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Biodiversity of Fungi on *Calamus* (Palmae) in Thailand

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Abstract – A study of saprotrophic microfungi associated with the palm *Calamus* sp. in Thai forests yielded 88 species, with 40 ascomycetes (45.5%), and 48 anamorphic taxa (54.5%) from 212 fungal collections. The most common fungi were *Tetraploa* sp. (14.1% of all records), *Morenoina palmicola* (11.8%), *Circinoconis paradoxa* (5.2%), *Diaporthe* sp. (4.7%), and *Helminthosporium* sp. (4.7%). The percentage of fungi occurring on dry versus damp materials were 68.5% and 31.5%, respectively, with 61% of fungi occurring on petioles and 39% on rachides. The fungi occurring on *Calamus* sp. are compared with those recorded on other palms in Australia, Brunei, Hong Kong and Thailand.

biodiversity / *Calamus* sp. / palm fungi / tissue preference.

INTRODUCTION

Several studies have been undertaken on saprotrophic fungi from Thai palms: Pilantanapak *et al*. (2005) reported 81 taxa from *Nypa fruticans* of which 22 were new records for the principality; Aramsiriujiwet (1996) collected 29 hyphomycetes from terrestrial palms in Southern Thailand (*Borassus flabellifer, Caryota* sp., *Cocos nucifera, Cyrostachys lakka, Corypha lecomtei, Elaeis guineensis*, and *Roystonea regina*), while Sarapat (2003) recorded

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111 species from twelve palm species sampled in Sirindhorn peat swamp forest. Hidiyat *et al.*, 2006 reported 4 species of *Oxydothis* from palms in Chiang Mai Province, and three of these were new to science. Subsequently Pinnoi *et al.*, 2006 and Pinruan *et al.*, 2007 documented fungi on the peat swamp palms *Eleiodoxa conferta* and *Licuala longicalycata,* respectively, from the peat swamp at Sirindhorn, yielding 114 and 147 species. Objectives of this study are to document the diversity of fungi occurring on *Calamus* spp. and, compare fungal diversity in this study with other palms from different habitats.

MATERIALS AND METHODS

Sample collection

Four collections of *Calamus* spp. were made in January, April, July and November 2006. Material was divided into 2 parts: palm rachides and petioles under 2 conditions: aerial (dry) and ground contact (damp). Collections were made at Khoa Yai National Park, Thaleban National Park and Klong Tom hot waterfall in Thailand. Samples were placed in plastic bags and the dates and locations recorded. Samples were returned to the laboratory and incubated in moist plastic boxes at 25°C for 1 week and observed.

Fungal isolation

Sporulating fungi were observed under a stereomicroscope and isolated into axenic culture using a single spore technique (Choi *et al.*, 1999). The isolation medium was corn meal agar (CMA), with added antibiotics (streptomycin 0.5 g/l, penicillin G 0.5 g/l), and germinating spores transferred to potato dextrose agar (PDA), and incubated at room temperature until good growth was established. Cultures and dry material are deposited in BIOTEC Culture Collection (BCC) and BIOTEC Bangkok Herbarium (BBH), respectively.

Data analyses

Percentage abundance of taxa was calculated according to the following formula:

$$\text{Percentage abundance of taxon A} = \frac{\text{Occurrence of taxon A} \times 100}{\text{Occurrence of all taxon}}$$

RESULTS

Abundance of occurrence of fungi on *Calamus*

Two-hundred and twelve fungal records made from the four field collections yielded 88 species (Ascomycota 40 species, 45.5% and anamorphic fungi 48 species, 54.5%). The most common fungi were *Tetraploa* sp. (14.2% of all records), *Morenoina palmicola* (11.8%), *Circinoconis paradoxa* (5.2%), *Diaporthe* sp. (4.7%), and *Helminthosporium* sp. (4.7%) (Table 1).

The percentage occurrence of fungi on different parts of *Calamus* spp. was as follows: dry material supported 68.5% of the fungi recorded, and damp material had 31.5%, with 61% on the petioles, and 39% on the rachides.

