# รายงานฉบับสมบูรณ์

โครงการ Biodiversity of Fungi on Palms in Sirindhorn Peat Swamp Forest, Narathiwat, Thailand

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31 ตุลาคม 2547

# รหัสโครงการ BRT R\_145008

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โครงการ Biodiversity of Fungi on Palms in Sirindhorn Peat Swamp Forest, Narathiwat,

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# BIODIVERSITY OF FUNGI ON PALMS IN SIRINDHORN PEAT SWAMP FOREST, NARATHIWAT, THAILAND

## BRT R 145008

FINAL REPORT (November 2001 – October 2004)

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**Duration:** Three years (November 2001 – October 2004)

Date: 31<sup>st</sup> December, 2004.

A. ...

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#### 1. SUMMARY

The biodiversity of fungi on selected palms: Eleiodoxa conferta, Licuala longecalycata, Metroxylon sagus, and Nenga pumila was studied. One thousand and eighty two collections of fungi were made following nine field collections, May; June; September; and November 2001, February; May; September; and December 2002; and April 2003. Three hundred and twenty eight fungi were recorded with 161 Ascomycota, 15 Basidiomycota and 152 anamorphic fungi. Two hundred and fifty five species were identified to genic level while the remaining taxa have yet to be fully identified.

Collections of *Eleiodoxa conferta* material was divided into 3 parts, which yielded the following percentage fungal occurrence: petioles with 67%; leaves 11% and rachides 22%. Palm material collected under different conditions were also sampled: dry material yielded 17% of the fungi; damp/moist material 26%, and wet material 57%.

On *Licuala longecalacata* material collected from 3 parts of the palm: yielded 62% on petioles, 19% on leaves and 19% on trunks. Palm material collected from different habitats were also sampled: dry aerial material with 49% of the fungi, wet material 33%, and damp/moist material 18%.

On *Metroxylon sagus* a total of 43 taxa (21 Ascomycomcota, 10 Basidiomycota, and 12 anamorphic fungi). Samples were collected from 3 parts of the palm: with 47% of the fungi recorded from petioles; 19% from leaves and 34% from rachis. Material collected from different habitats was: 47% on dry aerial material; 19% on wet material and 34% on damp/moist material.

Nenga pumila palm yielded 48 fungi (21 Ascomycota, and 27 anamorphic fungi) with supportive: petioles 23% of the fungi; leaves 3%; rachides 25% and the sheath 49%, while dry material yielded 50% of fungi; damp/moist material 31% and wet material 19%.

The most common genera included *Massarina*, *Astrosphaeriella*, *Oxydothis* and *Linocarpon*, while 53 new taxa have been collected, with 17 described. Three hundred and forty four axenic strains were isolated and deposited in the BIOTEC Culture Collection (BCC).

Endophytic fungi from *Licuala longecalycata* petioles were isolated with a recovery rate of 45%. Cultures on PDA and CMA were examined periodically for reproductive structures and identified as they sporulated. Many cultures did not sporulate but their distinctive colony of sterile stromata suggested they were xylariaceous species.

Thesis entitled "Biodiversity and antifungal production by fungi on the palm *Eleiodoxa conferta* in Sirindhorn Peat Swamp Forest, Narathiwat, Thailand" by A. Pinnoi and "Comparative Study of The Distribution of Fungi on Aerial and Submerged Fronds of Ka Pho Deang (*Licuala longecalycata*) and Amylase and Protease Enzymes Production" by Umpava Pinruan were approved for the M.Sc. degree of Chiang Mai University.

An analysis of the factors affecting colonisation of different palm parts identified drying out of the samples, volume of sample, and nutrient availability as the key elements. Like-wise drying out of the palm part was the single most important factors in the colonisation of samples under different environmental conditions.

Fungal diversity on the four palms in the peat swamp forest was high (328 species), with little overlap in fungal communities on each palm. This aspect warrants further investigation in order to resolve the results obtained.

#### 2. OBJECTIVES OF THIS STUDY:

- 1. To document the diversity of fungi occurring on selected palms.
- 2. To isolate the fungi identified into axenic culture and deposit in the BIOTEC Culture Collection (BCC).
- 3. To determine if there is host specificity or host occurrence of the fungi on the selected palms.
- 4. To compare fungi occurring on the four palms.
- 5. To examine the endophytic fungi of *Licuala longecalycata*.

