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Phylogenetic placement of Micropeltidaceae

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Abstract

"Microthyriaceae-like" taxa are fungal epiphytes which appear as black dots on the host surface. The families Micropeltidaceae and Microthyriaceae have been poorly studied, particularly with molecular data, due to the difficulty in obtaining pure cultures. The two families were placed in Microthyriales in many studies based on thyriothecial characters. Two species of Micropeltidaceae (*Micropeltis dendrophthoes* and *M. zingiberacicola*) clustered at the base of Dothideomycetes and were unrelated to Microthyriaceae in previous phylogenetic trees. Their placements were treated as unresolved. We restudied sequence data of Micropeltidaceae and its related strains to clarify the current placement using authentic strains available in GenBank. Phylogenetic analyses generated from maximum likelihood and Bayesian analyses (used ITS, LSU, RPB2, SSU and TEF1 sequence data) including Blast results, indicate that the current placement of Micropeltidaceae is in Ostropales (Lecanoromycetes), although it is not strongly supported. A revised phylogenetic tree for Micropeltidaceae and selected families from Dothideomycetes, Eurotiomycetes, Lecanoromycetes, Leotiomycetes and Sordariomycetes is provided with discussions in this paper.

Key words – Dothideomycetes– Lecanoromycetes– Microthyriales – Phylogeny

Introduction

The family Micropeltidaceae was introduced by Clements & Shear in 1931 as 'Micropeltaceae'. The family represents foliar, biotrophic epiphytes, which mostly can be found on the lower leaf surface as small black dots. Species in this family are characterized by flattened, black-blue or greenish to black thyriothecia, easily removed from the host surface, poorly developed at the base, comprising interwoven hyphae, with a central ostiole, and up to multi trans-septate, hyaline ascospores (Clements & Shear 1931, Batista 1959, von Arx & Müller 1975, Barr 1987, Kirk et al. 2008, Lumbsch & Huhndorf 2010, Wu et al. 2011, Hyde et al. 2013, Hongsanan et al. 2015a). Micropeltidaceae and Microthyriaceae were placed in Microthyriales (Dothideomycetes) based on their flattened, easily removed thyriothecia, and poorly-developed base. However, thyriothecium of Microthyriaceae comprise cuboid or angular cells, arranged in parallel rows from the prominent central ostiole and uniseptate ascospores (Doidge 1942, Müller & von Arx 1962, Luttrell 1973, Barr 1987, Hofmann & Piepenbring 2006, Hofmann 2010, Wu et al. 2011, Hyde et al. 2013, Hongsanan et al. 2015a).

Most of species with Microthyrium-like characters cannot be cultured; therefore, little molecular data is available until now. Schoch et al. (2009) provided a single strain of *Microthyrium microscopicum* (type species of Microthyriaceae) which represented the order Microthyriales as a

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long branch sister to *Stomiopeltis betulae* (considered as Micropeltidaceae) in phylogenetic trees (Wu et al. 2011, Hyde et al. 2013). *Micropeltis zingiberacicola*, *Neomicrothyrium siamense*, *Paramicrothyrium chinensis* and *Stomiopeltis versicolor* were introduced to accommodate as Microthyriaceae-like taxa, but they did not cluster with *Microthyrium microscopicum* in the phylogenetic tree (Wu et al. 2011). Therefore, the placement of Microthyriaceae was not confirmed, while the placement of Micropeltidaceae was unresolved (Wu et al. 2011). Hongsanan et al. (2014a) and Ariyawansa et al. (2015) added a new genus and species in Microthyriaceae based on morphology and molecular data; this supported the status of Microthyriaceae in Dothideomycetes. Although, Hongsanan et al. (2015a) established a new species in Micropeltidaceae with sequence data, their phylogenetic tree only distinguished the new species. Presently, there are only two species of Micropeltidaceae that were established with sequence data, thus the placement of Micropeltidaceae has not been well resolved until now.

In this study, we use sequence data from previous studies to restudy the placement of Micropeltidaceae by Blast searches and phylogenetic analysis of combined ITS, LSU, RPB2, SSU, and TEF1 sequence data. The placement of this family is presented in the phylogenetic tree (Figs 1, 2), and the discussion of the new placement is provided.

Materials & Methods

Phylogenetic analyses

Sequence data of Micropeltidaceae from previous studies were downloaded from GenBank to supplement the dataset (Table 1), and representative strains of major classes in Ascomycota were also downloaded. The data sets were aligned for each gene partition using MAFFT (Katoh et al. 2009), and checked manually using Bioedit (Hall 1999). *Schizosaccharomyces pombe* was selected as the outgroup taxon. Maximum likelihood analysis was performed by using raxmlGUIv.0.9b2 for each gene partitioned and combined dataset (Silvestro & Michalak 2012), using GTRGAMMA model of nucleotide substitution, and the search strategy was set to rapid boot strapping. Maximum likelihood bootstrap values equal or greater than 70% are shown as the first set of numbers at the nodes (Fig. 1). The best fit model of evolution was performed by MrModeltest 2.2 (Nylander et al. 2008). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were performed in MrBayes v 3.1.2 by Markov Chain Monte Carlo sampling (MCMC) (Huelsenbeck & Ronquist 2001). The six simultaneous Markov chains were run for 1,000,000 generations, with sampling frequency at 100. The total 10,000 trees were obtained; however, the first 2,000 trees were discarded based on the suggestion from Tracer software. The remaining 8,000 trees were calculated for posterior probabilities (Cai et al. 2006, 2008). The posterior probabilities (PP) equal or greater than 0.90 are given as the second set of numbers at the nodes (Figs 1, 2).