Fungi found only on petioles were: *Melanographium citri* (4 records), *Astrosphaeriella* sp. 1 (4). *Astrosphaeriella vesuvius* (4), AOM 324 (3), and *Coleodictyospora micronesica* (2) while only *Lachnellula* sp. (2) occurred on the rachides. Fungi found on both petioles and rachides included: *Anthostomella* sp., *Circinoconis paradoxa*, *Diaporthe* sp., *Diplococcium* sp., *Exserticlava vasiformis*, *Goidanichiella fusiformis*, *Helminthosporium* sp., *Linocarpon* sp., *Morenoina palmicola*, *Pheosphaeria* sp., and *Sporidesmium* sp.

Forty–two taxa were found only on dry material, but only 15 taxa on damp material. Fungi occurring in both micro–habitats included: *Anthostomella* sp., *Diaporthe* sp., *Exserticlava vasiformis*, *Goidanichiella fusiformis*, *Helmithosporium* sp., *Linocarpon* sp., *Morenoina palmicola*, *Phaeosphaeria* sp., and *Sporidesmium* sp.

DISCUSSION

Hawksworth (1991) estimated fungal diversity at 1.5 million species worldwide, but to date only approximately 80,000 species have been described (Kirk *et al.*, 2001). This prompted Hyde (2001) to speculate as to where these missing fungi might be found. Many locations, habitats and substrata have not been examined for the occurrence of fungi, many may occur as endophytes (Sánchez Márquez *et al.*, 2007; Wei *et al.*, 2007), while others are non culturable (Duong *et al.*, 2006). This has led to intensive studies on fungal diversity worldwide, in particular Asian and South American regions (e.g. Desjardin & Ovrebo, 2006; Nuytinck *et al.*, 2006; Le *et al.*, 2007).

In a survey of fungal communities on *Calamus* in northern Queensland, Australia (Fröhlich & Hyde, 2000) and the present study, 17 and 88 genera, respectively were reported, but only six genera were common to both localities: *Anthostomella*, *Diaporthe*, *Lachnellula*, *Linocarpon*, *Morenoina* and *Oxydothis*. Eighty-five genera occurred only on the peat swamp palms *Eleiodoxa conferta* and *Licuala longicalycata* when compared to those on *Calamus* species (Table 2). This indicates the great variation of diversity that occurs between the different palms and their habitats as seen in previous studies (Fröhlich & Hyde, 2000, Taylor & Hyde, 2003).

The fungal community on *Calamus* spp. in this study also differs from that on the terrestrial palms from Brunei and Hong Kong SAR, in having more ascomycetes than anamorphic fungi (Table 3). Only *Astrosphaeriella* and *Helminthosporium* were common to *Calamus* spp. in this study and terrestrial palms in Hong Kong and Brunei (Table 3). One reason that more ascomycetes may occur on palm material is their ability to withstand desiccation, larger size of the resource allowing for a wide variety of taxa to colonize it. Often the fronds remain attached to the tree in an aerial position where they decay. The ascomycete fruiting bodies are usually covered in hardened clypei (e.g. *Oxydothis* spp; *Astrosphaeriella* spp.) which reduces drying out of the substratum. Subsequently when the fronds become wet ascomata absorb water and start to release spores (Hyde, pers. obers.).

Table 1. Percentage abundance of saprotrophic fungi on the terrestrial palm *Calamus*