## 3. MATERIAL AND METHODS

#### 3.1. Location:

Sirindhorn Peat Swamp Forest (Pru To Daeng) Narathiwat Province; 06°12'N 101°57'E. Wildlife Sanctuary, and is the largest remaining peat swamp forest in Thailand, situated in the extreme south, located in the area of 3 districts, Amphur Tak Bai, Amphur Su-Ngai Padi and Amphur Su-Ngai Ko-Lok. This is more than 80,000 acres in area.

## 3.2. Sample collection:

No new collections have been made during the past year as time was focused on identification of taxa, writing up an M.Sc. thesis and analyzing the data. One visit was made in April to undertake an experimental of study of the drying out of palm material under different environmental conditions.

## 3.3. Sampling Palm Material

## Description of Eleiodoxa conferta

A prickly palm which forms thickets in swampy lowland areas. The trunks are subterranean and branch freely to form spreading clumps. Each trunk, at its apex, bears a tuft of tall, erect, willowy fronds which have coarse. The petioles, rachides and even the midribs of the leaflets are liberally coated with long, sharp, black spines, and the fruits are pear-shaped with a cover of overlapping scales.

## Description of Licuala longecalycata Furt.

A small fan palm, forming large thickets in the peat swamp forest. Stem is erect, 3-5(-8) m high, 4-5 cm across. Leaves palmately compound; blade suborbicular, 1-1.5 m diam.; leaflets many, green; petioles orange-yellow, 1-2 m long, with curved spines at margins; spines 1-3 mm long, rather evenly arranged, gradually from large to small. Flowers small, greenish-white, with 3 free carples, in axillary panicles 1-2 m long, with 3-5 tubular sheaths. Fruits globose, 5-6 mm diam., red to black at maturity.

## Description of Metroxylon sagus Rottb.

A monocarpic feather palm, forming a thicket in fresh water swamps, and riverside. Stems at first prostrate-ascending, then erect, up to 8-10(-15) m high. Leaves pinnately compound, alternate, 4-5 by 2-3 m long. Leaflets linear acuminate, numerous, close and evenly arranged.

## Description of Nenga pumila

A feather palm, forming thickets in lowland, fresh water and peat swamp forests. Stem 3-5 m high, 5-8 cm across; crown shaft, enveloping with greenish-yellow to bronze leaf sheath. Leaves pinnately compound, alternate, 2-3 by 0.5-1 m. Leaflets numerous, linear with pointed or toothed apex.

Collections of palm material were made and divided into 3 parts: palm leaves, petioles and rachides/trunks and from 3 conditions: wet material (constantly submerged), damp material (moist and on the surface of the soil), and dry material (aerial part), and placed in plastic bags and the date of collection recorded. Samples were returned to BIOTEC. Moist tissue paper was placed in the base of plastic boxes to create humid conditions. All the samples were examined under the microscope. The fungi appearing on the samples were isolated into axenic culture using a single spore technique.

## 3.4. Habitats sampled

- 1. Dry material = decaying palms collected under dry conditions-usually aerial
- 2. Damp material = decaying palms collected under damp conditions-general on the surface of the soil
- 3. Wet material = decaying palms totally submerged

## 3.4. Isolation of fungi:

#### Media:

Corn meal agar (CMA) supplemented with added antibiotics (streptomycin sulfate 0.5g/L, penicillin G 0.5 g/L) was used as a standard medium for isolation and sixteen squares are marked on the bottom of the agar plate. Spore suspension were transferred using a sterile Pasteur pipette onto the surface of the CMA plate, with a drop placed above each of the drawn aquares and checked for spore germination on a daily basis.

## Fungal isolation:

Single spore isolations were made from sporulating structures on material incubated in the laboratory or fresh material when isolated in the field laboratory. Isolates were transferred to PDA plates when the fungi had germinated and incubation was at room temperature until growth was observed (Choi et al., 1999). Axenic cultures are maintained in the BIOTEC Culture Collection (BCC).

## 3.6. Endophyte Study

Healthy fan-leaves of *Licuala longecalycata* were collected from Sirindhorn Peat Swamp Forest, Narathiwat, southern Thailand. Palms of about the same size and age were chosen, leaf with parts of the petiole were collected, placed in plastic bags and processed within 5 days of collecting.

Discs approximately 5 mm were cut from leaf tissues with a razor blade. Four discs were cut so as to include a major vein and four were cut from the tissue between the veins, therefore, taken from (A) near the leaflet base, (B) approximately 15 cm from A towards the apex, (C) approximately 15 cm from B towards the apex and (D) near the leaflet tip.