Phylogenetic analysis

The combined analyses of LSU, RPB2, SSU and TEF1 sequence data from representative strains of Dothideomycetes, Eurotiomycetes, Geoglossomycetes, Lecanoromycetes, Leotiomycetes and Orbiliomycetes (Fig. 1) and combined analyses of ITS, LSU, SSU and TEF1 sequence data from Stictidaceae and related families (Fig. 2) were used in this study. The topology of each gene partition is mostly similar to the combined data set, except some unstable strains. Lecanoromycetes Clade includes representative strains of families in Lecanoromycetes. *Micropeltis zingiberacicola* is closely related to *M. dendrophthoes* (100% ML and 1.0 PP), they cluster in Ostropales within Lecanoromycetes and closely related to *Cyanodermella asteris* strain 03HOR06-2-4 and *Cyanodermella oleoligni* strain DTO 301-G1 (100% ML and 1.0 PP). In the Dothideomycetes clade, Microthyriaceae represented by *Microthyrium microscopicum* and *M. buxicola* clusters as a sister group to the clade containing strains from Natipusillales, Venturiales and Zeloasperisporiales (0.9% PP).

Table 1 Taxa used in the phylogenetic analysis and GenBank accession numbers (ITS, LSU, RPB2, SSU and TEF1) and species voucher/culture numbers.

Species	Voucher/culture	*Accession numbers*				
		LSU	SSU	*ITS*	*RPB2*	*TEF1*
Acarospora laqueata	AFTOL-ID 1007	AY640943	AY640984	DQ842014	-	-
Acarosporina microspora	AFTOL-ID 78	AY584643	AY584667	DQ782834	AY584682	DQ782890
Agonimia sp.	AFTOL 684	DQ782913	DQ782885	DQ826742	DQ782874	DQ782917
Agyrium rufum	Wedin 7931	EF581826	EF581823	JX000097	-	-
Agyrium rufum	Buck 48698	EF581825	EF581824	-	-	-
Aigialus grandis	JK 5244A	GU301793	GU296131	-	GU371762	-
Anzia colpodes	Lumbsch 4.VI.04	DQ923651	DQ923622	-	-	-
Apiospora montagnei	AFTOL-ID 951	DQ471018	-	-	DQ470921	DQ842027
Arctomia delicatula	P166	KR017191	KR017335	-	-	KR017563
Arthrorhaphis alpina	Spribille 26526	KP794950	-	-	-	-
Arthrorhaphis citrinella	AFTOL-ID 2341	AY853356	-	-	-	-
Aspergillus fumigatus	ATCC 1022	AY660917	AB008401	KF314726	-	-
Aspergillus nidulans	ATCC 16855	AF454167	ENU77377	-	-	-
Asterina melastomatis	VIC 42822	KP143739	-	-	-	-
Aureobasidium pullulans	CBS 584.75	FJ150942	EU682922	NR_144909	KT693976	FJ157895
Australiasca queenslandica	BRIP 24607	HM237324	-	HM237327	-	-
Baeomyces rufus	P78	KJ462341	KR017260	KJ462264	-	KR017567
Bombardia bombarda	AFTOL-ID 967	DQ470970	DQ471021	-	DQ470923	DQ471095
Botryosphaeria dothidea	AFTOL-ID 946	DQ678051	FJ190612	-	DQ677944	DQ767637
Byssoloma subdiscordans	AFTOL-ID 4884	KJ766538	KJ766696	-	-	-
Calicium salicium	CBS 100898	KF157982	KF157970	-	KF157998	
Calicium viride	10-VII-1997 (DUKE)	AF356670	AF356669	-	-	-
Calycidium cuneatum	Wedin 8034 (S)	JX000083	-	JX000114	-	-
Candelaria concolor	AFTOL-ID 1706	DQ986791	DQ986806	-	-	-
Candelariella reflexa	AFTOL-ID 1271	DQ912331	DQ912309	-	DQ912380	-
Canoparmelia caroliniana	AFTOL-ID 6	AY584634	AY584658	DQ782833	-	DQ782889
Capnodium coartatum	MFLUCC10-0069	JN832614	JN832599	-	-	-
Capnodium coffeae	CBS 147.52	DQ247800	DQ247808	AJ244239	KT216519	
Capronia pilosella	AFTOL-ID 657	DQ823099	DQ823106	DQ826737	DQ840561	DQ840565

Table 1 Continued.