Fungus	*Percentage abundance*	*Fungus*	*Percentage abundance*
Tetraploa sp.	14.2	*Dictyosporium* sp. 1	0.5
Morenoina palmicola	11.8	*Dictyosporium* sp. 2	0.5
Circinoconis paradoxa	5.2	*Diplocladiella* sp.	0.5
Diaporthe sp.	4.7	*Ellisembia* sp. 1	0.5
Helminthosporium sp.	4.7	*Ellisembia* sp. 2	0.5
Linocarpon sp.	3.8	*Helicoma* sp.	0.5
(AOM 301)	3.8	*Helminthosporium*	0.5
Phaeosphaeria sp.	2.8	*senseletii*	
Anthostomella sp.	1.9	*Helminthosporium* sp.	0.5
Astrosphaeriella sp. 1	1.9	*Hyphodiscova jaipurensis*	0.5
Goidanichiella fusiformis	1.9	*Linocarpon* sp.	0.5
Melanographium citri	1.9	*Orbilia* sp.	0.5
Diplococcium sp.	1.4	*Oxydothis* sp. 1	0.5
(AOM 238)	1.4	*Oxydothis* sp. 2	0.5
(AOM 329)	1.4	*Oxydothis* sp. 3	0.5
(AOM 324)	1.4	*Oxydothis* sp. 4	0.5
Coleodictyospora micronesica	0.9	*Oxydothis* sp. 5	0.5
Cordana triseptata	0.9	*Pithomyces* sp.	0.5
Exserticlava vasiformis	0.9	*Thozetella* sp.	0.5
Lachnellula sp.	0.9	*Sporidesmium altum*	0.5
Sporidesmium sp.	0.9	*Sporidesmium* sp. 1	0.5
Acrodictys erecta	0.5	*Sporidesmium* sp. 2	0.5
Astrosphaeriella sp. 2	0.5	*Sporidesmium* sp. 3	0.5
Astrosphaeriella vesuvius	0.5	*Sporidesmium* sp. 4	0.5
Berkleasmium micronesicum	0.5	*Sporoschisma* sp.	0.5
Berkleasmium crunisia	0.5	*Stictis* sp. 1	0.5
Berkleasmium sp.	0.5	*Stictis* sp. 2	0.5
Brachysporiella gayana	0.5	Unidentified (27 taxa)	13.5
Capnodiastrum sp.	0.5	*Verticillium* sp.	0.5
Chaetosphaeria sp.	0.5	*Volutella ramkumarii*	0.5
Cylindrocladium sp.	0.5	**Anamorphic fungi**	54.5
Dactylaria sp.	0.5	**Ascomycetes**	45.5

Table 2. A comparison of fungi reported from this study with terrestrial and peat swamp palm at the genera level

Genus name	*1**	*2**	*3**	*4**	*Genus name*	*1**	*2**	*3**	*4**
Acrocalymma	–	–	+	–	*Cylindrocladium*	–	+	–	–
Acrodictys	–	+	–	–	*Dactylaria*	–	+	+	+
Annulatascus	–	–	+	+	*Delortia*	–	–	+	+
Anthostomella	+	+	+	+	*Diaporthe*	+	+	+	–
Apioclypea	–	–	+	–	*Dictyochaeta*	–	–	–	+
Arecomyces	+	–	–	+	*Dictyosporium*	–	–	–	+
Arecophila	+	–	–	+	*Dictyosporium*	–	+	–	–
Arthrinium	–	–	–	+	*Didymobotryum*	–	–	+	–
Arthrobotrys	–	–	+	+	*Didymosphaeria*	–	–	–	+
Ascominuta	–	–	–	+	*Diplocladiella*	–	+	–	–
Aspergillus	–	–	–	+	*Diplococcium*	–	+	+	–
Astrocystis	+	–	–	+	*Durispora*	+	–	–	–
Astrosphaeriella	–	+	+	+	*Ellisembia*	–	+	–	–
Bactrodesmium	–	–	–	+	*Endocalyx*	–	–	–	+
Baipadsphaeria	–	–	–	+	*Eutypa*	+	–	–	–
Berkleasmium	–	+	+	+	*Exserticlava*	–	+	–	–
Bionectria	–	–	+	–	*Fasciatispora*	+	–	–	–
Brachysporiella	–	+	+	–	*Flammispora*	–	–	–	+
Canalisporium	–	–	–	+	*Fluviatispora*	–	–	+	–
Cancellidium	–	–	+	+	*Frondisphaeria*	+	–	–	–
Candelabrum	–	–	–	+	*Gaeumannomyces*	–	–	+	–
Capnodiastrum	–	+	+	–	*Gliocladium*	–	–	–	+
Capsulospora	+	–	+	–	*Glomerella*	–	–	–	+
Carinispora	–	–	–	+	*Glonium*	–	–	–	+
Caryospora	–	–	–	+	*Gnomonia*	–	–	+	–
Cenangiumella	+	–	–	–	*Goidanichiella*	–	+	+	–
Chaetoporthe	–	–	+	–	*Gonytrichum*	–	–	+	+
Chaetopsina	–	–	+	–	*Guignadia*	+	–	+	+
Chaetospermum	–	–	–	+	*Haematonectria*	+	–	+	–
Chaetosphaeria	–	+	–	+	*Haplographium*	–	–	+	–
Chalara	–	–	+	+	*Helicoma*	–	+	+	+
Chloridium	–	–	+	–	*Helicomyces*	–	–	+	–
Circinoconis	–	+	–	–	*Helicosporium*	–	–	+	+
Coleodictyospora	–	+	+	–	*Helicoubisia*	–	–	+	–
Cordana	–	+	–	–	*Helminthosporium*	–	+	–	–
Cosmospora	+	–	–	–	*Herpotrichia*	+	–	–	–
Craspedodidymum	–	–	–	+	*Heteroconium*	–	–	+	–

