMCC.

Notes: *Delonicicola siamense* is phylogenetically related to *Asteromella pistaciarum*, *Liberomyces* and endophytes of angiosperms (Fig 1). Both *Liberomyces* and *Asteromella* are coelomycetous genera with pycnidial conidiomata and allantoid, bacilloid or filiform, hyaline conidia. *Delonicicola siamense* is the only sexual morph known in *Delonicicolaceae*. The morphology of the sequenced endophytes was not described in the original publications (Vega *et al.* 2010; Lin *et al.* 2010). However, phylogenetic analysis supports that *D. siamense* is distinct from those sequenced endophyte sequences (Fig. 1).

DISCUSSION

The phylogenetic placement of *Liberomyces* was discussed by Pažoutov *et al.* (2012). They pointed out the possibility of the existence of a new family of Xylariales or even a new related order, that is shown by its sister position to the


a
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entire Xylariales clade (Pažoutov *et al.* 2012). They emphasized the absence of tandem repeats of the CTACCCTGTAG type in the ITS region of *Liberomyces.* Those were found in ITS region of all xylariaceous families (Platas *et al.* 2001). By considering the molecular data analysis and morphological and life mode resemblance to Xylariales, here we introduce the new order Delonicicolales with a new family *Delonicicolaceae* to accommodate the taxa in the *Liberomyces-Delonicicola* clade. In our analysis, sequence data of *Asteromella pistaciarum* clustered with the *Liberomyces-Delonicicola* clade. A new order Delonicicolales is introduced in this study based on phylogenetic analysis (Fig. 1) and molecular clock analysis, the latter providing additional support (Fig. 3). The use of divergence times as a universal criterion for ranking taxa (temporal banding) was suggested and applied in several recent studies including fungi (Lücking 1999; Beimforde *et al.* 2014; Phukhamsakda *et al.* 2016; Samarakoon *et al.* 2016; Hongsanan *et al.* 2016; Liu *et al.* 2017; Hyde *et al.* 2017).

The close phylogenetic relationship between *Liberomyces* and *Asteromella pistaciarum* was previously shown by Pažoutová *et al.* (2012) based on ITS sequence data. However, *Asteromella pistaciarum* has smaller conidiomata than *Liberomyces*, viz. 45-90 µm vs. 100-500 µm diam. (Bremer & Petrak 1947; Pažoutová *et al.* 2012). Another sequenced *Asteromella* species, *A. tiliae* (CBS 265.94), has been placed in Pleosporales based on LSU/SSU data analysis by de Gruyter *et al.* (2009), suggesting the genus *Asteromella* is polyphyletic. There is already a family available for *Asteromella* (*Asteromellaceae* Melnik, Mikol. Fitopatol. 20(2): 101 (1986)). The type species of *Asteromella* is however, *Asteromella ovata* Thüm. which was described from a dead leaf of *Acer* sp. (as *pseudoplatani*) collected in Austria and is a coelomycete. There is presently no way to establish if *A. ovata* is related to *A. pistaciarum*. Thus, as the genus is polyphyletic and until the former species is epitypified and sequenced it is advisable to assume they are unrelated. Therefore, we introduce a new family and order to accommodate our new genus, *Delonicicola*.

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◀ Fig. 4. ***Delonicicola siamense*** (MFLU 17-0739, **holotype**) **a-b.** Appearance of ascomata on host substrate. **c.** Vertical section through pseudostroma with ascomata. **d.** Vertical section through ascoma. **e.** Ostiole. **f.** Close up of the peridium. **g.** Paraphyses **h-j.** Asci. **k-o.** Ascospores. **p.** Germinating ascospore. **q, r.** Colony on MEA. Scale bars: b = 1 mm, c, d = 100 µm, e = 50 µm, f, g = 20 µm, h-j = 10 µm, k-p = 5 µm.

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