Species	Voucher/culture	*Accession numbers*				
		LSU	SSU	*ITS*	*RPB2*	*TEF1*
Carestiella social	Gilenstam 2437a	AY661682	-	AY661682	-	-
c	MFLUCC 10-0008	HQ895835	-	NR_137768	-	-
Chaenothecopsis montana	HT435	KF157987	KF157975	-	KF158002	-
Chaenothecopsis sitchensis	HT22	KF157988	KF157976	-	KF158003	-
Chaetomidium galaicum	CBS 113678	FJ666361	-	-	FJ666392	-
Chaetosphaerella fusca	GKML124N	FJ968967	-	-	-	FJ969002
Chaetosphaeria innumera	SMH 2748	AY017375	-	-	-	-
Chlorociboria aeruginosa	AFTOL-ID 151	AY544669	AY544713	DQ491501	DQ470886	DQ471053
Cladophialophora carrionii	CBS 260.83	KF928518	AY554285	KF928454	-	EU137234
Clypeosphaeria uniseptata	HKUCC 6349	DQ810219	DQ810255	-	-	-
Coccomyces dentatus	AFTOL-ID 147	AY544657	AY544701	DQ491499	DQ247789	DQ497605
Coccomycetella richardsonii	Baloch SW068	HM244761	HM244737	-	HM244785	-
Coccotrema cucurbitula	-	AF274092	AF274114	AF329162	-	-
Coccotrema maritimum	-	AF329164	AF329163	AF329165	-	-
Coccotrema pocillarium	-	AF274093	AF274113	AF329167	-	-
Cochliobolus heterostrophus	AFTOL-ID 54	AY544645	AY544727	DQ491489	DQ247790	DQ497603
Coenogonium disjunctum	-	AF465443	AF465458	-	-	-
Coenogonium leprieurii	-	AF465442	AF465457	-	-	-
Colletotrichum gloeosporioides	LC0555	JN940412	JN940356	JN943090	-	-
Conidiocarpus caucasicus	GUMH937	KC833050	KC833051	-	-	-
Coniocessia nodulisporioides	Co108	GU553349	-	GU553338	-	-
Crocynia pyxinoides	AFTOL-ID 111	AY584653	AY584677	-	DQ883748	DQ883767
Cryptadelphia groenendalensis	SH12	EU528007	-	-	-	-
Cryptodiscus cladoniicola	RP159	KY661652	KY661694	KY661619	-	-
Cryptodiscus foveolaris	EB88	FJ904671	-	-	-	-
Cryptodiscus gloeocapsa	Vezda 1198	AF465440	AF465456	-	-	-
Cryptodiscus tabularum	EB87	FJ904688	-	FJ904688	-	-
Curvularia brachyspora	AFTOL-ID 354	AF279380	L36995	-	-	-
Curvularia fallax	FSU<DEU>:5293	KF157989	KF157977	-	KF158004	-
Cyanodermella asteris	03HOR06-2-4	KT758843	-	KT758843	KU934214	-

Table 1 Continued.

Species	Voucher/culture	*Accession numbers*				
		LSU	SSU	*ITS*	*RPB2*	*TEF1*
Cyanodermella oleoligni	DTO 301-G1	KX950461	-	KX950434	KX999147	-
Cyphelium inquinans	Tibell 22283	AY453639	U86695	AY450584	-	-
Cyphelium tigillare	Tibell 22343	AY453641	AF241545	AY452497	-	-
Cyphellophora reptans	CBS 120903	KF928542	KF155209	KF928478	-	-
Cyphellophora sessilis	CBS 238.93	KF928523	KC455309	KF928459	KC455289	-
Dermatocarpon miniatum	AFTOL-ID 91	AY584644	AY584668	DQ782837	DQ782863	DQ782893
Dermea acerina	CBS 161.38	DQ247801	DQ247809	-	-	-
Diaporthe eres	AFTOL-ID 935	AF408350	DQ471015	DQ491514	DQ470919	DQ479931
Dissoconium aciculare	CBS 342.82	EU019266	GU214524	NR_119427	-	-
Dothidea sambuci	AFTOL-ID 274	AY544681	AY544722	DQ491505	DQ522854	DQ497606
Elsinoe centrolobi	CBS 222.50	DQ678094	DQ678041	KX887206	KX887089	KX886852
Elsinoe phaseoli	CBS 165.31	DQ678095	DQ678042	KX887263	KX887144	KX886908
Exophiala dermatitidis	AFTOL-ID 668	DQ823100	DQ823107	DQ826738	DQ840562	DQ840566
Glyphopeltis ligustica	Brusse 4947	AY756337	AY756399	-	-	-
Gnomonia gnomon	CBS 199.53	AF408361	DQ471019	AY818956	-	EU221885
Gregorella humida	Pykala 23630	EU360846	EU360867	-	-	-
Harknessia eucalypti	CPC 13643	JQ706215	-	JQ706089	-	-
Hymenelia epulotica	AFTOL-ID 1844	KJ766569	KJ766404	-	KJ766975	-
Hypocrea americana	AFTOL-ID 52	AY544649	-	DQ491488	-	DQ471043
Hysterium angustatum	CBS 236.34	FJ161180	GU397359	-	FJ161117	FJ161096
Hysterobrevium smilacis	CBS:114601	FJ161174	FJ161135	-	FJ161114	FJ161091
Icmadophila ericetorum	AFTOL-ID 4846	KJ766573	KJ766729	-	-	-
Ingvariella bispora	BCNLich 17183	HQ659185	-	-	-	-
Jahnula aquatica	R68-1	EF175655	EF175633	JN942354	-	-
Jattaea mookgoponga	STE-U 6184	EU367458	EU367463	EU367449	-	-
Kellermania yuccigena	CBS 131727	KF766356	KF766466	KF766186	-	KF766417
Lachnum virgineum	AFTOL-ID 49	AY544646	AY544688	DQ491485	DQ470877	DQ497602
Lembosia abaxialis	VIC 42825	KP143737	-	-	-	-
Leotia lubrica	AFTOL-ID 1	AY544644	AY544687	DQ491484	DQ470876	DQ471041
Leptosphaerulina australis	CBS 317.83	EU754166	EU754067	GU237829	-	GU349070