* 1 = *Calamus* (Fröhlich and Hyde, 2000)
2 = *Calamus* (this study)
3 = *Eleiodoxa conferta* (Pinnoi *et al.*, 2006)
4 = *Licuala longicalycata* (Pinruan *et al.*, 2007)

Table 2. A comparison of fungi reported from this study with terrestrial and peat swamp palm at the genera level *(suite)*

Genus name	*1**	*2**	*3**	*4**	*Genus name*	*1**	*2**	*3**	*4**
Cryptophailoidea	–	–	–	+	*Hydropisphaera*	+	–	–	–
Custingophora	–	–	+	–	*Hyphodiscova*	–	+	–	–
Cyanopulvis	+	–	–	–	*Hypoxylon*	+	–	–	–
Ijuhya	+	–	–	–	*Phaeodothis*	+	–	–	+
Jahnula	–	–	+	+	*Phaeoisaria*	–	+	+	+
Koorchaloma	–	–	–	+	*Phialogeniculata*	–	–	+	–
Lachnellula	+	+	–	–	*Phomatospora*	–	–	–	+
Lachnum	+	–	–	–	*Phruensis*	–	–	–	+
Lanceispora	–	–	–	+	*Pithomyces*	–	+	–	–
Lasiodiplodia	–	–	–	+	*Pleurophragmium*	–	–	+	–
Lasionectria	+	–	–	–	*Pseudorobillarda*	–	–	–	+
Linocarpon	+	+	+	+	*Rosellinia*	–	–	–	+
Lophiostoma	–	–	+	+	*Septomyrothecium*	–	–	+	–
Lophodermium	–	–	+	–	*Solheimia*	–	–	–	+
Manokwaria	+	–	–	–	*Sorokinella*	+	–	–	–
Massarina	–	–	–	+	*Spadicoides*	–	–	–	+
Melanographium	–	+	+	+	*Sporidesmiella*	–	–	–	+
Microthyrium	–	–	+	+	*Sporidesmium*	–	+	+	–
Mollisia	+	–	–	–	*Sporoschisma*	–	+	–	–
Monotosporella	–	–	+	+	*Stachybotrys*	–	–	+	+
Morenoina	+	+	+	–	*Stictis*	–	+	+	–
Munkovalsaria	–	–	+	–	*Stilbohypoxylon*	+	–	+	+
Mycomicrothelia	+	–	–	–	*Strossmayeria*	+	–	–	–
Myelosperma	+	–	–	+	*Submersisphaeria*	+	–	+	+
Nawawia	–	–	+	+	*Terriera pandani*	+	–	–	–
Nectria	–	–	–	+	*Tetraploa*	–	+	–	–
Nemania	–	–	+	–	*Thailandiomyces*	–	–	–	+
Niesslia	–	–	–	+	*Thozetella*	–	+	+	+
Ochronectria	+	–	–	–	*Trichoderma*	–	–	+	+
Ophioceras	+	–	–	+	*Tubeufia*	+	–	+	+
Ophiostoma	–	–	+	–	*Unisetosphaeria*	–	–	+	–
Orbilia	–	+	+	+	*Vanakripa*	–	–	+	–
Ornatispora	–	–	+	–	*Verticillium*	–	+	+	+
Oxydothis	+	+	+	+	*Volutella*	–	+	–	–
Pemphidium	+	–	–	–	*Wiesneriomyces*	–	–	–	+
Penicillium	–	–	+	+	*Xylaria*	+	–	–	–
Pestalosphaeria	–	–	+	–	*Xylomyces*	–	–	+	+
Petrakiopsis	–	–	–	+	**Total species**	40	37	68	75

