Mycosphere 7 (11): 1690–1712 (2016) www.mycosphere.org ISSN 2077 7019

Article – special issue
Doi 10.5943/mycosphere/7/11/6

The evolution of fungal epiphytes

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Hongsanan S, Sánchez-Ramírez S, Crous PW, Ariyawansa HA, Zhao RL, Hyde KD. 2016 – The evolution of fungal epiphytes. Mycosphere 7 (11), 1690–1712, Doi 10.5943/mycosphere/7/11/6

Abstract

Fungal epiphytes are a polyphyletic group found on the surface of plants, particularly on leaves, with a worldwide distribution. They belong in the phylum Ascomycota, which contains the largest known number of fungal genera. There has been little research dating the origins of the common ancestors of fungal epiphytes. This study uses a molecular clock to provide a rough time frame for the origins of fungal epiphytes in the orders Asterinales, Capnodiales, Meliolales, Microthyriales and Zeloasperisporiales. LSU, SSU, RPB1 and RPB2 sequence data from representative strains of the major classes of Ascomycota are used to represent internal calibration points in the phylogenetic tree, to estimate divergence times of fungal epiphyte lineages. The estimated date crowns of fungal epiphytes included in the orders Asterinales, Capnodiales, Meliolales occur in the middle or the end of Jurassic, with Meliolales and Zeloasperisporiales occurring in the Cretaceous. Foliar epiphytes placed in totally unrelated classes evolved as early as the Permian (298.9 to 252.17 Mya) based on sequence data from representative foliar epiphytes and fossil calibrations. The evolution of the most closely related groups of fungi and foliar epiphytes occurred during the Triassic to Jurassic. Phylogenetic relationships, evolution of morphological characters and nutritional mode of foliar epiphytes are discussed.

Key words – BEAST – Fossil fungi – MrBayes – Phylogeny – Sooty molds – Taxonomy

Introduction

Fungal epiphytes are a polyphyletic group with a worldwide distribution (Schoch et al. 2009, Wu et al. 2011, Hyde et al. 2013, Hongsanan et al. 2014a, 2015a, b, c, Li et al. 2016). They are defined as specialized nutritional guilds found on the surface of living plant parts, particularly on leaves; including saprobes, plant parasites, fungal parasites and lichens (Gilbert & Reynolds 2002, 2005). Many fungal epiphytes are obligate parasites (Wu et al. 2011, Hongsanan et al.

Submitted 13 October 2016, Accepted 11 December 2016, Published online 29 December 2016
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2015a), which can damage the host plants by penetrating host cells for the uptake of nutrients (Ariyawansa et al. 2015, Hongsanan et al. 2014a, 2015a, b). Some species are saprobes and cause marketability problems, due to the black hyphae coating the surface of plants, especially economic fruits. Furthermore, they reduce photosynthetic ability of plants through the hyphal cover; they can also cause chlorosis under the hyphae and can cause plant-stunting disease and lower yield (Chomnunti et al. 2014, Hongsanan et al. 2015c, d). Some fungal epiphytes cause sooty blotch and flyspeck disease on surface of apple or other host plants such as mango and pears (Ismail et al. 2016).

Molecular clock methods have been used in several studies to date the origin and subsequent evolution of lineages in many groups of micro- and macrofungi (Berbee & Taylor 1993, 2007, 2010, Heckman et al 2001, Sanderson 2003, Taylor & Berbee 2006, Vijaykrishna et al. 2006, Beimforde et al. 2014, Zhao et al. 2016). The first application of the molecular clock to fungal groups was provided by Simon et al. (1993). Fossil evidence is essential for dating (Benton et al. 2009, Hedman 2010, Inoue et al. 2010, Magallon 2010, Pyron 2010, Wilkinson et al. 2011, Lukoschek et al. 2012, Sauquet et al. 2012). Fossil evidence is used as the minimum age, which results in calibration points in the phylogenetic tree (Marshall 2008, Forest 2009, Parham et al. 2012, Sauquet et al. 2012, Beimforde et al. 2014). Despite several recent studies dating fungi, molecular clock dating and studies on the evolution of fungal epiphyte lineages has been poorly studied.

In this study, we focus on the evolution of fungal epiphytes using molecular clock dating. The fungal epiphytes are black mildews, black dots, and sooty moulds, mostly on plant leaves and belong in the orders Asterinales, Capnodiales, Microthyriales and Zeloasperisporiales of Dothideomycetes (Hyde et al. 2013, Chomnunti et al. 2011, 2014, Hongsanan et al. 2014a, b, 2015a, b, c, d), and Meliolales of Sordariomycetes (Kirk et al. 2001, Justavino et al. 2015, Hongsanan et al. 2015a, Maharachchikumbura et al. 2015, 2016). We used multi-calibrations distributed across the Ascomycota as used in Pérez-Ortega et al. (2016), and in addition we used fossil evidence for fungal epiphytes from previous studies to strengthen the calibration.

Fossil studies on fungal epiphytes

Asterinales

Species in Asterinales are pathogenic biotrophs, appearing as black colonies on the surface of plants, particularly on leaves, and are common in tropical and subtropical regions and have a worldwide distribution)Hyde et al. 2013, Hongsanan et al. 2014a(. Although Asterinales appear to be similar to sooty moulds when observed with the unaided eye, they produce black, web-like colonies on leaves (Fig. 1), and cause minor damage to host plants by penetrating host cells for the uptake of nutrients. Sooty moulds however, feed on honeydew excreted from insects)Hughes 1976, Reynolds 1998, Chomnunti et al. 2012, 2014(. Members of Asterinales are host-specific biotrophs (Hongsanan et al. 2014a). A recent monograph of Asterinales was provided by Hongsanan et al. (2014a). The order presently comprises three families based on phylogenetic inferences: Asterinaceae, Melaspileaceae, and Parmulariaceae (Guatimosim et al. 2015).

Engelhardt & Kinkelin (1908) studied fossils of leaves of *Ilex* (Aquifoliaceae), *Sequoia* (Cupressaceae), *Sapindus* (Sapindaceae), and *Chrysobalanus* (Chrysobalanaceae) and dated them to the Pliocene. They found taxa typical of *Asterina* on the fossil specimens. Thus, they concluded that the genus *Asterina* existed during the Pliocene (5.333 to 2.58 Mya). Dilcher (1965) found *Asterina* species on angiosperm leaves collected from lower Eocene deposits (56 to 33.9 Mya) in western Tennessee (USA), and introduced two new species, *Asterina nodosaria* Dilcher and *A. eocenica* Dilcher from *Sapindus* and *Chrysobalanus*, respectively. Dilcher (1965) also compared these asterina-like taxa from fossil specimens with modern specimens, and concluded that the genus *Asterina* originated at least as early as the Eocene. There are very few studies on both fossil and modern specimens of Asterinales. Consequently, they are poorly studied from an evolutionary point of view.

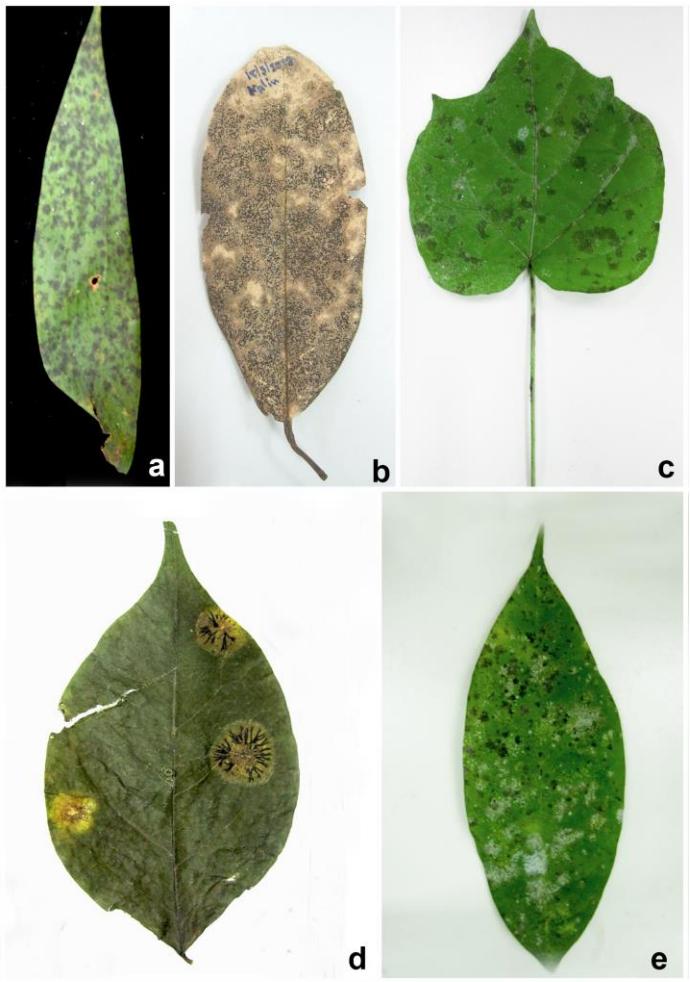

Fig. 1 − Asterinales on the surface of various hosts. **a** *Asterina* species (Asterinaceae) on *Acacia* sp. **b** *Asterina* species (Asterinaceae) on unidentified leaves. **c** *Asterina* species (Asterinaceae) on *Tinospora* sp. **d** *Aldona* species (Parmulariaceae) on *Pterocarpus draco*. **e** *Asterina* species (Asterinaceae) on *Pterocarpus* sp.