Table 1 Continued.

Species	Voucher/culture	*Accession numbers*				
		LSU	SSU	*ITS*	*RPB2*	*TEF1*
Leptoxyphium cacuminum	MFLUCC10-0049	JN832602	JN832587	-	-	-
Lichenoconium aeruginosum	CBS 129239	HQ174269	HQ174268	-	-	-
Lichenoconium erodens	JL363-09	HQ174267	HQ174266	-	-	-
Lobothallia radiosa	AFTOL-ID 1860	KJ766596	KJ766746	-	KJ766935	-
Loxospora cismonica	AFTOL-ID 878	DQ986749	DQ986742	HQ650640	DQ992433	-
Loxosporopsis corallifera	T1087	KR017219	KR017264	KR017089	KR017516	KR017572
Lulworthia fucicola	ATCC 64288	AY878965	AY879007	-	-	
Magnaporthe salvinii	M21	JF414887	JF414862	JF414838	-	JF710406
Manglicola guatemalensis	BCC20157	FJ743450	FJ743444	JN819282	-	-
Massaria inquinans	CBS 122369	GU456322	GU456300	-	-	GU456282
Megalaria grossa	AFTOL-ID 4916	KJ766597	KJ766747	-	-	-
Megaspora verrucosa	L009	HM060725	HM060687	-	-	-
Microascus trigonosporus	AFTOL-ID 914	DQ470958	DQ471006	-	-	-
Micropeltis dendrophthoes	MFLUCC:15-0599	KT588595	KT588597	-	-	-
Micropeltis zingiberacicola	IFRDCC 2264	JQ036227	JQ036222	-	-	-
Microthyrium buxicola	MFLUCC 15-0213	KT306552	KT306550	-	-	-
Microthyrium microscopicum	CBS 115976	GU301846	GU296175	-	-	GU349042
Miltidea ceroplasta	-	HQ391558	HQ391557	-	-	-
Mollisia cinerea	AFTOL-ID 76	DQ470942	DQ470990	DQ491498	DQ470883	DQ471051
Monascus purpureus	AFTOL-ID 426	DQ782908	DQ782881	DQ782847	DQ782869	-
Montagnula opulenta	CBS 168.34	NG_027581	-	-	-	-
Mycoblastus affinis	AFTOL-ID 1047	KJ766601	-	-	KJ766958	-
Mycoblastus sanguinarius	AFTOL-ID 4865	KJ766602	KJ766440	-	-	-
Mycocalicium polyporaeum	ZW-Geo60-Clark	AY789362	AY789361	AY789363	-	-
Myeloconis erumpens	MPN778	KJ449338	-	-	-	-
Myeloconis fecunda	MPN759	KJ449337	-	-	-	-
Myriangium duriaei	CBS 260.36	NG_027579	AF242266	-	KT216528	-
Mytilinidion mytilinellum	CBS 303.34	FJ161184	FJ161144	-	FJ161119	FJ161100
Natipusilla decorospora	L_A236_1A	HM196369	HM196376	-	-	-
Natipusilla naponensis	AF217-1a	HM196371	HM196378	-	-	-

Table 1 Continued.