To investigate the endophytes living within the petioles, sections were reassembled in the correct order and were then cut into 5 cm long pieces. A 5 mm segment of tissue was then cut from the apical end of each piece of petiole.

Surface-sterilization techniques which have been widely used for isolation of endophytic fungi involve a sequence of alcohol and sodium hypochlorite: each leaf disc taken from the frond blade was surface sterilized by dipping in 95% ethanol for 1 min, then soaked in sodium hopochlorite (5% available chlorine) for 10 min with a second immersion in 95% ethanol for 30 s then washed with sterile distilled water. The leaf discs were then transferred into Petri dishes (9 cm diam) containing potato dextrose agar (PDA) with added streptomycin sulphate. Four discs were placed in each dish.

The same proceduce was applied to the 5 mm wide petiole segments except that they were dipped in 95% ethanol for 90 s, Chlorax for 15 min, then 95% ethanol for 30 s and washed with sterile distilled water.

Petri dishes were incubated at 25°C. Fungi that grew from the tissue fragments were subcultured on to PDA and corn meal agar (CMA) in 6 cm diam Petri dishes and incubated as above. Living cultures are deposited at BIOTEC Culture Collection (BCC)

## 3.7. Identification and nomenclature of organisms:

Most of the fungi were identified with the help of Professor Gareth Jones, Professor Kevin D. Hyde, Dr. Eric McKenzie and Ms. Umpava Pinruan based on the morphology and sporulation on media and fresh material.

The following texts were consulted for basic identification:

Ascomycetes: Hyde et al. (2000), Fröhlich & Hyde (2000).

Coelomycetes: Ainsworth et al. (1973), Nag Raj (1993) and Sutton (1980).

Hyphomycetes: Ainsworth et al. (1973), Carmichael, Kendrick, Conners & Sigler (1980), Ellis (1971; 1976) and Matsushima (1975; 1980; 1989; 1993; 1995).

#### 4. RESULTS

## 4.1. Taxonomy

Some 53 new fungal taxa have been reported as the result of this study. Seventeen have been published and copies are to be found in Appendix 3, while others await publication (Appendix 4).

## 4.2. Ecological observations

Four palms were studied and 328 fungi found and the common species on each palm are listed in Table 4.

The key observations of this study have been that each palm supported its own unique fungal community with little overlap in taxa. Furthermore, each microhabitat in the peat swamp supported its own fungal population. Like wise, each part of the palm supported a characteristic fungal community. These observations are detailed below.

In order to account for the differences in fungal communities on palm parts we examined various factors that might be responsible for this. These results indicate that drying out of palm material was one key factor, along with the anatomical structure of the different parts: leaves, rachides and petiole.

## 4.3. Eleiodoxa conferta:

#### General survey

Three hundred and fifty five fungal collections were made from six field collections: May; June; September; November (2001), February; May (2002). One hundred and seventy four collections were identified to species level, 148 collections were identified to generic level while 33 collections were unidentified. A total of 114 species were identified representing ascomycetes (45: 48%), anamorphic fungi (47: 50%) and basidiomycetes (2: 2%) (Figure 1). The percentage of fungi occurring on different parts of the *E. conferta* palm were as follows: dry material supported 17% of the fungi recorded, damp material had 36% while the wet material supported the most fungi with 47% (Figure 2). The percentage occurrence of fungi on different parts of *E. conferta* was petioles 56%, leaves 17%, and rachides 27% (Figure 3). The most common fungi were *Astrosphaeriella aquatica*-like, *Cancellidium applanatum*,

*Xylomyces* sp., *Stilbohypoxylon moelleri*, *Lophiostoma fronsisubmersum*, *Microthyrium* sp. and *Morenoina* sp.

## **Quantitative survey**

One hundred and three fungal samples were made from 3 field collections: Fortyfour collections were identified to species level, 57 collections to generic level with 2 basidiomycetes. A total of 25 species were identified representing ascomycetes (14: 56%), anamorphic fungi (10: 40%) and basidiomycetes (1: 4%) (Figure 4). The percentage fungi occurring on different parts of the E. conferta palm were as follows: dry material supported 18% of the fungi recorded; damp material had 31% while the wet material supported the most fungi with 51% (Figure 5). The percentage occurrence of fungi on different parts of E. conferta was, petioles 41%, leaves 20%, and rachides 39% (Figure 6). The most common fungi were Cancellidium applanatum, Xylomyces sp., Microthyrium sp., Morenoina sp., Lophiostoma fronsisubmersum, Stilbohypoxylon moelleri, Phaeoisaria clematidis, Astrosphaeriella aquatica-like, Jahnula appendiculata, and Astrocystis eleiodoxae.