Families of sooty moulds

Sooty moulds are saprobes, which indirectly damage host plants by reducing photosynthesis. Sooty moulds species normally feed on carbon sources, such as sugars (honeydew) excreted from sap-feeding insects; aphids, and whiteflies)Hughes 1976, Reynolds 1998, Chomnunti et al. 2011, 2012, 2014(. These taxa appear as a black mold covering the host surface with dark hyphae (Fig. 2); they can affect many economic crops, e.g. *Capnodium citri* on *Citrus* spp. (Reynolds 1999) and *Sorias* spp. on mangoes. Species that occur on the surface of plants especially on fruits can reduce their quality, have important implications for import and export of fruits and reduce the sale of fruits in markets)Chomnunti et al. 2014(. Sooty mould species are polyphyletic and comprise seven families based on morphology and phylogeny (Reynolds 1998, Winka et al. 1998, Hughes & Seifert 2012, Hyde et al. 2013, Chomnunti et al. 2001, 2012, 2014); Antennulariellaceae, Capnodiaceae, Euantennariaceae, Metacapnodiaceae (Dothideomycetes) and Chaetothyriaceae, Coccodiniaceae, and Trichomeriaceae (Eurotiomycetes). Although they belong in two classes, they live differently and are unrelated. The ascomata of Chaetothyriales are surrounded by a pellicle of superficial mycelium, and are often multilocular. However, some taxa in Capnodiales have also been isolated as rock-dwelling fungi and were noted that some of these taxa perhaps evolved from capnodiaceous species (Ruibal et al. 2009, Selbmann et al. 2014). These questions need to be clarified by using the life cycle of all the families of sooty moulds. In this study, we focus on the foliar epiphytes of Dothideomycetes and Sordariomycetes, and *Chaetothyriales* will be studied in the future.

The oldest known fossils of sooty moulds belong to the Metacapnodiaceae (Capnodiales) and were found in France in Early Cretaceous Charentes amber (145 ± 4 to 66 Mya) and were dated to be at least 100 Mya (Néraudeau et al. 2002, Perrichot et al. 2010, Beimforde et al. 2014, Schmidt et al. 2014, Pérez-Ortega et al. 2016). Some of the taxa isolated as rock-dwelling fungi probably evolved from capnodiaceous sooty moulds (Ruibal et al. 2009, Selbmann et al. 2014). This can be clarified by molecular data when sufficient informative data become available.

Fig. 2 – Sooty moulds on various hosts. **a** *Coffea arabica*. **b** *Mangifera indica*. **c** *Psidium guajava*. **d** *Citrus maxima*. **e** *Heliconia* sp. **f** *Lansium domesticum*.

Meliolales

The order Meliolales accommodates species of biotrophic epiphytes (Hansford 1961, Hongsanan et al. 2015a), which may cause leaves to become stunted and pale in colour. Some species do not produce pathogenic effects, but reduce photosynthetic efficiency and aesthetic beauty of the host plant (Fig. 3) (Thomas et al. 2013, Hongsanan et al. 2015a). Hongsanan et al. (2015a) monographed the order based on morphology and phylogeny and concluded that Meliolales presently contains Armatellaceae and Meliolaceae. The family Armatellaceae comprises a single genus, while Meliolaceae contains seven genera (Hongsanan et al. 2015a). Species are believed to

be host-specific, however, Hongsanan et al. (2015a) have shown that *Meliola thailandicum* can occur on at least two host families. Phylogenetic analysis indicates that Meliolales is a subclass of Sordariomycetes, Meliolomycetidae (Kirk et al. 2001, Justavino et al. 2015, Hongsanan et al. 2015a, Maharachchikumbura et al. 2015, 2016), and this was confirmed by Maharachchikumbura et al. (2015). Meliolales species are biotrophic and cannot be cultured, thus there are few sequences for the species in GenBank.

There is little fossil evidence for Meliolales (Dilcher 1965). *Meliola ellis* Roum. was introduced as a species from fossils in northern England dated to the Holocene (0.0117 to 0 Mya) (Roumeguère 1880). Köck (1939) described meliola-like taxa from Eocene fossils (56 to 33.9 Mya) on *Taxus* L. (Taxaceae), *Sapindus* L. (Sapindoideae), *Chrysobalanus* L. (Chrysobalanaceae) and unidentified leaves. Dilcher (1965) established two new species, *Meliola spinksii* Dilcher and *M. anfractus* Dilcher, based on the comparison between fossil specimens discovered from the Eocene of Tennessee deposits.

Fig. 3 – Meliolales on various hosts. **a** *Litchi chinensis*. **b** *Citrus* sp.

Microthyriales

Species in Microthyriales are fungal epiphytes including biotrophs and saprotrophs, usually found on leaves or fruits (Wu et al. 2011, Hongsanan et al. 2014b). They indirectly affect host plants by penetrating and extracting nutrients, but do not cause much damage. Species of Microthyriales appear as on leaves as small, circular, flattened, black dots, with a prominent central ostiole, and are poorly developed at the base. They are easily removed from the surface of the host. Asci are bitunicate and ascospores are 1-septate, some with appendages (Arnaud 1918, Luttrell 1973, von Arx & Müller 1975, Barr 1987, Kirk et al. 2008, Wu et al. 2011, Hyde et al. 2013). The appearance of Microthyriales species on fruits can reduce marketability. Wu et al.)2011(recognized seven genera of Microthyriaceae, while a further four genera were added by Hyde et al.)2013(. A further two new genera were added by Hongsanan et al.)2014b(and Ariyawansa et al. (2015) with evidence from morphology and phylogeny. A few species in *Microthyriales* have been sequenced and higher level placement of the order has partially been resolved. However, there are many species in Microthyriales that have not been sequenced. Frantz (1959) reported microthyriaceous species on *Sapindus*, *Pityophyllum* (Gnetopsida) and numerous unidentified leaves from the fossil Tertiary (66 to 2.58 Mya). Fossilized *Microthyrium* on *Buxus protojaponica* was found in Japan in the Miocene (23.03 to 5.332 Mya) by Doi and Uemura (1985). Szafer (1961) and Lancucka-Srodoniowa (1966) described Microthyriales on *Buxus* sp. from the fossil Miocene of Europe. Godwin and Andrew (1951) reported *Microthyrium* on a *Buxus* fossilized leaf in England from the Plaeistocene (2.58 to 0.0117 Mya). Some fossil specimens were described from the Plaeistocene under the form-name Microthyriacites or Phragmothyrites (Givulescu 1971, Cooksoon 1947, Selkirk 1975). Microthyrialean taxa are common in the Eocene (56 to 33.9 Mya)

or younger than Eocene (Germeraad 1979, Doi & Uemura 1985). However, the oldest fossil of the order Microthyriales was recorded in Colorado from the upper Cretaceous (145 ± 4 to 66 Mya) (Eriksson 1978).

Zeloasperisporiales

The order was established to accommodate a single family Zeloasperisporiaceae by Hongsanan et al. (2015b). Species of Zeloasperisporiaceae appear as small black dots on the surface of the host, often similar to species in Microthyriales. The generic type of this family is *Zeloasperisporium* which was introduced based on asexual characters. The life cycle of *Zeloasperisporium* species are remarkable as they have been isolated from the air and leaves and may obtain nutrients from plant cells using appressorium-like, inflated hyphopodia, which are slightly warted to lobed at the apex (Castañeda et al. 1996). However, Crous et al. (2007) recognized this structure as conidiogenous cells of a synanamorph forming a second conidial type. The sexual and asexual morphs of Zeloasperisporiaceae species were clarified and discussed by Hongsanan et al. (2015b). There is no fossil evidence for this order. Some taxa were discovered on amber fossil and resemble to Zeloasperisporiales species such as *Callimothallus pertusus* Dilcher which has flattened stromata on upper epidermis of leaf of *Sapindus* sp. (Sapindaceae), but associated with hyphae of *Shortensis memorabilis* Dilcher. Thus, we cannot be sure that they belong in Zeloasperisporiales due to the poor condition of specimens.

Fig. 4 – Zeloasperisporiales species on living leaves. **a** On *Wrightia religiosa*. **b** On unidentified leaves

Materials & Methods

Phylogenetic analyses

LSU, SSU, and RPB2 sequence data from the representative major orders in Dothideomycetes and Sordariomycetes were obtained from GenBank (Table. 1). The molecular clock tree was divided into two trees, which are fungal epiphytes in Sordariomycetes and Dothideomycetes.