Species	Voucher/culture	*Accession numbers*				
		LSU	SSU	*ITS*	*RPB2*	*TEF1*
Neofracchiaea callista	SMH2689	AY695269	-	-	FJ968941	FJ969020
Neophyllis melacarpa	-	AY340556	-	-	-	-
Nitschkia tetraspora	GKML148N	FJ968987	-	-	FJ968936	FJ969011
Ochrolechia tartarea	DNA7	JN941358	-	JN943620	-	-
Odontotrema phacidiellum	Gilenstam 2625	HM244769	-	-	HM244802	-
Ophioceras commune	M92	JX134688	JX134662	JX134676	-	JX134702
Ophiocordyceps sinensis	YN09_64	JX968033	JX968028	JQ325141	JX968013	JX968018
Ophiostoma piliferum	AFTOL-ID 910	DQ470955	DQ471003	-	DQ470905	DQ471074
Orbilia auricolor	AFTOL-ID 906	DQ470953	DQ471001	DQ491512	DQ470903	DQ471072
Orbilia vinosa	AFTOL-ID 905	DQ470952	DQ471000	DQ491511	-	DQ471071
Orceolina kerguelensis	-	AF274116	-	-	-	DQ366254
Paramicrothyrium chinensis	IFRDCC2258	KF636760	JQ036224	KM246198	-	-
Parmelia saxatilis	Wedin 5051	AY300849	AF117985	-	-	-
Parmularia styracis	VIC 42447	KP143728	-	KP273230	-	KP289325
Peltula auriculata	AFTOL-ID 892	DQ832330	DQ832332	DQ832329	DQ832331	-
Peltula umbilicata	AFTOL-ID 891	DQ832334	DQ782887	DQ832333	DQ832335	DQ782919
Pertusaria dactylina	AFTOL-ID 224	DQ782907	DQ782880	DQ782843	DQ782868	DQ782899
Phaeosaccardinula ficus	MFLUCC 10-0009	HQ895837	-	HQ895840	-	KF791915
Phaeosphaeria oryzae	CBS 110110	GQ387591	GQ387530	KF251186	KF252193	-
Phlyctis argena	AFTOL-ID 1375	DQ986771	DQ986880	-	KJ766940	-
Phyllobaeis imbricata	AFTOL-ID 852	DQ986781	DQ986895	HQ650635	-	-
Physcia aipolia	AFTOL-ID 84	DQ782904	DQ782876	DQ782836	DQ782862	DQ782892
Platystomum scabridisporum	BCC 22835	GQ925844	GQ925831	-	GU479830	GU479857
Plectosphaerella cucumerina	DAOM 226828	GU180647	GU180612	GU180630	GU180663	-
Pleospora herbarum	CBS 191.86	DQ247804	DQ247812	KC584239	KC584471	KC584731
Pleurostomophora richardsiae	CBS H-7595	AY761080	AY761066	-	-	-
Porina farinosa	MPN36	KJ449333	KJ449312	-	-	-
Porina nucula	MPN13B	KJ449331	KJ449310		-	-
Potebniamyces pyri	AFTOL-ID 744	DQ470949	DQ470997	DQ491510	DQ470900	DQ471068
Protothelenella corrosa	2002 Palice	AY607734	-	-	-	-

Table 1 Continued.

Species	Voucher/culture	*Accession numbers*				
		LSU	SSU	*ITS*	*RPB2*	*TEF1*
Protothelenella sphinctrinoidella	Lumbsch 19031d (F)	AY607735	AY607747	-	-	-
Pseudovalsa longipes	AR 3541	EU683072	-	-	-	-
Psora decipiens	AFTOL-ID 4857	KJ766640	KJ766778	-	-	-
Pyrenula pseudobufonia	Reeb VR 14-VI-02/5 (DUKE)	AY640962	AY641001	-	-	-
Pyrgillus javanicus	AFTOL-ID 342	DQ823103	DQ823110	DQ826741	DQ842009	-
Ramalina farinacea	AFTOL-ID 1965	KJ766646	KJ766783	-	KJ766963	-
Ramichloridium anceps	AFTOL-ID 659	DQ823102	DQ823109	DQ826740	DQ840564	DQ840568
Schaereria corticola	Toensberg 28432	AY300859	AY300909	-	-	-
Schizoparme straminea	CBS 149.22	AF362569	-	-	-	-
Schizosaccharomyces pombe	Z19136	X54866	-	-	-	-
Siphula ceratites	P110	KR017179	KR017288	KR017095	KR017498	KR017625
Sordaria fimicola	HP153	KT323354	-	KT323211	-	-
Sphaerophorus fragilis	AFTOL-ID 226	DQ986805	DQ983487	HQ650600	-	-
Sphinctrina turbinata	AFTOL-ID 1721	EF413632	EF413631	-	EF413633	-
Spiromastix warcupii	CBS 576.63	AB040679	AB015768	LN867609	-	-
Stachybotrys chlorohalonata	UAMH6417	AY489712	AY489680	AF206273	-	AY489607
Staurothele frustulenta	AFTOL-ID 697	DQ823098	DQ823105	DQ826736	DQ840560	-
Stegonsporium protopyriforme	CBS 117041 or D30	EU039992	-	NR_126119	-	EU040017
Stictis confusum	Wedin 7070	DQ401143	-	DQ401143	-	-
Stictis confusum	Gilenstam 2610a	AY527327	-	AY527327	-	AY527306
Stictis radiata	Palice (ESS 21520)	AY300864	AY300914	-	-	-
Sydowiella stellatifolii	CBS:119342	EU552156	-	EU552156	-	-
Sympoventuria capensis	CPC 12840	DQ885904	-	DQ885904	-	-
Tephromela atra	AFTOL-ID 780	DQ986764	-	HQ650606	-	-
Teratosphaeria associata	CBS 112224	KF901827	GU296200	DQ302968	KF902183	KF903090
Thelenella antarctica	-	AY607739	KU358979	-	-	-
Trapelia placodioides	KS163	KU844623	KU844691	KU844758	-	KU844395
Trichomerium gleosporum	MFLUCC10-0087	JX313662	-	JX313656	-	-
Tubeufia chiangmaiensis	MFLUCC110514	KF301538	KF301543	KF301530	-	KF301557
Umbilicaria hyperborea	Wiklund 25	AY853399	AY853349	-	-	-

Table 1 Continued.