## 4.4. Licuala longecalycata

## General survey

A total of three hundred and fifty eight fungal collections were made from six field collections: May; June; September; November (2001), February; May (2002). One hundred and seventy seven collections were identified to species level, one hundred and fifty three collections to generic level while twenty eight collections remaimed unidentified. A total of 147 species were identified, including 78 ascomycetes from 50 genera (53%), 66 anamorphic fungi species from 53 genera (45%) and 3 basidiomycetes species from 3 genera (2%) (Figure 7). Of these 9 ascomycetes and 5 anamorphic fungi were new to science. New genera are proposed for three taxa: Baipadicola siamense, Flammispora bioteca, and Phruensis brunniespora. A further eleven new species have also been encountered: Astrosphaeriella sp., Caryospora sp., Craspedodidymum licualae, C. microsporum, C. siamense, Diaportae setulae, Dictyosporium siamense, Jahnula appendiculata, Massarina sp., Oxydothis atypical and Stachybotrys palmae. The percentage of fungi occurring on different parts of the L. longecalycata palm were as follows: dry material supported the most fungi with 40%, wet material had 32% while the damp material supported the least number of fungi with 28% (Figure 8). The percentage occurrence of fungi on different tissues of L. longecalycata was: petioles

61%, trunks 24%, and leaves 15% (Figure 9). The most common fungi were Annulatascus velatispora, Microthyrium sp., Phaeoisaria clamatidis, Massarina bipolaris, Phruensis brunneispora, Diaporthe setulae, and Solheimia costaspora.

## Quantitative survey

A total of one hundred and twenty four fungal collections were made from 3 field collections: February, May, and September (2002). Eighty-nine collections were identified to species level, thirty eighty collections were to generic level while seven collections were unidentified. A total of 44 species were identified, including 25 ascomycetes from 21 genera (57%), 18 anamorphic fungi from 17 genera (41%) and 1 basidiomycete from 1 genus (2%) (Figure 10). The percentage of fungi occurring in different habitats were as follows: dry material supported the most fungi with 41%, submerged material had 33% of species while damp material supported the least number (26%) (Figure 11). The percentage occurrence of fungi on different tissues of *L. longecalycata* was petioles 61%, trunks 28%, and leaves 11% (Figure 12). The most common fungi were *Phaeoisaria clamatidis, Astrosphariella malayensis, Massarina bipolaris, Nectria* sp., *Diaporthe setulae, Solheimia costaspora, Submersisphaeria palmae, Dactylaria flammulicornuta*, and *Microthyrium* sp.

## 4.5. Metroxylon sagus

Two collecting trips have been made to Sirindhorn Peat Swamp Forest, Narathiwat. When material of *M. sagus* was sampled, a total of 33 taxa (67 occurences of taxa), including 21 ascomycetes (49%), 10 basidiomycete (23%) and 12 anamorphic fungi (28%) were recorded (Figure 13). The percentage of fungi occurring on different parts of the palm were as follows: dry aerial material with 47% of the fungi; damp material 34%, and wet submerged material 19% (Figure 14). The percentage of fungi recorded from petioles was 47%, leaves 19%, and rachides 34% (Figure 15). The most common fungi were *Anthostomella bipapillisspora*, *Apioclypea eccentricospora*, *Nawawia fusiformis*, *Arecophila* sp., *Apiospora* sp., *Oxydothis angustispora*, and *Astrocystis rachides*.

## 4.6. Nenga pumila

Two collecting trips have been made to Sirindhorn Peat Swamp Forest, Narathiwat for the collection of *N. pumila* material. Anamorphic fungi were dominant on collections on *N. pumila* (59 %), ascomycetes 41 % and basidiomycetes 0 % (Figure

16). The percentage of fungi on different parts of the *N. pumila* palm was: dry material 50 % of the fungi; damp material supported 31 % and wet material had 19 % of the fungi (Figure 17). The percentage of fungi on different parts of the palm was as follows: petioles supported 23 % of the fungi; leaves 3 %, rachides 25 %, and sheaths 49 % (Figure 18). The most common fungi were: *Diplococcium stoveri*, *Dinemasporium* sp., *Arecomyces* sp., *Linocarpon* sp., *Lophodermium* sp., and *Dactylaria palmae*.