The representative strains from Dothideomycetes were downloaded from GenBank. The representative strains from Leotiomycetes were selected as outgroup. The data set was aligned by using MAFFT (Katoh et al. 2009), checked and aligned manually using Bioedit (Hall 1999). The jModeltest was used to perform to select the best-fit models of nucleotide substitution for each gene. Initial phylogenetic tree was performed by using MCMC sampling in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001, Zhaxybayeva & Gogarten 2002), following Cai et al. (2006, 2008). The analysis used 4 nchains, and run for 10,000,000 generations. Trees were sampled every 1000th generation which produces 10,000 trees. The first 2,000 trees are known as burin-in phase, and were discarded, and the remaining 8,000 trees were used to calculate the posterior probabilities.

The representative strains from Sordariomycetes were downloaded from GenBank. The representative strains from Lecanoromycetes were selected as outgroup taxon. The data set was aligned using the same methods with the Dothideomycetes tree. The jModeltest was used in combination with AIC to estimate the best nucleotide substitution model the recommended models in the dataset of Sordariomycetes were GTR+I+G for LSU and RPB2, SYM+I+G for SSU. An initial phylogenetic tree was prepared using the same methods mentioned above.

Fossil calibrations

Divergence time estimation analyses were performed using the fossil calibrations as in Beimforde et al. (2014) and Pérez-Ortega et al. (2016). The results from Pérez-Ortega et al. (2016) were used as secondary calibration in this study. Estimating divergence time of the common ancestor of fungal epiphytes in Dothideomycetes and Sordariomycetes was performed separately using BEAST for evolution analysis.

To estimate the molecular clock tree of fungal epiphytes in the Dothideomycetes, the Dothideomycetes crown group was calibrated using the result from Pérez-Ortega et al. (2016) as secondary calibration (normal distribution, mean = 290, SD = 30, with providing 95% credibility interval of 339 Mya). Metacapnodiaceae was used as a minimum age of Capnodiales (normal distribution, mean = 100, SD = 150, with providing 95% credibility interval of 346 Mya.). *Microthyrium microscopicum* and *M. buxicola* are typical of the oldest fossil of *Microthyrium*, thus used as the oldest fossil to be the minimum age of the common ancestor of Microthyriales (gamma distribution, shape = 1, scale = 50, offset = 65, with providing 95% credibility interval of 215 Mya.). The divergence times of the genus *Calicium* was estimated by Pérez-Ortega et al. (2016) and it was used as the secondary calibration in this study (Gamma, mean = 35, SD = 40, with providing 95% credibility interval of 155 Mya).

To estimate the molecular clock tree of fungal epiphytes in the Sordariomycetes, the rootHeight parameter was calibrated from the split of Leotiomycetes and Sordariomycetes (gamma distribution, shape = 1, scale = 50, offset = 300). The Sordariomycetes crown group was calibrated to be 256 Mya (202–306) by Pérez-Ortega et al. (2016), (normal distribution, mean = 250, SD = 50, with providing 95% credibility interval of 332 Mya). Dilcher (1965) studied the *Meliola* species on fossil Eocene, *Meliola spinksii*, and it is similar to modern specimens of *M. thailandicum*. However, setae with branches at the apex are present in *M. thailandicum*, while they are undetermined in fossil specimens. Based on the similarity of fossil specimens and modern specimens, we assumed that the genus *Meliola* had existed in the Eocene (gamma distribution, shape = 1, scale = 50, off set = 35).

Molecular clock analysis

Molecular clock analyses were performed using BEAST 1.8.0. Aligned sequence data were partitioned separately for each LSU, SSU and RPB2 data set, and loaded to BEAUti 1.8.0. The data partitions were set with unlinked substitution, models and unlinked clock model and linked tree based on jModeltest results. Taxa sets were created for each interested groups and calibration of the common ancestor nodes, statistics associated with the most recent common ancestor (TMRCA). Substitution model was specified for each of data partitions (GTR+I+G for all genes used in Dothideomycetes, GTR+I+G for LSU and RPB2 and SYM+I+G for SSU used in Sordariomycetes). We used a lognormal distribution of rates for each gene estimated during the analyses with uncorrelated relaxed clock model (ucld). The tree prior was shared by all tree models, and consisted in a birth/death incomplete sampling tree prior was used to model the speciation of nodes in the topology. The analyses were performed for 50 million generations for both Dothideomycetes and Sordariomycetes, and sampling parameters every 1000 generations. Tracer v.1.6 was used to check the effective sample sizes, acceptable values were higher than 200. The first 50,000 trees representing the burn-in phase were discarded. The remaining trees were combined in LogCombiner 1.8.0. A maximum clade creditability (MCC) tree was given by summarized data, tree was estimated in TreeAnnotator 1.8.0. The tree was then viewed in FigTree (Rambaut 2006).

Table 1 Taxa used in the phylogenetic analysis and GenBank accession numbers (LSU, SSU, RPB2) and species voucher/culture numbers.

Species	Voucher/culture	Accession numbers		
		LSU	SSU	RPB2
Acrospermum compressum	M151	EU940084	EU940237	EU940301
Acrospermum gramineum	M152	EU940085	EU940238	EU940302
Alternariaster bidentis	CBS 134021	KC609341		KC609347
Amphibambusa bambusicola	MFLUCC 11-0617	KP744474		
Annulusmagnus triseptatus	CBS:128831	JQ429242		JQ429258.1
Antennariella placitae	CBS 124785	GQ303299		
Ascovaginospora_stellipala	P5-13A	U85088	U85087	
Asteridiella obesa	VIC:31239	JX096809		
Asterina fuchsiae	TH 590	GU586216	GU586210	
Asterina phenacis	TH 589	GU586217	GU586211	
Bambusicola massarinia	MFLUCC 11-0389	JX442037	JX442041	KP761716
Bambusicola splendida	MFLUCC 11-0439	JX442038	JX442042	
Bombardia bombarda	AFTOL_ID_967	DQ470970	DQ471021	DQ470923
Botryosphaeria agaves	MFLUCC 11-0125	NG_042723	JX646825	
Botryosphaeria dothidea	CBS 115476	DQ377852	DQ677998	DQ677944
Botryosphaeria tsugae	AFTOL-ID 1586	DQ767655		DQ767644
Botryotinia fuckeliana	spat 03-11	AY544651	AY544695	DQ247786
Calicium salicium	CBS:100898		KF157982	KF157998
Calicium viride	U. Soechting 7475	AF356670		
Camarosporium_quaternatum	CBS 483.95	GU301806	GU296141	
Capnodium coartatum	MFLUCC10-0069	JN832614	JN832599	
Capnodium salicinum	AFTOL-ID937	DQ678050	DQ677997	
Caryospora minima	-	EU196550	EU196551	
Catabotrys deciduum	SMH3436	AY346268		AY780158
Cephalotheca foveolata	UAMH11631	KC408398		KC408404
Chaetomidium galaicum	CBS:113678	FJ666361		FJ666392
Chaetosphaerella fusca	GKML124N	FJ968967		
Chaetosphaeria innumera	SMH 2748	AY017375		
Chromendothia citrina	AFTOL-ID 2121		DQ862046	
Conidiocarpus caucasicus	GUMH937	KC833050	KC833051	
Coniochaeta ligniaria	C8	AY198388		
Coniochaeta ostrea	AFTOL-ID 915	DQ470959	DQ471007	DQ470909
Cordana abramovii	PE 0053-24a	KF833358		
Cordana inaequalis	CBS 508.83	HE672157		
Coronophora gregaria	ANM1555			FJ968938.1
Corynascella inaequalis	CBS 284.82			HQ871839
Corynespora cassiicola	CBS 100822	GU301808	GU296144	GU371742
Corynespora smithii	CABI 5649b	GU323201		GU371783
Cryptendoxyla hypophloia	WM10.89	HQ014708		
Cryptodiaporthe aesculi	AFTOL_ID_1238	DQ836905	DQ836899	DQ836892
Cryptosphaerella elliptica	SMH4722	FJ968974		FJ968944
Cucurbitaria berberidis	MFLUCC 11-0387	KC506796	KC506800	
Curvularia brachyspora	MFLU 14-0013	KU746805	KU746807	KU746809
Cyphelium inquinans	Tibell 22283 (UPS)	AY453639	U86695	
Cyphelium tigillare	Tibell 22343 (UPS)	AY453641	AF241545	
Cyphellophora laciniata	AFTOL-ID 1033	EF413619	EF413618	
Cystocoleus ebeneus	L161	EU048578	EU048571	
Cytospora elaeagni	CFCC_89633	KF765693		KU710956
Dermea acerina	CBS 161.38	DQ247801	DQ247809	DQ247791
Diaporthe eres	AR3519	AF362565		
Diaporthe phaseolorum	NRRL_13736			AY641036
Diatrype disciformis	AFTOL-ID 927	DQ470964	DQ471012	DQ470915
Diatrype palmicola	MFLUCC 11-0020	KP744482	KP753950	
Didymella exigua	CBS 183.55	JX681089	EU754056	GU371764
Didymosphaeria rubi-ulmifolii	MFLUCC 14-0023	KJ436586	KJ436588	
Dothiora cannabinae	AFTOL-ID 1359	DQ470984	DQ479933	DQ470936
Echinosphaeria_canescens	SMH 4791	AY436403		