* 1 = *Calamus* (Fröhlich and Hyde, 2000)
2 = *Calamus* (this study)
3 = *Eleiodoxa conferta* (Pinnoi *et al.*, 2006)
4 = *Licuala longicalycata* (Pinruan *et al.*, 2007)

Table 3. Comparison of fungi on terrestrial palms with those on *Calamus* spp.

Calamus *spp.*	Arenga engleri *(Hong Kong SAR)*	Livistona chinensis *(Hong Kong SAR)*	Phoenix hanceana *(Hong Kong SAR)*	Arenga undulatifolia *(Brunei)*	Oncosperma horridum *(Brunei)*	Salacca affinis *(Brunei)*
Tetraploa sp.	*Piricauda cochinensis*	*Astrosphaeriella bakeriana*	*Diplococcium stoveri*	*Piricauda cochinensis*	*Linocarpon livistinae*	*Zygosporium minus*
Morenoina palmicola	*Diplococcium stoveri*	*Lachnum palmae*	*Endocalyx cinctus*	*Melanographium selemiodes*	*Craspedodydimum nigroseptatum*	*Linocarpon livistinae*
Circinoconis paradoxa	*Helminthosporium solani*	*Appendicospora hongkongensis*	*Cryptophiale udagawae*	*Trichoderma harzianum*	*Zygosporium minus*	*Peltistromella anomala*
Diaporthe sp.	*Melanographium palmicola*	*Monodictys putredinis*	*Penzigomyces nodipes*	*Zygosporium minus*	*Monotosporella setosa* var. *macrospora*	*Helicosporium griseum*
Helminthosporium sp.	*Melanographium selenioides*	*Oxydothis elaeicola*	*Thozetella effusa*	*Pleurophragmium* sp.	*Neolinocarpon australiense*	*Volutella ciliata*
Linocarpon sp.	*Monodictys putredinis*	*Trichoderma harzianum*	*Pseudospiropes simplex*	*Helmithosporium velutimum*	*Trichoderma harzianum*	*Oxydothis luteaspora*
(AOM 301)	*Oxydothis ragai*	*Neolinocarpon australiense*	*Dictyochaeta simplex*	*Volutella ciliate*	*Oxydothis luteaspora*	*Periconiella* sp.
Phaeosphaeria sp.	*Pestalotiopsis palmarum*	*Fasciatispora petrakii*	*Serenomyces shearii*	*Peltistromella anomala*	*Oxydothis licualae*	*Arecomyces bruneiensis*
Anthostomella sp.	*Guignardia manokwaria*	*Corynesporopsis isabelicae*	*Capsulospora brunneispora*	*Stachylidium* sp.	*Oxydothis elaeicola*	*Sporidesmium parvum*
Astrosphaeriella sp.	*Dischoridium roseum*	*Dictyosporium elegans*	*Harknessia globosa*	*Anthostomella minutoides*	*Brachysporiella gayana*	*Codinaea intermedia*
Ascomycetes = 6 species	Ascomycetes = 2 species	Ascomycetes = 6 species	Ascomycetes = 2 species	Ascomycetes = 1 species	Ascomycetes = 5 species	Ascomycetes = 3 species
Anamorphic fungi = 4	Anamorphic fungi = 8	Anamorphic fungi = 4	Anamorphic fungi = 8	Anamorphic fungi = 9	Anamorphic fungi = 5	Anamorphic fungi = 7
Total = 10	Total = 10	Total = 10	Total = 10	Total = 10	Total = 10	Total = 10