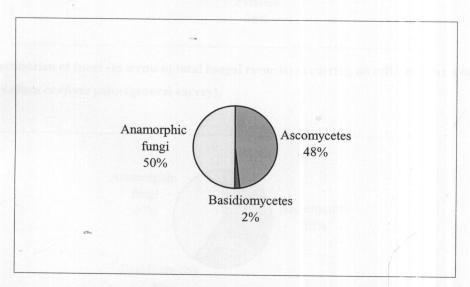


Figure 1. Percentage of ascomycetes, anamorphic fungi and basidiomycetes collections occurring on samples of the palm *Eleiodoxa conferta* (general survey).

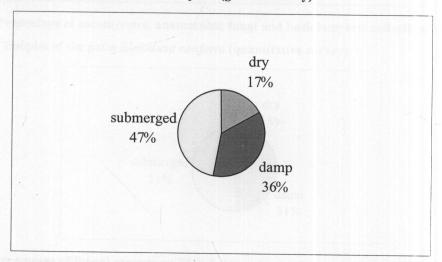


Figure 2. Percentage of fungal records on *Eleiodoxa conferta* palm material from different habitats (general survey).

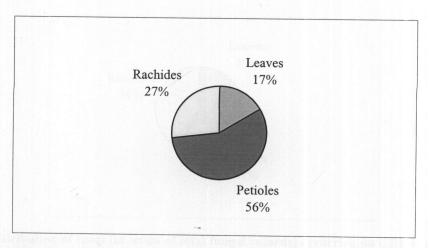


Figure 3. Distribution of fungi (in terms of total fungal records) occurring on different parts of the *Eleiodoxa conferta* palm (general survey).

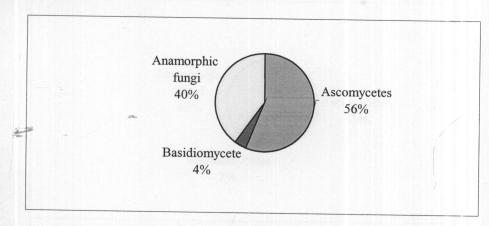


Figure 4. Percentage of ascomycetes, anamorphic fungi and basidiomycete collections occurring on samples of the palm *Eleiodoxa conferta* (quantitative survey).

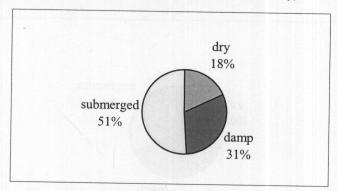


Figure 5. Percentage of fungal records on *Eleiodoxa conferta* palm material from different habitats (quantitative survey).

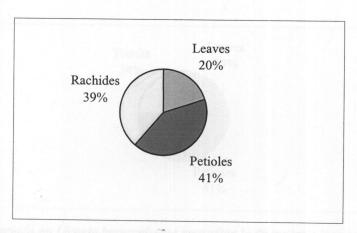


Figure 6. Distribution of fungi (in terms of total fungal records) occurring on different parts of the *Eleiodoxa conferta* palm (quantitative survey).

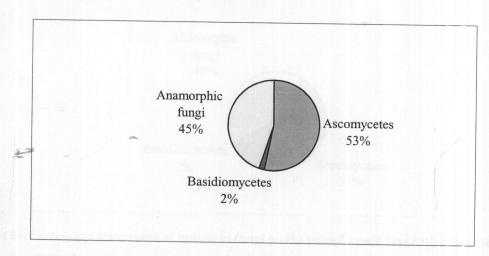


Figure 7. Percentage occurrence of groups of fungi on the palm *Licuala longecalycata* (general survey).

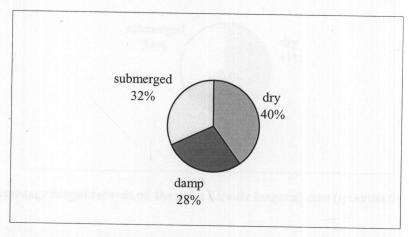


Figure 8. Percentage fungal records on the palm *Licuala longecalycata* under different conditions (general survey).