Species	Voucher/culture	Accession numbers		
		LSU	SSU	RPB2
Elsinoe centrolobi	CBS 222.50	DQ678094	DQ678041	
Elsinoe fawcettii	CPC 18535	JN940382	JN940559	
Elsinoe phaseoli	CBS 165.31	DQ678095	DQ678042	KT216560
Elsinoe verbenae	CPC 18561	JN940391	JN940562	
Endomeliola dingleyae	PDD 98304	GU138866		
Endothia gyrosa	AFTOL-ID 1223	DQ470972	DQ471023	DQ470926
Exserticlava vasiformis	TAMA 450	AB753846		
Extremus antarcticus	CCFEE 5312	KF310020		KF310086
Gelasinospora tetrasperma	CBS 178.33	DQ470980	DQ471032	DQ470932
Gnomonia gnomon	CBS 199.53	AF408361	DQ471019	DQ470922
Gondwanamyces capensis	CMW997	KM495391		
Gondwanamyces proteae	CMW738	KM495393		
Helicascus nypae	BCC 36751	GU479788	GU479754	GU479826
Helminthosphaeria hyphodermae	SMH4192	KF765608		
Hydropisphaera erubescens	ATCC 36093	AY545726	AY545722	AY545731
Hypocrea americana	AFTO-ID 52	AY544649	AY544743	
Irenopsis walsurae	MFLU13-0621	KT021648	KT021648	
Irenopsis cornuta	VIC32058	KC618642	KC618657	
Irenopsis vincensii	VIC:31751	JX133163		
Jobellisia guangdongensis	GD14-4	JN936990		
Jobellisia luteola	SMH2753	AY346286		
Jugulospora rotula	ATCC 38359	AY346287		AY780178
Julella avicenniae	BCC 20173	GU371822	GU371830	GU371786
Karschia cezannei	Cezanne-Eichler 7453	KP456153		
Katumotoa bambusicola	KT 1517a	AB524595	AB524454	AB539095
Kellermania yuccigena	CBS 131727	KF766356	KF766272	
Kylindria peruamazonensis	CBS 838.91	GU180638	GU180609	GU180656
Labrocarpon canariense	Ertz 16308 (BR)	KP456157		
Lachnum virgineum	CBS:122031	AY544646	AY544688	DQ470877
Lentithecium fluviatile	CBS 123090	FJ795450	FJ795492	FJ795467
Leptosphaeria doliolum	MFLUCC:151875	KT454719	KT454734	
Leptosphaerulina australis	CBS 317.83	EU754166	GU296160	GU371790
Leptoxyphium cacuminum	MFLUCC10-0049	JN832602	JN832587	
Leptoxyphium cacuminum	MFLUCC10-0049	JN832602	JN832587	
Leucostoma niveum	AR3413	AF362558		
Lindra thalassiae	JK 5090A	DQ470947	DQ470994	DQ470897
Lophiotrema nucula	CBS 627.86	GU301837	GU296167	FJ795463
Lophium mytilinum	AFTOL-ID 1609	DQ678081	DQ678030	DQ677979
Lulworthia fucicola	ATCC 64288	AY878965	AY879007	
Magnaporthe salvinii	M21	JF414862	JF414887	
Manglicola guatemalensis	BCC20157	FJ743450	FJ743444	
Massarina eburnea	CBS 473.64	GU301840	GU296170	GU371732
Mazzantia napelli	AR3498	AF408368		
Melanomma pulvis-pyrius	CBS 371.75	GU301845	FJ201989	GU371798
Melanospora tiffanii	ATCC 15515	FJ748915	AY015619	AY015637
Melanospora zamiae	ATCC 12340	U17405	AY046578	AY046580
Melaspileopsis cf. diplasiospora	Ertz 16247 (BR)	KP456164		
Meliola centellae	VIC:31244	JQ734545		
Meliola thailandicum	MFLU 15-0379	KR868696		
Microascus trigonosporus	AFTOL-ID 914	DQ470958	DQ471006	DQ470908
Microsphaeropsis_olivacea	CBS 233.77	GU237988		KT389643
Microthyrium buxicola	MFLUCC 15-0212	KT306551	KT306549	
Microthyrium microscopicum	CBS 115976	GU301846	GU296175	GU371734
Mollisia cinerea	AFTOL-ID 76	DQ470942	DQ470990	DQ470883
Monilinia fructicola	AFTOL-ID 169	AY544670	AY544714	DQ470889
Murispora rubicunda	IFRD 2017	FJ795507	GU456308	
Muyocopron dipterocarpi	MFLUCC:14-1103	KU726966	KU726969	
Muyocopron lithocarpi	MFLUCC:14-1106	KU726967	KU726970	
Myriangium duriaei	CBS 260.36	NG_027579	AF242266	KT216528
Myriangium duriaei	CBS 260.36	NG_027579	AF242266	KT216528

Species	Voucher/culture	Accession numbers		
		LSU	SSU	RPB2
Myriangium hispanicum	CBS 247.33	GU301854	GU296180	GU371744
Mytilinidion rhenanum	CBS 135.45	FJ161175		
Natarajania indica	GUFCC_5240	HM171321		
Natipusilla decorospora	AF236-1a	HM196369	HM196376	
Natipusilla naponensis	AF217-1a	HM196371	HM196378	
Nectria cinnabarina	CBS 114055			DQ522456
Neocylindroseptoria pistaciae	CBS 471.69	KF251656		KF252161
Nitschkia tetraspora	GKML148N	FJ968987		
Ophioceras aquaticus	IFRDCC 3091	JQ797433	JQ797435	
Ophioceras commune	M91	JX134687	JX134661	
Ophiocordyceps sinensis	YN09 64	JX968033	JX968028	JX968013
Ophiostoma piliferum	AFTOL-ID 910	DQ470955	DQ471003	DQ470905
Parabambusicola thailandica	MFLUCC 11-0183	KP744490	KP753955	
Phaeodimeriella cissampeli	MFLU 16-0558	KU746806	KU746808	KU746810
Phaeotrichum benjaminii	CBS 541.72	AY004340	AY016348	GU357788
Phyllachora graminis	UME 31349		AF064051	
Phyllopsora sp.	AFTOL-ID 84	KF157990	KF157978	
Piedraia hortae	CBS 480.64	GU214466		
Plagiostoma euphorbiae	CBS 340.78	AF408382	DQ862055	DQ368643
Platystomum crataegi	MFLUCC 14-0925	KT026109	KT026113	
Pleomassaria siparia	AFTOL-ID 1600	DQ678078	DQ678027	DQ677976
Pleospora herbarum	IT 956	KP334709	KP334729	KP334733
Pleurostoma ootheca	CMU 23858	AY761079	AY761074	
Pleurostomophora ochracea	CBS 131321	JX073274	JX073269	
Pleurostomophora richardsiae	CBS 270.33	AY761080	AY729812	HQ878607
Podosordaria tulasnei	CBS 128.80	KT281897		
Preussia funiculata	CBS 659.74	GU301864	GU296187	GU371799
Proxipyricularia zingiberis	HYZiM201-1-1-1	KM484986		
Pseudallescheria boydii	CBS 108.54	EF151315		
Pseudomassariosphaeria bromicola	IT-1333	KT305994	KT305996	
Pseudoproboscispora caudae-suis	A336-2D	AY094192		
Pseudostrickeria muriformis	MFLUCC_13-0764	KT934254	KT934258	
Pyricularia borealis	CBS 461.65	KM009150	DQ341489.1	
Ramularia endophylla	CBS 113265	KF251833	EU167569	KF252332
Rasutoria pseudotsugae	rapssd	EF114704	EF114729	
Rasutoria tsugae ratstk	ratstk	EF114705	EF114730	
Remispora maritima	BBH28309	HQ111012	HQ111002	HQ111041
Salsuginea_ramicola	KT 2597.1	GU479800	GU479767	GU479833
Schizothyrium pomi	CBS 406.61	EF134949	EF134949	
Scortechinia acanthostroma	SMH1143	FJ968988		FJ968948
Scortechiniellopsis leonensis	GKM1269	FJ968993	FJ968933	
Sillia ferruginea	AR_3440	AR 3440		
Slopeiomyces cylindrosporus	CBS 609.75	KM485040		KM485158
Sordaria fimicola	CBS 508.50	AY681160		
Stachybotrys chlorohalonata	UAMH6417	AY489712	AY489680	
Stictographa lentiginosa	Ertz 17447 (BR)	KP456169		
Sympoventuria capensis	CBS 120136	KF156104	KF156094	
Teratosphaeria fibrillosa	CBS 121707	KF902075	GU296199	
Tirisporella beccariana	BCC36737	JQ655450	JQ655454	
Trichodelitschia munkii	Kruys 201 (UPS)	DQ384096	DQ384070	
Tubeufia chiangmaiensis	MFLUCC 11-0514	KF301538	KF301543	
Uwebraunia commune	NC1 32C1d	JQ622093		
Valsa_ambiens	AR3516	AF362564		
Venturia inaequalis	CBS 476.61	GU456336		
Vialaea mangifia	MFLUCC 12-0808	KF724975		
Xenolophium_applanatum	CBS 123127	GU456330	GU456313	GU456355
Xylaria hypoxylon	CBS 122620	KM186301		
Zeloasperisporium hyphopodioides	CBS 218.95	EU035442		
Zeloasperisporium siamemse	IFRDCC 2194	JQ036228	JQ036223	
Zeloasperisporium wrightiae	MFLUCC 15-0225	KT387737	KT387738	