Species	Voucher/culture	*Accession numbers*				
		LSU	SSU	*ITS*	*RPB2*	*TEF1*
Umbilicaria mammulata	AFTOL-ID 645	DQ782912	-	DQ782851	DQ782873	DQ782903
Varicellaria rhodocarpa	AFTOL-ID 4890	KJ766675	KJ766814	-	-	-
Venturia inaequalis	CBS 476.61	GU456336	-	EU282478	-	GU456288
Vonarxia vagans	CPC 15152	FJ839673	-	FJ839637	-	-
Xylaria acuta	AFTOL-ID 63	AY544676	AY544719	DQ491493	DQ247797	DQ471048
Xylaria hypoxylon	AFTOL-ID 51	AY544648	AY544692	DQ491487	DQ470878	DQ471042
Xyloschistes platytropa	AFTOL-ID 4891	KJ766680	-	-	-	-
Zeloasperisporium cliviae	CPC 25145	KR476781	KR476748	KR476748	-	-
Zeloasperisporium hyphopodioides	CBS 218.95	EU035442	-	EU035442	-	-
Zeloasperisporium siamemse	IFRDCC 2194	JQ036228	JQ036223	-	-	-
Zeloasperisporium wrightiae	MFLUCC 15-0225	KT387737	KT387738	-	-	-

Discussion

Sequence data of Microthyrium-like taxa were established recently to clarify the classification of some species (Hongsanan et al. 2014a,b, 2015a,b, Ariyawansa et al. 2015). The molecular data has been used to confirm the placement of Microthyriaceae in Dothideomycetes and to establish a new family/order (Hongsanan et al. 2014a, 2015a,b, Ariyawansa et al. 2015, Hongsanan et al. 2017). *Neomicrothyrium siamense* was established with Microthyrium-like taxa (Fig. 3), and treated as genus *incertae sedis* in Microthyriales because it clustered with freshwater genus *Natipusilla* without similar morphological characters (Hyde et al. 2013). Hongsanan et al. (2015b) introduced two new species of the asexual genus *Zeloasperisporium*, which is linked to *Neomicrothyrium* and therefore *Neomicrothyrium* was synonymized under *Zeloasperisporium* in the new order *Zeloasperisporiales* (Hongsanan et al. 2015b). *Chaetothyrina* was placed in Micropeltidaceae based on its flattened thyriothecium, comprising interwoven hyphae, but it has 1-septate ascospores. Sequence data of two new *Chaetothyrina* species indicated that the genus belongs to Capnodiales as a new family, Phaeothecoidiellaceae (Hongsanan et al. 2017).

Micropeltis is the generic type of Micropeltidaceae with the type species *M. applanata* Mont. Wu et al. (2011) provided sequence data of *M. zingiberacicola*, however, it clustered separately from Microthyriaceae (represented by *Microthyrium microscopicum*). Therefore, Wu et al. (2011) concluded that the placement of Micropeltidaceae was uncertain, but suggested that this family probably can be placed in Microthyriales based on its morphology (Wu et al. 2011). *Micropeltis zingiberacicola* clustered as basal to Dothideomycetes without statistical support in previous studies (Wu et al. 2011, Hyde et al. 2013, Hongsanan et al. 2014a), however, it clustered together with Zeloasperisporiales and Natipusillales in some studies. *Micropeltis dendrophthoes* was established by Hongsanan et al. (2015a), and it is closely related to *M. zingiberacicola* in their phylogenetic tree. The placement of Micropeltidaceae in Dothideomycetes was not supported in their phylogenetic tree (Hongsanan et al. 2015a).