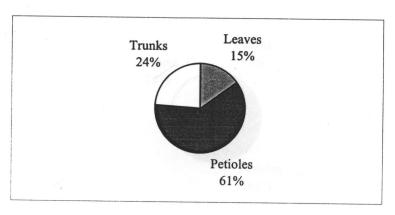


Figure 9. Fungal records on Licuala longecalycata according to tissue type (general survey).

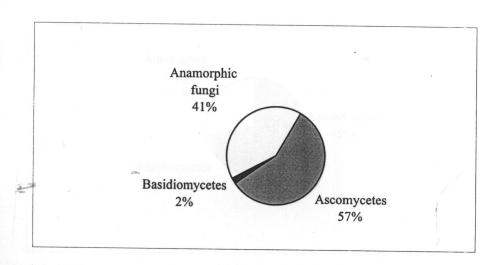


Figure 10. Percentage occurrence of groups of fungi on the palm *Licuala longecalycata* (quantitative survey).

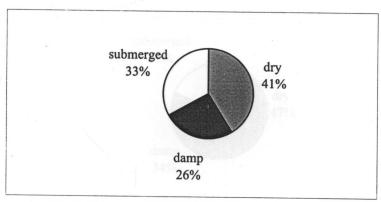


Figure 11. Percentage fungal records on the palm Licuala longecalycata (quantitative survey).

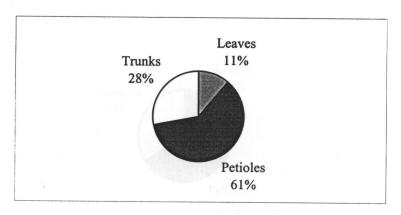


Figure 12. Fungal records from Licuala longecalycata by tissue types (quantitative survey).

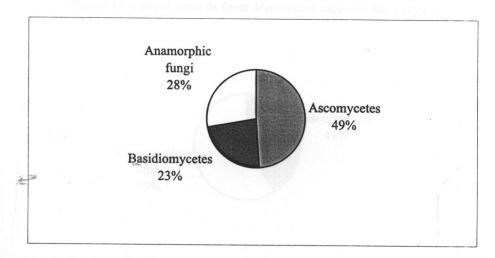


Figure 13. Percentage occurrence of groups of fungi on the palm Metroxylon sagus.

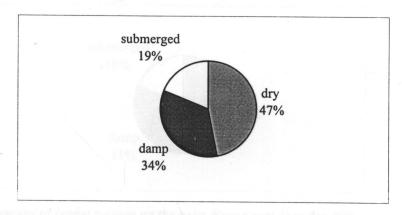


Figure 14. Percentage of fungal records on the palm Metroxylon sagus under different conditions.

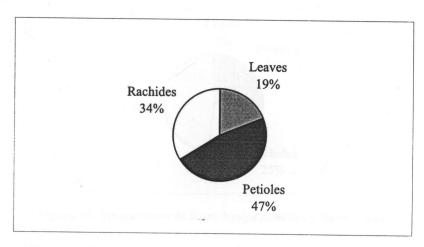


Figure 15. Fungal records from Metroxylon sagus by tissue types.

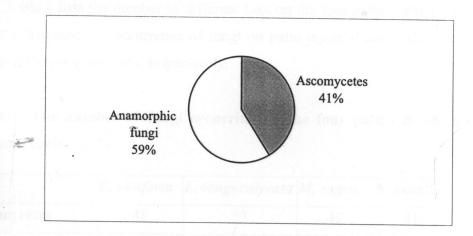


Figure 16. Percentage occurrence of groups of fungi on the palm Nenga pumila.

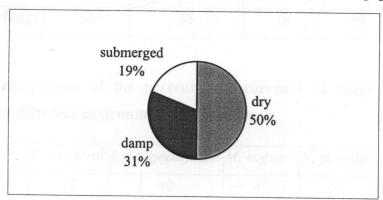


Figure 17. Percentage of fungal records on the palm Nenga pumila under different conditions.

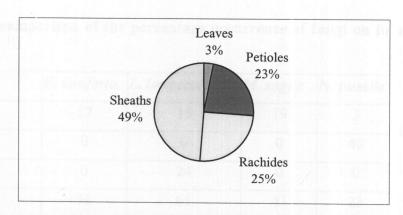


Figure 18. Fungal records from Nenga pumila by tissue types.

## 4.7. FREQUENCY OF OCCURRENCE OF FUNGI ON PALM MATERIAL

Table 1 lists the number of different taxa on the four palms, while Tables 2 and 3 give the frequency of occurrence of fungi on palm material under different conditions and on different palm parts, respectively.