Results

Phylogenetic analyses

Phylogenetic analyses based on the LSU, SSU, and RPB2 sequence data of Dothideomycetes (Fig. 5) indicate that the species in Asterinales is placed within Dothideomycetes, and is distinct from other orders in the Dothideomycetes with high support (100% ML). Asterinales is closely related to Botryosphaeriales, but this relationship is not well-supported. The order Capnodiales contains the largest family of sooty moulds which is Capnodiaceae. The order Capnodiales was represented by 12 strains in this study, consequently appear to be well-resolved within Dothideomycetes with high bootstrap support (100% ML), and they formed a sister group to Myriangiales (83% ML). The order *Microthyriales* comprises four representative strains, which clustered within the Dothideomycetes (100% ML). *Microthyriales* is related to the clade of *Natipusillales*, which is fresh water fungi and fungal epiphytes *Zeloasperisporiales*, but its affinities to other orders are not well-resolved. Zeloasperisporiales species clustered with 100% ML support and are closely related to Natipusillales (84% ML), but as a distinct lineage from Microthyriales. However, Natipusillales and Zeloasperisporiales have very different morphology and habitats (Hongsanan et al. 2015b). The Sordariomycetes tree generated by maximum likelihood analysis from combined LSU, SSU, and RPB2 sequence data indicates that the Meliolales clade includes seven species from the family Meliolaceae, which are grouped and placed in Sordariomycetes with high support (100% ML), which is congruent to the results of Hongsanan et al. (2015a) and Maharachchikumbura et al. (2015, 2016). Meliolales is closely related to the family Cephalothecaceae, which is placed as family *incertae sedis* in Sordariomycetes.

Divergence time estimates

In this study, the mean estimated dates are quite similar to previous studies (Beimforde et al. 2014; Pérez-Ortega et al. 2016). The MCC tree of Dothideomycetes provided by BEAST indicates that the divergence time estimated for Dothideomycetes and Lecanomycetes is 334 Mya (320−368), in the Carboniferous. The strains of *Calicium*, *Cyphelium* and *Phycia* grouped to represent the class Lecanoromycetes, which has an estimated date of 96 Mya (62−138) (Fig. 7). The calibrations for Lecanoromycetes in our analysis resulted in an estimated crown date for Dothideomycetes at 315 Mya (278−348), during the Carboniferous. The divergence times of fungal epiphytes within the class Dothideomycetes estimated in this study are shown in Table 2. The order Asterinales was estimated with a crown date at 188 Mya (130−248), during the Jurassic. Furthermore, our analysis demonstrated that Asterinales shared the most common ancestor with Capnodiales, Dothideales, and Myriangiales at 270 Mya (226−315), in the Permian. The order Capnodiales diverged from Myriangiales at 205 Mya (166−248), in the terminal of the Triassic, with an estimated crown date at 166 Mya (127−205), during the Jurassic. Microthyriales split from Venturiales at 242 Mya (199−285), in the Triassic, with an estimated crown date at 181 Mya (133−230), in the Jurassic. The split between Zeloasperisporiales and Natipusillales is estimated at 195 Mya (148−243), in the early Jurassic. Their life styles are very different, Zeloasperisporiales are fungal epiphytes, while Natipusillales are freshwater fungi. The estimated crown date at 73 Mya (38−113) for Zeloasperisporiales and 69 Mya (34−111) for Natipusillales, is during the Cretaceous; however, they can be older once sufficient data is available. The MCC tree of Sordariomycetes provided by BEAST dates the split between Sordariomycetes and Leotiomycetes at 314 Mya (300−355), during the Carboniferous. The estimated date crowns at 283 Mya (235−331) for Sordariomycetes, in the Permian. The Meliolales crowns group shared the most common ancestor with Cephalothecaceae, Chaetosphaeriales, Coniochaetales, Cordanales, and Phyllachorales at 203 Mya (157−247), at the end of the Triassic. The Meliolales crown group is estimated at 135 Mya (93−177), during the Cretaceous.

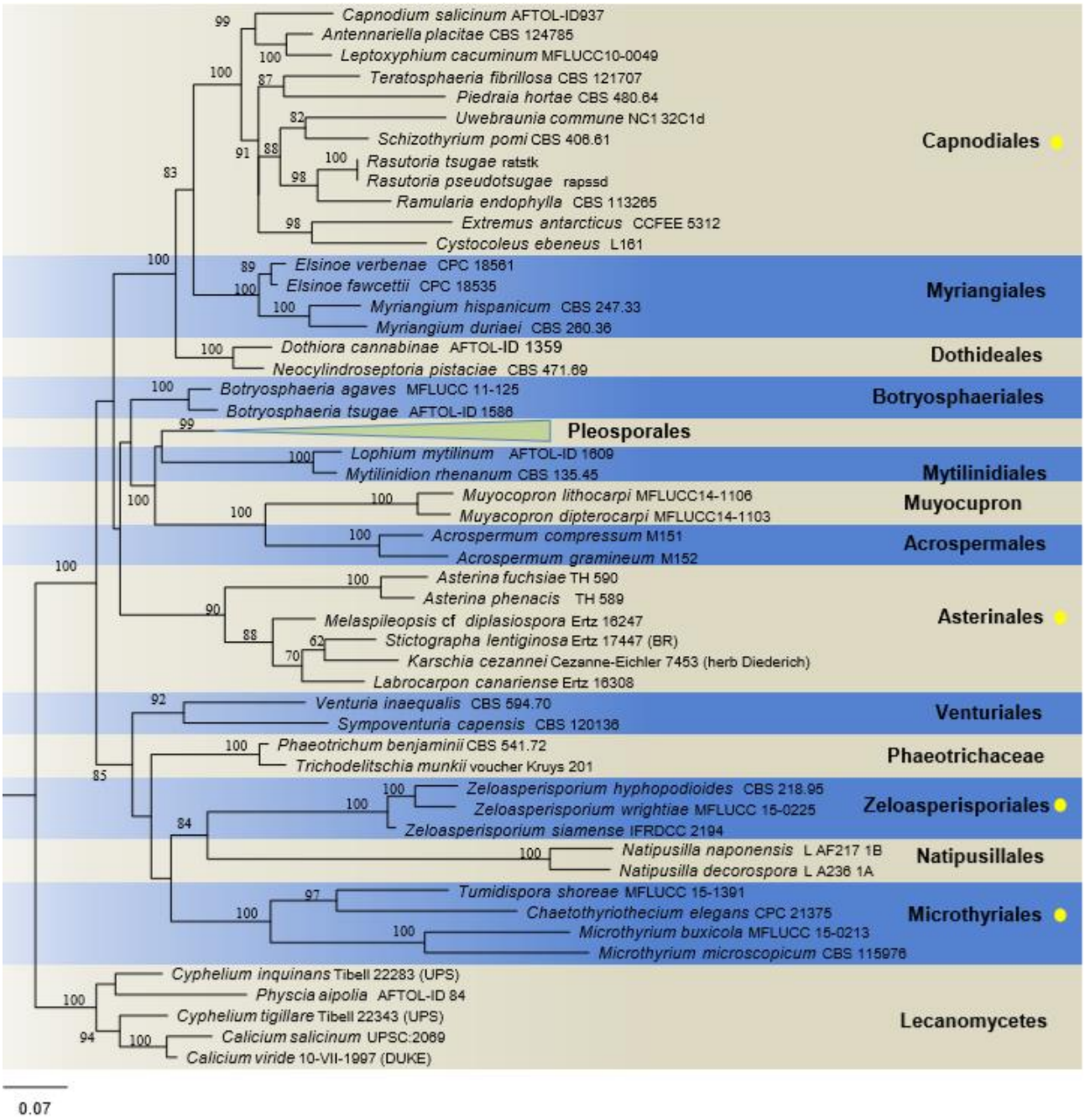

Fig. 5 – Phylogenetic tree for taxa of Dothideomycetes generated from maximum likelihood analysis of LSU, SSU, and RPB2 sequence data, including representative strains of fungal epiphytes. Bootstrap values above 50 are shown. Strain numbers are indicated after species names. The foliar epiphytes discussed in this study are marked with yellow dots.