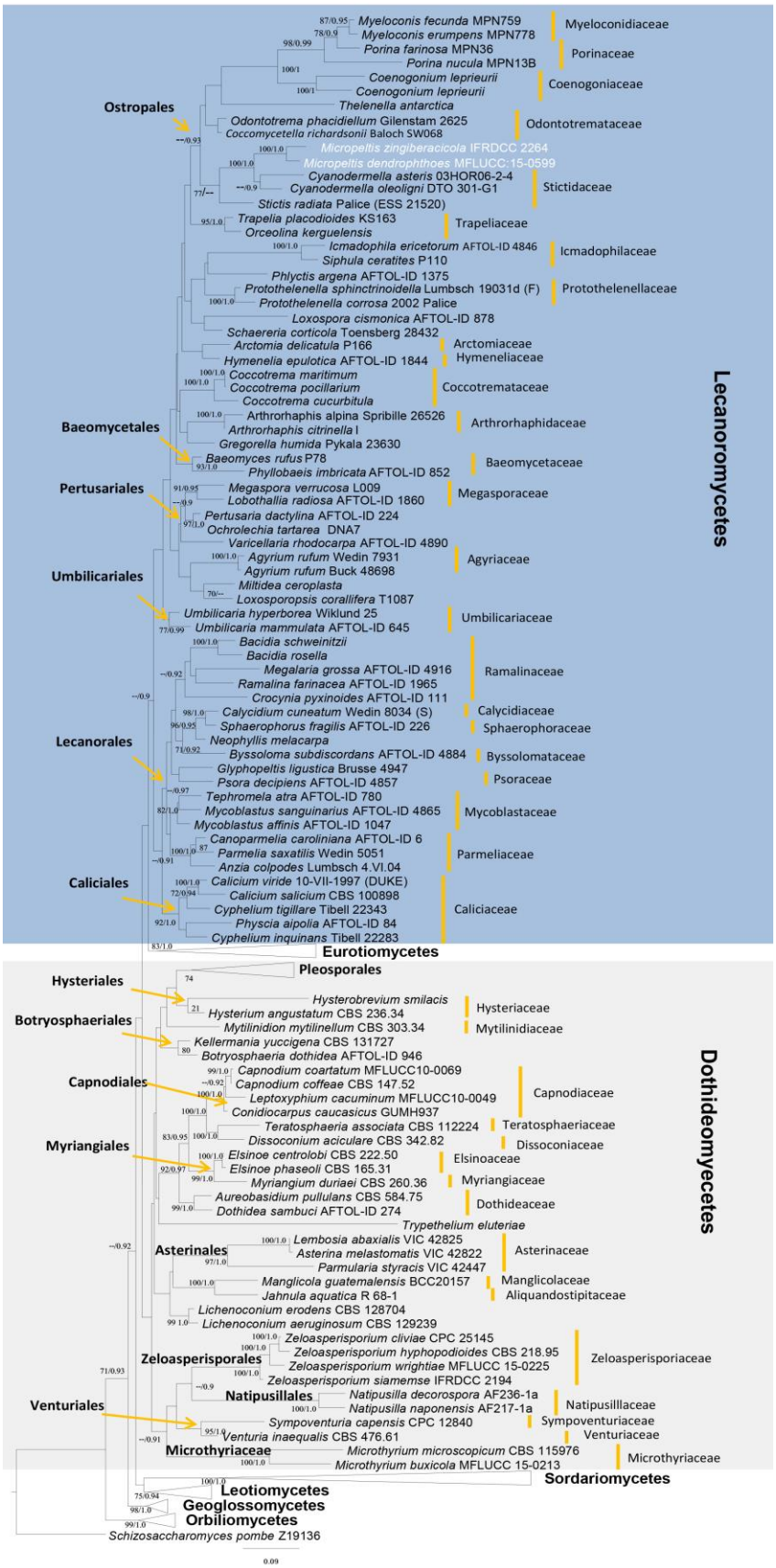

Figure 1 – RAxML maximum likelihood phylogenetic tree (LSU, RPB2, SSU and TEF1). The first set of numbers above the nodes are RAxML value expressed from 1,000 repetitions with values above 70% shown. The second set of numbers above the nodes are Bayesian posterior probabilities, with values above 0.90 shown. Strain numbers are indicated after species names. Micropeltidaceae strains are in white.