Table 1. The number of fungi occurring on the four palms in Sirindhorn Peat Swamp Forest.

	E. conferta	L. longecalycata	M. sagus	N. pumila
Ascomycetes	48	53	49	41
Basidiomycetes	2	2	23	0
Anamorphic fungi	50	45	28	59

Table 2. A comparison of the percentage occurrence of fungi on four palm material under different environmental conditions.

	E. conferta	L. longecalycata	M. sagus	N. pumila
Dry	17	40	47	50
Damp	36	28	34	31
Submerged	47	32	19	19

Table 3. A comparison of the percentage occurrence of fungi on four palm tissue types.

	E. conferta	L. longecalycata	M. sagus	N. pumila
Leaves	17	15	19	3
Sheaths	0	0	0	49
Trunks	0	24	0	0
Petioles	56	61	47	23
Rachides	27	0 ·	34	25

\*

Slides of all the fungi collected have been made and photographs taken of key species for future publications. Dried material has also been prepared for deposition in the BIOTEC Herbarium.

A number of new taxa were collected and these will be described as new species as soon as their study is complete. Most common species of both palms are presented in Table 4.

Table 4. The ten most common fungi collected on selected palms in Sirindhorn Peat Swamp Forest, Thailand.

Licuala longecalycata	
Annulatascus velatispora	
Microthyrium sp.	
Phaeoisaria clamatidis	
Massarina bipolaris	
Phruensis brunneispora	
Diaporthe setulae	
Solheimia costaspora	
Helicoma sp. 1	
Nectria sp. 1	
Astrosphaeriella aquatica	
Nenga pumila	
Diplococcium stoveri	
Dinemasporium sp.	
	Annulatascus velatispora  Microthyrium sp.  Phaeoisaria clamatidis  Massarina bipolaris  Phruensis brunneispora  Diaporthe setulae  Solheimia costaspora  Helicoma sp. 1  Nectria sp. 1  Astrosphaeriella aquatica  Nenga pumila  Diplococcium stoveri

Capsulospora sp.	Arecomyces sp.	
Dictyochaeta ramulosetulae	Linocarpon sp.	
Arecophila sp.	Lophodermium sp.	
Astrocystis rachisdis	Dactylaria palmae	
Apioclypea eccentricospora	Jahnula appendiculata	
Solheimia sp.	Lophiostoma sp.	
Astrosphaeriella lophiostomopsis	Oxydothis sp.	
Cylindrocladium sp.	Cryptophialoidea sp.	

## 4.8. ENDOPHYTES

Endophytic fungi were recovered from 91 of the 200 discs (colonization rate 45%). Total number of isolates were 130 (isolation rate 58.5%). The identification of endophytic fungi has proved to be difficult, largely because of the lack of information on the cultural characters of species already described and the none sporulation of many of the isolates. Cultures on PDA and CMA were examined periodically for reproductive structures and identified as they sporulated.

## 4.9. ISOLATIONS

Three hundred and forty four saprophytic and endophytic fungi have been isolated and deposited in the BIOTEC Culture Collection (Table 5).

Table 5. List of axenic strains deposited in BIOTEC Culture Collection (BCC).

No.	Original code.	BCC No.	Name of fungus	Substratum
1	PF1	9880	Helicoubisia cornata	Eleiodoxa conferta
2	PF 2	9881	Custingophora undulatophora	Eleiodoxa conferta
3	PF3	9882	Chalara siamense	Eleiodoxa conferta
4	PF4	9883	Dactylaria uliginicola	Eleiodoxa conferta
5	PF5	11397	Vanakripa minutellipsoidea	Eleiodoxa conferta
6	PF6	11398	Vanakripa minutellipsoidea	Eleiodoxa conferta
7	PF7	11399	Vanakripa minutellipsoidea	Eleiodoxa conferta