Discussion

Reconstruction of the evolutionary lineages

The relaxed molecular clock allows more elastic modeling of rate heterogeneity, thus providing well-resolved phylogenetic results (Drummond et al. 2006). In this study, we have used only the fossils and calibration points estimated from previous studies that are reliable for our objectives. According to our target groups belonging to Dothideomycetes and Sordariomycetes, the divergence times of both classes were estimated by Pérez-Ortega et al. (2016). Thus, we used some calibration points from Pérez-Ortega et al. (2016) in our analysis, and also used the calibrations from fossil records in Dilcher (1965) and Beimforde et al. (2014). Although, there are 13 available fossil records for Ascomycota, we could not use them all in these analyses because each is suitable

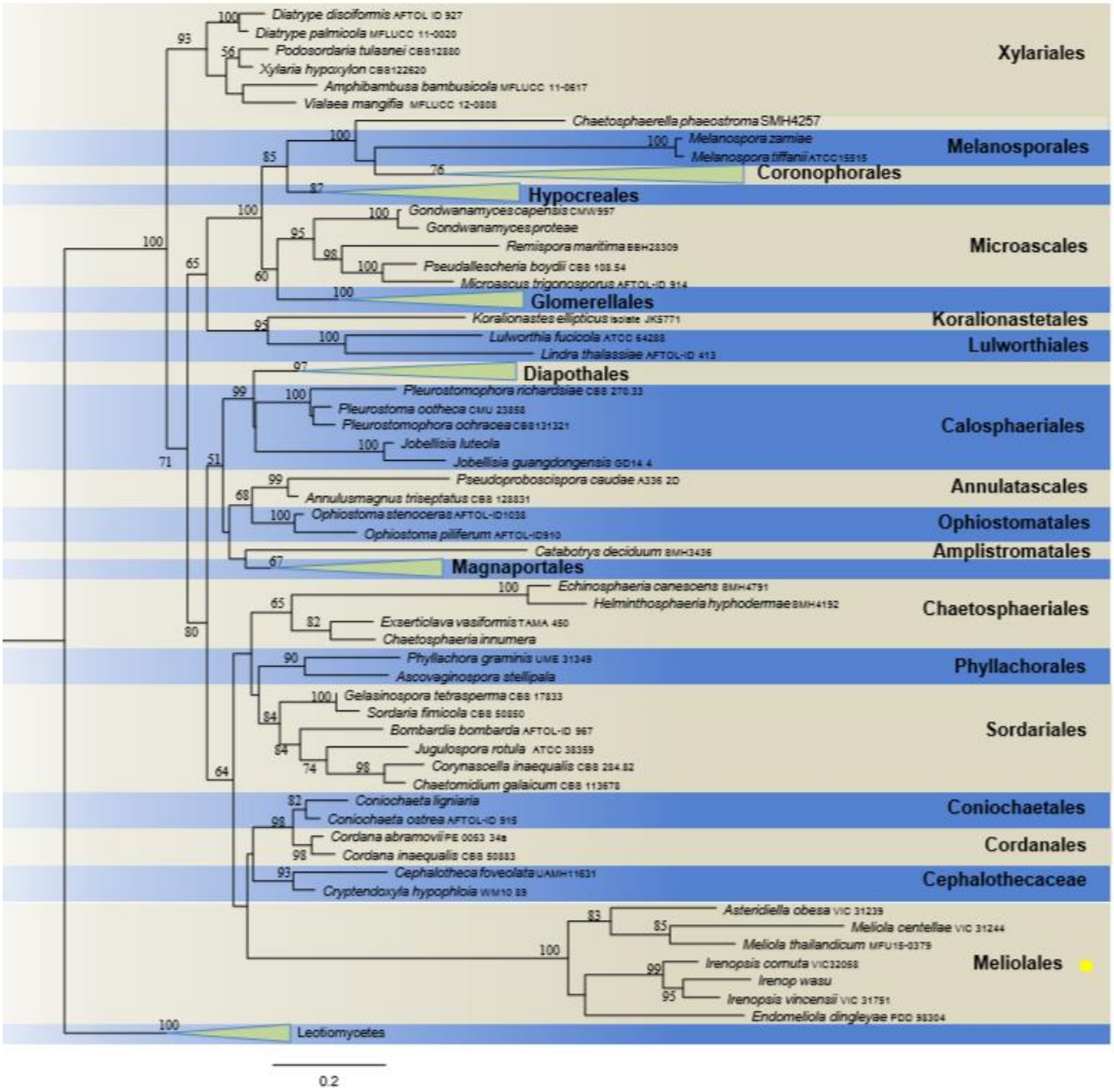

Fig. 6 – Phylogenetic tree of Sordariomycetes generated from maximum likelihood analysis of LSU, SSU, RPB1 and RPB2 sequence data, including representative strains of fungal epiphytes. Bootstrap values above 50 are shown. Strain numbers are indicated after species names. The foliar epiphytes discussed in this study are marked with yellow dot.

for focusing on individual groups of Ascomycota. They will however, provide potential calibrations when sufficient molecular data is available in the future (Beimforde et al. 2014). Therefore, we only used fossils that appeared to be identical to the modern specimens. Hence, we used fossils calibrations from Asterinales, Capnodiales and Microthyriales, and *Calicium* in Lecanoromycetes, plus the calibration points estimated for the Dothideomycetes crown group (Pérez-Ortega et al. 2016) in the maximum clade credibility (MCC) tree for Dothideomycetes. We used fossil calibrations for Meliolales, including the calibration points estimated for Sordariomycetes crown group and the split between Sordariomycetes and Leotiomycetes from Pérez-Ortega et al. (2016) in the MCC tree for Sordariomycetes.

Table 2 Maximum likelihood analysis using internal calibrations from the fossils evidence and previous calibrations. Divergence times are listed in millions of years (Mya).

	Time (Mya)	Geological period	Pérez-Ortega et al. (2016)	Beimforde et al. (2014)
Asterinales crown group	188 (130−248)	Jurassic	-	-
Capnodiales	166 (127−205)	Jurassic	-	-
Capnodiales + Myriangiales	205 (166−248)	Triassic−Jurassic	-	-
Dothideomycetes crown group	315 (278−348)	Carboniferous	290 (241–349)	350 (273–459)
Meliolales crown group	135 (93−177)	Cretaceous	-	-
Microthyriales crown group	181 (133−230)	Jurassic	-	-
Sordariomycetes crown group	283 (235−331)	Permian	256 (202–306)	260 (207–339)
Sordariomycetes + Leotiomycetes	314 (300−355)	Carboniferous	290 (242–353)	315 (255–414)
Zeloasperisporiales crown group	73 (38−113)	Cretaceous	-	-
Zeloasperisporiales + Natipusillales	195 (148−243)	Jurassic	-	-

The phylogenetic tree with divergence estimation was topologically quite similar to the maximum likelihood phylogenetic tree in most of the major lineages within Dothideomycetes and Sordariomycetes (Figs. 5, 6). According to our target groups, topological differences were found in the clade of Asterinales and Microthyriales, but did not affect to the position of each species in other target groups. By using the maximum likelihood analysis (ML), Asterinales shared the most recent common ancestor (MRCA) with Botryosphaeriales, although such relationships were not clearly statistically supported, and are probably due to inadequate taxon sampling in the dataset (Fig. 7). The molecular clock tree provided by BEAST suggested that Asterinales is closely related and shared the most common ancestor with Dothideales, Myriangiales and Capnodiales in the Permian (Fig. 7), but it was unique, a supposition supported by moderate Bayesian posterior probability, based on available sequence data and fossil records. The speciation event in Asterinales and in Dothideales, Myriangiales and Capnodiales occurred in a different geological period. This is probably because numerous fungal taxa of Dothideomycetes have not yet been discovered and sequenced. More collections of species in Dothideomycetes are needed to resolve their evolutionary relationships. The Capnodiales split from Myriangiales at the end of the Triassic and the beginning of the Jurassic; however, the Myriangiales crown group is much younger than the Capnodiales crown group. The order Microthyriales is morphologically similar to Zeloasperisporiales in having thyriothecia and in addition, they are foliar epiphytes. On the other hand, Venturiales are saprobes or parasites on various plants with ascomata. The order Microthyriales shared the most common ancestor with Venturiales in the MCC tree, while sharing the most common ancestor with Natipusillales and Zeloasperisporiales in maximum likelihood analysis based on available fossil records and sequence data. The moderate support for both Asterinales and Microthyriales is mainly due to the insufficient taxon sampling. More collections are needed to fulfill the incomplete data.