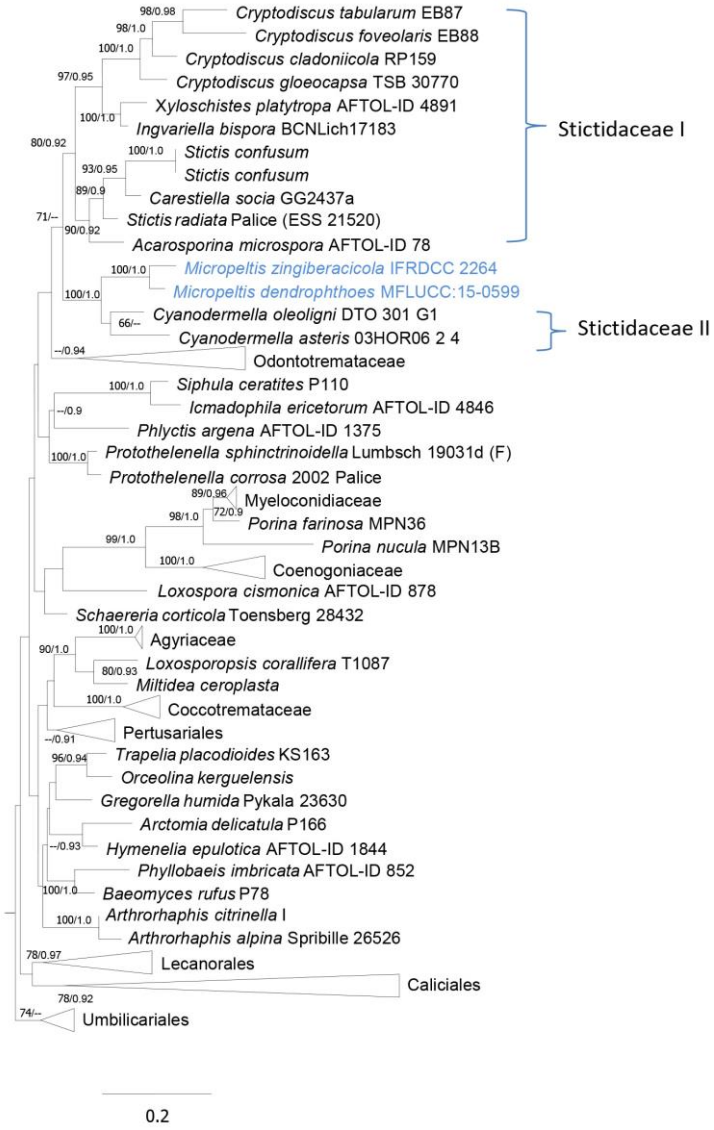

Figure 2 – RAxML maximum likelihood phylogenetic tree of Stictidaceae (ITS, LSU, SSU and TEF1). The first set of numbers above the nodes are RAxML value expressed from 1,000 repetitions with values above 70% shown. The second set of numbers above the nodes are Bayesian posterior probabilities, with values above 0.90 shown. Strain numbers are indicated after species names. Micropeltidaceae strains are in blue.

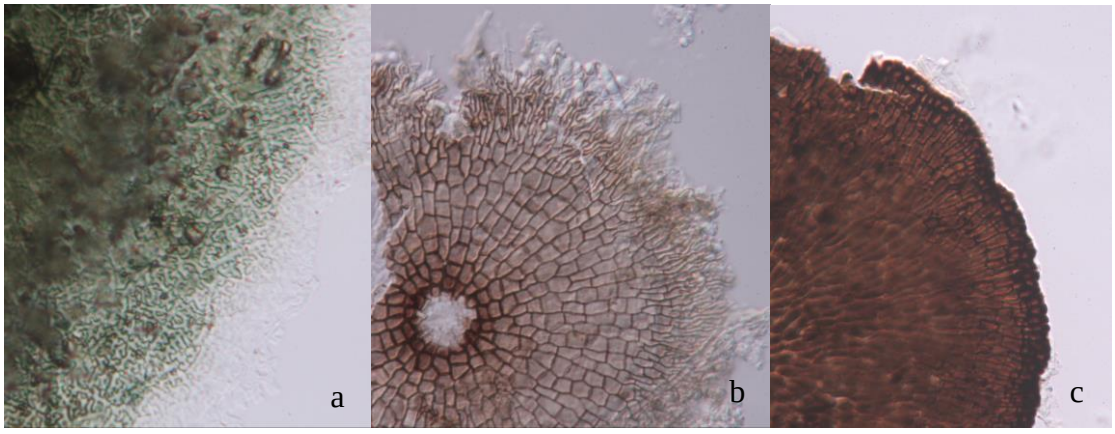

Figure 3 – a Thyriothecium of Micropeltidaceae. b Thyriothecium of Microthyriaceae. c Thyriothecium of Zeloasperisporiaceae.

From Blast searches of both LSU and SSU gene regions in GenBank, we found that both species of *Micropeltis* are closely related to *Cyanodermella oleoligni* (DTO 301-G1) (Stictidaceae, Ostropales, Lecanoromycetes), other related species are members of Lecanoromycetes (Blast search November 2017). Phylogenetic analyses based on combined LSU, RPB2, SSU and TEF1 gene regions in this paper confirm that Microthyriaceae, represented by *Microthyrium buxicola* and *Microthyrium microscopicum* are placed in Dothideomycetes. This conforms with previous studies (Wu et al. 2011, Hyde et al. 2013, Hongsanan et al. 2014, 2015a,b). The two species from Micropeltidaceae clustered in Stictidaceae within Ostropales (Lecanoromycetes), this conforms to the same result as the Blast search. The placement of Micropeltidaceae in Dothideomycetes in previous studies was probably because they only used sequence data from Dothidiomycetes and a small number of sequences from other classes, thus, Micropeltidaceae was presented at the base of Dothidiomycetes with no statistical support. Although, phylogenetic analyses indicate that the two strains of *Micropeltis* species are placed with other members of Stictidaceae (Ostropales), their morphological characters are very different from other species in Ostropales. Therefore, we suggest the current placement of Micropeltidaceae as family *incertae sedis* in Lecanoromycetes based on its placement in phylogenetic trees and the morphological uniqueness. Further sequence data of Microthyrium-like taxa and other epiphytes (e,g Asterinaceae, Microthyriaceae, Micropeltidaceae, Phaeothecoidiellaceae, Zeloasperisporiaceae) are needed to clarify their natural classification (Wu et al. 2011, Hyde et al. 2013, Hongsanan et al. 2014b, 2015a, 2017).

We suggest that careful checking of sequence data by Blast searches is required in the future studies, not only to check the most closely related strain, but also representative strains from major classes which should be included in the first analysis to make sure the placement matches the Blast result.

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