Several studies, of different habitats and hosts show dissimilar fungal communities (Goh & Hyde, 1996; Wong *et al.*, 1998; Ho *et al.*, 2000; Kane *et al.*, 2002; Tsui & Hyde, 2003; Tsui *et al.*, 2003; Shearer *et al.*, 2007; Kodsueb *et al.*, 2008a,b). Of key importance is the low overlap between different habitats (Cai *et al.*, 2006; Pinnoi *et al.*, 2006, Pinruan *et al.*, 2007; Kodsueb *et al.*, 2008a,b). Fungal colonization may depend on environmental conditions such as climate, temperature, humidity, and these usually differ between different habitats (Baker & Meeker, 1972).

Fungal diversity in tropical regions is greater than temperate regions (Goh & Hyde, 1996; Wong *et al.*, 1998; Ho *et al.*, 2000; Kane *et al.*, 2002; Tsui & Hyde, 2003; Tsui *et al.*, 2003; Hyde *et al.*, 2007; Shearer *et al.*, 2007). Pinruan (2004) suggested that a number of factors can affect fungal diversity including: number of samples collected, portion of plant material sampled (such as rachis, petiole or inflorescence), collecting times, different hosts, different habitats, climate, nutrient status of host, presence of inhibitory compounds, fungal competition for resource, and the status of the host in the country.

In the present study, approximately an equal number of ascomycetes and anamorphic fungi were recorded. Ascomycetes are prevalent on peat swamp palms; where the relative humidity of the habitat may be a key factor in determining the fungal community. The anatomy and structure of ascomycetes are more complicated than those of anamorphic fungi, so they may need a longer time and suitable environment to produce ascomata. Consequently the nature of the substratum in terms of dimension, composition and size of resource is also relevant. Therefore the combination of a large resource combined with suitable conditions are important to determine the fungal community.

Calamus petioles supported a greater species diversity than rachides and this may be accounted for by the larger surface area, and tissues composed of lignocellulose. There is a marked difference in the anatomical structure of palm tissues: thin walled parenchymatous cells in leaves and thick-walled cells in petioles and rachides that are cellulose rich and contain lignin (Pinruan *et al.*, 2007; Hyde *et al.*, 2007).

Petioles contain vascular bundles and a larger surface area that may take up water and retain moisture for a longer time. Tran *et al.* (2006) suggest that a large leaf retains more moisture than a similar layer of small leaves. This may affect tissue-specificity, a topic rarely discussed (Yanna *et al.*, 2001a; Paulus *et al.*, 2003). Host substrata contain a wide variety of compounds, some of which may attract fungal colonization (Boddy & Watkinson, 1995; Pinruan *et al.*, 2007), and some may inhibit or are toxic for fungal growth e.g. phenolic compounds (Yanna *et al.*, 2001a; Pinruan *et al.*, 2007).

The overlap in fungal diversity on different hosts is quite low (Cai *et al.*, 2006; Pinnoi *et al.*, 2006, Pinruan *et al.*, 2007; Kodsueb *et al.*, 2008a,b) and it may be due to tissue-specificity, or recurrence (Fröhlich & Hyde 2000; Yanna *et al.* 2001a,b; Zhou & Hyde, 2001; Taylor & Hyde, 2003). Hyde & Alias (2000) report 41 fungi that are unique to *Nypa fruticans,* with different parts of the palm supporting different fungi. A similar observation was made by Pinruan *et al.* (2007) for the palm *Licuala longicalycata* and equally different palm tissues and microhabitats supported distinct fungal communities. Hyde *et al.* (2007) suggested that "currently, lack of knowledge of the full extent of fungal specificity or recurrence because of incomplete sampling and because no systematically collected data is available for microfungal assemblages on other closely related plant taxa", may account for the observation made.

Competition between fungi is another factor that may account for the observed specificity. Dix & Webster (1995) observed stronger competition between species occurring on the lower than the upper portions of grasses after stem collapse. Pinnoi (2004) reported some ascomycetes, such as *Stilbohypoxylon eleiodoxae* produced inhibition zones with other fungi.

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