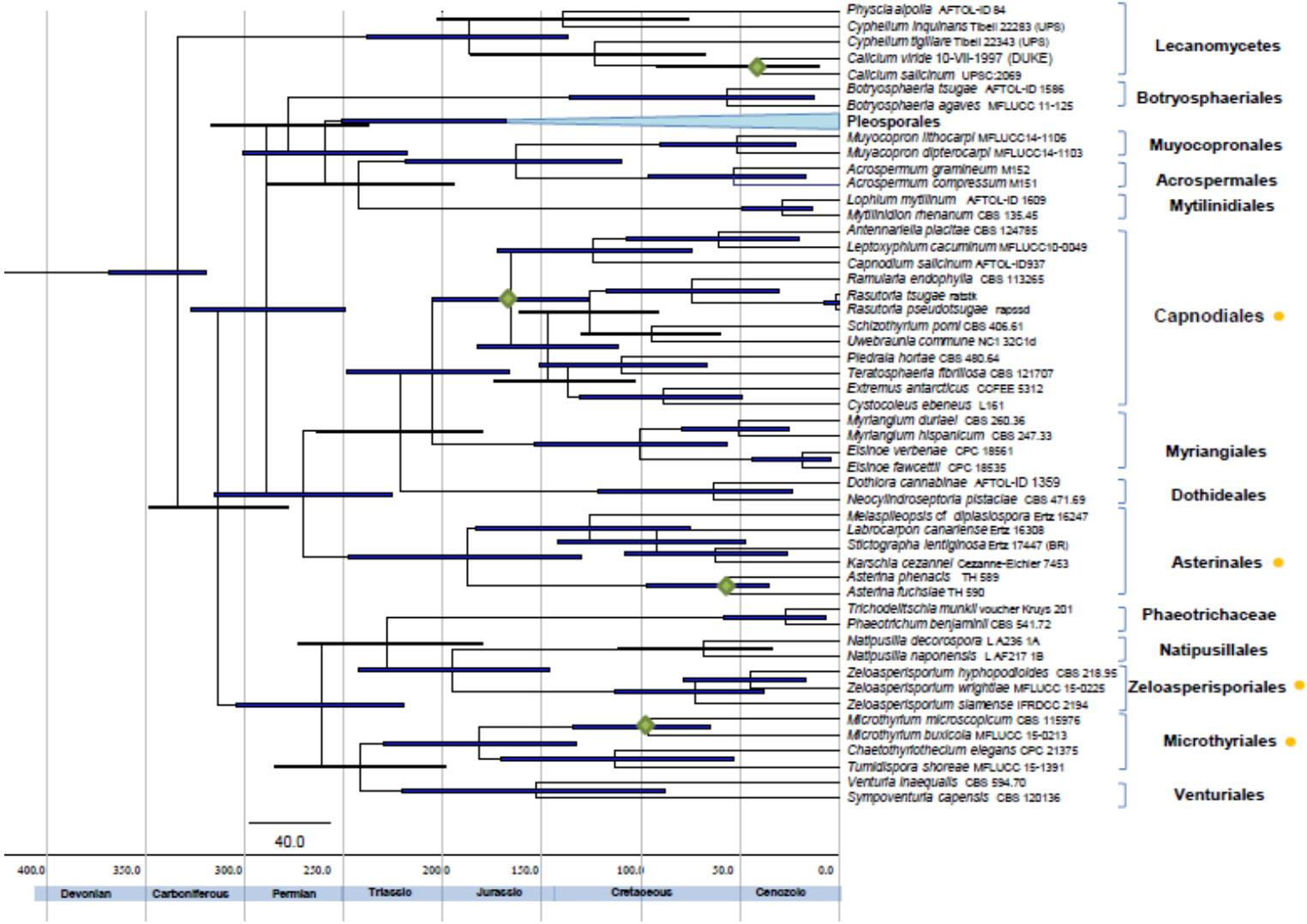

Fig. 7 – *Divergence time estimations* of Dothideomycetes tree obtained from a Bayesian approach (BEAST) using internal calibrations from fossil minimum age constraints and previous studies. Bars correspond to the 95% highest posterior density (HPD) intervals. The fossil minimum age constraints and second calibrations used in this study are marked with green dots. Geological periods are indicated at the base of the tree. The foliar epiphytes discussed in this study are highlighted in purple.

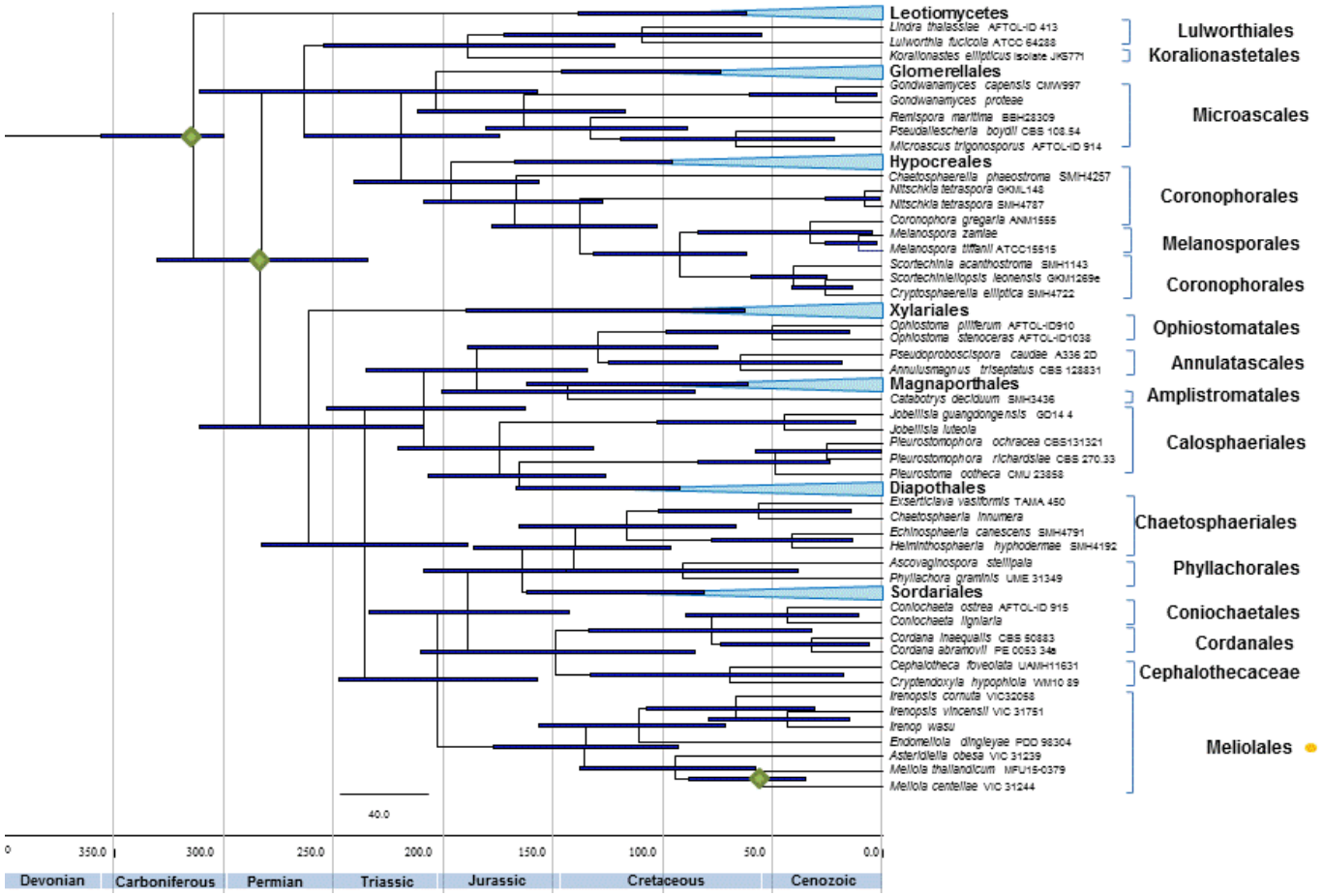

Fig. 8 – *Divergence time estimations* of Sordariomycetes tree obtained from a Bayesian approach (BEAST) using internal calibrations from fossil minimum age constraints and previous studies. Bars correspond to the 95% highest posterior density (HPD) intervals. The fossil minimum age constraints and second calibrations used in this study are marked with green dots. Geological periods are indicated at the basal of the tree. The foliar epiphytes discussed in this study are highlighted in purple.

The phylogenetic tree generated by maximum likelihood analysis of Sordariomycetes indicated that Meliolales is closely related to the clade comprising Cephalothecaceae, Coniochaetales and Cordanales, but is a distinct order, but the relationships are weakly supported. This result is similar to those of Hongsanan et al. (2015a) and Maharachchikumbura et al. (2015, 2016). The MCC tree for Sordariomycetes indicates that the Sordariomycetes crown group existed in the Permian. The Meliolales crown group is estimated to have evolved in the Cretaceous and its most common ancestors are Cephalothecaceae, Chaetosphaeriales, Coniochaetales, Cordanales, Phyllachorales, and Sordariales. However, these five orders clustered together, with Meliolales forming a distinct adjacent lineage. Accordingly, the Meliolales can be considered as relatively quite young, the crown group of this order is slightly distant from others.

Divergence time estimates

Divergence times estimated from our study used five calibration points for Dothideomycetes and three calibrations points for Sordariomycetes and generally correspond with the results of Beimforde et al. (2014). In the analysis of Dothideomycetes, our data included more calibration points within Dothideomycetes and Sordariomycetes. Furthermore, we did not use the external taxon in another phylum (e.g. *Basidiomycetes*), which may result to high ages in some lineages of Ascomycota. Compared with other studies, our results indicate neither much younger nor older when compared to Beimforde et al. (2014) and Pérez-Ortega et al. (2016).

Earlier studies used few representative strains from *Dothideomycetes* and *Sordariomycetes* to estimate the divergence times as aims of the studies were different. Thus, we are unable to compare individual lineages between our analysis and earlier studies. Divergence times estimated in this study correspond to Beimforde et al. (2014) and Pérez-Ortega et al. (2016) in the estimated date for the Dothideomycetes crown group. However, our analysis produced an older origin than Pérez-Ortega et al. (2016), while younger than Beimforde et al. (2014). The divergence time estimated for the Sordariomycetes crown group in our analysis was older than in Beimforde et al. (2014) and Pérez-Ortega et al. (2016), but younger than the estimated date of Gueidan et al. (2011). However, the Sordariomycetes crown group in the Permian in our analysis was the same as Beimforde et al. (2014) and Pérez-Ortega et al. (2016). The split between Leotiomycetes and Sordariomycetes estimated date in our analysis is most similar to Beimforde et al. (2014) (see Table 2), it is however older than the results from Prieto and Wedin (2013) and Pérez-Ortega et al. (2016). It is difficult to compare inferred ages estimated in each study due to various reasons such as genes under study, model of evolutionary rates, and parameter setting. The dating is based on fossil records and therefore depends on the period that a fossil specimen was discovered. In fact, the group may have evolved long before this period. Similarly, the dating resolved by the molecular clock is an indication of which taxa evolved on a chronological scale, but may be inaccurate due to insufficient data.

Evolution of foliar epiphytes

Foliar epiphytes in totally unrelated classes evolved at least in Permian (298.9 to 252.17 Mya) (Figs 7, 8). This is based on evidence from sequence data from representative foliar epiphytes and fossil calibrations. The estimated crown dates of most fungal epiphytes are in the Jurassic, with only Meliolales and Zeloasperisporiales in the Cretaceous. The evolution of the most closely related groups of fungi and foliar epiphytes occurred during the Triassic to Jurassic.

The order Asterinales is represented in this study by two strains of foliar epiphytes from Asterinaceae, which shared the most recent common ancestor with four lichenicolous strains of Melaspileaceae. This clade was synonymized under Asterotexiales based on the phylogenetic analysis of Ertz et al. (2016). Asterinales was represented as a clade unrelated to Asterotexiales in their study. Hyde et al. (2016) noted that both clades contain members of Asterinaceae and Parmulariaceae, thus they treated the older clade as Asterinales *sensu stricto*. Asterinaceae and Parmulariaceae were reported as polyphyletic by Inácio and Cannon (2008) and Guatimosim et al. (2015). The group of Asterinales *sensu stricto* was included in our analyses.

The two families Asterinaceae and Melaspileaceae belong in Asterinales *sensu stricto* (≡ Asterotexiales) based on phylogenetic analysis (Ertz & Diederich 2015). This is similar to our analyses (Fig. 5). Although Melaspileaceae species are lichenicolous, many Melaspileaceae strains lacking sequence data are saprobic or weakly lichenized (Ertz & Diederich 2015). Ertz & Diederich (2015) expect that many of the remaining Melaspileaceae species will be placed in Asterinales and presumably shared some characters of ascomata and life style (Ertz & Diederich 2015).

The orders Asterinales, Capnodiales, Dothideales and Myriangiales have the same common ancestor, with possible origins in the Permian. The uniqueness of superficial hyphae with appressoria, and thyriothecia with "star"-like openings are typical of foliar epiphytes in Asterinales. The lichenicolous (Melaspileaceae) in Asterinales also share with Asterinaceae, some characters such as clavate with 8-spored asci and 1-septate, brown ascospores. Some species in Capnodiales and Myriangiales have similar flattened ascomata, as Asterinaceae species, but lack "star"-like openings and superficial hyphae with appressoria. However, they are unrelated to the Asterinales clade. The divergence time estimates for Asterinales are approximately in the Jurassic, while the split nodes of Dothideales, Capnodiales, and Myriangiales are approximately at the middle of Triassic, which suggest Asterinales evolved later.

The origin of land plants are major structural components of terrestrial ecosystems which led to important changes in the environment (Kenrick & Crane 1997, Lewis & McCourt 2004; O'Kelly 2007), and is dated approximately at 476−432 Mya (McCourt et al. 2004, Leliaert et al. 2011). Because the crown node estimated for *Asterina* appears in the Cretaceous to Cenozoic, thus we presume that foliar epiphytes in Asterinales may already have been associated with plants at least in the Cretaceous. No fossil for Asterinales has so far been discovered in pre-Cretaceous.

In our study, Capnodiales comprise three strains of Capnodiaceae, which are closely related to the clade containing Dissoconiaceae, Euantennariaceae, Extremaceae, Mycospherellaceae, Schizothyriaceae, and Teratosphaeriaceae. The family Capnodiaceae has unique morphological characters. Foliar epiphytes in Capnodiaceae feed on honey dew excreted by insects (Chomnunti et al. 2011, 2014). Others families within Capnodiales presumably evolved from Capnodiaceae (Fig. 7). Some groups in Capnodiales have the ability to reproduce and survive in specific habitats, such as the rock-inhabiting fungi (i.e. *Extremus antarcticus* and *Cystocoleus ebeneus*) occur at the base of Capnodiales. Over time, species might have diverged and adapted to changing environments several times.

Species of Capnodiales mostly have superficial ascomata, but they are immersed in some species of Mycosphaerellaceae and Teratosphaeriaceae (represented by *Ramularia endophylla* and *Teratosphaeria fibrillosa* in our analysis, Fig. 7). *Schizothyrium pomi* (Schizothyriaceae) and *Uwebraunia commune* (Dissoconiaceae) are flyspeck and sooty blotch fungi, with have completely superficial ascomata, appearing as small black dots, on the cuticle of plants. The superficial hyphae of foliar epiphytes from these families can coat the surface of plants. *Piedraia hortae* (Piedraiaceae) is a pathogen in humans causing 'black piedra' in hair. It is therefore important to understand how this species evolved and became pathogenic, but we are unable to establish the evolutionary relationships of Piedraiaceae with our dataset herein.

Sooty moulds in Capnodiaceae live on plant surfaces and feed on the honeydew from insects (Hughes 1976, Faull et al. 2002, Auclair 1963). The first aphid fossil was dated to the middle of Triassic (Szwedo & Nel 2011), and sucking insects with sucking beaks are known from the Carboniferous (Labandeira 2006, Nel et al. 2013). Thus, associations between sooty moulds and honeydew-producing insects may have evolved before the Cretaceous (Schmidt et al. 2014). Foliar epiphytes in Capnodiales were also presumably associated with plants at least in the early Cretaceous based on the oldest fossil evidence from Metacapnodiaceae. This family provides the fossil calibration for the crown node of Capnodiales with an estimated date in the Cretaceous (Fig. 7). No fossil evidence for Capnodiales has been found before the Cretaceous. Based on evidence from the earliest diverging lineages, *Phaeotheca* and *Comminutispora* (Crous et al. 2007), the ancestral nutritional mode of ancestors of Capnodiales are likely to have been saprobic. However, Ismail et al. (2016) indicated that the ancestral nutritional mode of Capnodiales ancestor is likely to

have been plant parasites. The different families of Capnodiales appear to have evolved several times, with different lifestyles (e.g. *Capnodium salicinum* as saprobes and *Piedraia hortae* as human pathogens), however, the taxa in the later diverging clades tend to be strictly necrotrophic plant pathogens (Crous et al. 2007). *Capnodiales* will probably comprise numerous divergent groups with different lifestyles, once adequate sequence data from a wider number of species are analysed.

The Meliolales crown group diverged in the Cretaceous (Fig. 8). Species in this order are mostly biotrophic parasites, as they have superficial hyphae with hyphopodia. They form a distinct lineage at the base of Sordariomycetes. Thus, they were represented as the subclass Meliolomycetidae (Maharachchikumbura et al. 2015, 2016). The ancestral nutritional mode of Meliolales is likely to have been saprobic or weakly parasitic; this presumption is based on the relationships between Meliolales and other ancestral saprobic orders of Sordariomycetes. The lineage of Meliolales has therefore evolved to be specific plant pathogens and are not saprobes. Within the Meliolales, species have evolved the unique characters of superficial hyphae with hyphopodia, which obtain nutrients from living plants (Hongsanan et al. 2015a). The genus *Meliola* and *Asteridella obesa* evolved from a common ancestor and the former have reduced outer, conical, peridial cells and produce setae on superficial hyphae. The associations between Meliolales and plants presumably evolved at least in the Cretaceous, based on the divergence estimates, although fossil specimens of Meliolales have only been found in the Eocene. This coincides with the major periods of radiation and spread of Angiosperms (Slippers et al. 2013).

In this study, the order Microthyriales form a sister group with Venturiales and share a common ancestor, but this relationship is not well-resolved (Fig. 7). The earlier ancestral node comprises Microthyriales, Natipusillales, Phaeotrichaceae and Zeloasperisporiales. Microthyriales and Zeloasperisporiales are foliar epiphytes, while Natipusillales and Phaeotrichaceae are freshwater fungi and dung fungi, respectively (Fig. 7). The appearance of freshwater and dung fungi in this node demonstrates that different lifestyles have evolved several times (Vijaykrishna et al. 2006). Since Zeloasperisporiales and Microthyriales are a recent lineage, we conclude that foliar epiphytes within this ancestral node have evolved the thyriothecia, later than other foliar epiphytes. As little sequence data is available for Microthyriales, thus we do not discuss the evolution of genus/species here.

Acknowledgements

Kevin D. Hyde thanks the Chinese Academy of Sciences, project number 2013T2S0030, for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany.

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