8	PF8	11400	Jahnula appendiculata	Eleiodoxa conferta
9	PF9	11445	Jahnula appendiculata	Eleiodoxa conferta
10	PF10	11401	Stillbohypoxylon moelleri	Eleiodoxa conferta
11	PF11	11402	Lophiostroma sp.	Eleiodoxa conferta
12	PF12	11403	Unidentified	Eleiodoxa conferta
13	PF13	11404	Unidentified	Eleiodoxa conferta
14	PF14	11405	Oxydothis sp.	Eleiodoxa conferta
15	PF15	11166	Ascominuta lignicola	Licuala longecalycata
16	PF16	11165	Unidentified	Licuala longecalycata
17	PF17	11167	Craspedodidymum licualae	Licuala longecalycata
18	PF18	11168	Diaporthe setulae	Licuala longecalycata
19	PF19	11169	Phruensis brunniespora	Licuala longecalycata
20	PF20	11170	Solheimia costospora	Licuala longecalycata
21	PF21		Jahnula appendiculata	Licuala longecalycata
22	PF22	11171	Zalerion sp.	Licuala longecalycata
23	PF23	11172	Spadicoides sp.	Licuala longecalycata
24	PF24	11173	Chalara siamense	Licuala longecalycata
25	PF25		Thozetella sp.	Licuala longecalycata
26	PF26	12538	Helicoma sp.	Licuala longecalycata
27	PF27	12539	Phaeoisaria clematidis	Licuala longecalycata
28	PF28	12540	Unidentified	Licuala longecalycata
29	PF29	12541	Canalisporium sp.	Licuala longecalycata
30	PF30		Stillbohypoxylon sp.	Licuala longecalycata
31	PF31	12542	Trichoderma sp.	Licuala longecalycata
32	PF32	12543	Dictyosporium sp.	Licuala longecalycata
33	PF33	12544	Nectria sp.	Licuala longecalycata
34	PF34	12492	Unidentified	Eleiodoxa conferta
35	PF35	12493	Unidentified	Eleiodoxa conferta
36	PF36	12494	Sporidesmium sp.	Eleiodoxa conferta
37	PF37	12495	Sporidesmium sp.	Eleiodoxa conferta

38	PF38	12496	Astrosphaeriella sp.	Eleiodoxa conferta
39	PF39	12491	Astrosphaeriella sp.	Eleiodoxa conferta
40	PF40	12497	Unidentified	Eleiodoxa conferta
41	PF41	12498	Unidentified	Eleiodoxa conferta
42	PF42	12499	Unidentified	Eleiodoxa conferta
43	PF43	12500	Helicosporium sp.	Eleiodoxa conferta
44	PF44	12501	Astrosphaeriella sp.	Eleiodoxa conferta
45	PF45	12502	Chloridium sp.	Eleiodoxa conferta
46	PF46	12503	Chloridium sp.	Eleiodoxa conferta
47	PF47	12504	Chloridium sp.	Eleiodoxa conferta
48	PF48	12505	Unidentified	Eleiodoxa conferta
49	PF49	12506	Unidentified	Eleiodoxa conferta
50	PF50	12507	Astrosphaeriella sp.	Eleiodoxa conferta
51	PF51	12508	Unidentified	Eleiodoxa conferta
52	PF52	12509	Astrosphaeriella sp.	Eleiodoxa conferta
53	PF53	12510	Astrosphaeriella sp.	Eleiodoxa conferta
54	PF54	12511	Stillbohypoxylon moelleri	Eleiodoxa conferta
55	PF55	12512	Stillbohypoxylon moelleri	Eleiodoxa conferta
56	PF56	12513	Unidentified	Eleiodoxa conferta
57	PF57	12514	Unidentified	Eleiodoxa conferta
58	PF58	12515	Stictis sp.	Eleiodoxa conferta
59	PF59	12516	Dactylaria uliginicola	Eleiodoxa conferta
60	PF60	12517	Helicosporium sp.	Eleiodoxa conferta
61	PF61	12518	Helicosporium sp.	Eleiodoxa conferta
62	PF62	12519	Berkleasmium sp.	Eleiodoxa conferta
63	PF63	12520	Unidentified	Eleiodoxa conferta
64	PF64	12521	Sporidesmium sp.	Eleiodoxa conferta
65	PF65	12522	Sporidesmium sp.	Eleiodoxa conferta
66	PF66	12523	Sporidesmium sp.	Eleiodoxa conferta
67	PF67	12524	Sporidesmium sp.	Eleiodoxa conferta

68	PF68	12525	Vanakripa sp.	Eleiodoxa conferta
69	PF69	12526	Vanakripa sp.	Eleiodoxa conferta
70	PF70	12527	Astrosphaeriella sp.	Eleiodoxa conferta
71	PF71	12528	Unidentified	Eleiodoxa conferta
72	PF72	12529	Trichoderma sp.	Nenga pumila
73	PF73	12530	Trichoderma sp.	Nenga pumila
74	PF74	12775	Submersisphaeria sp.	Eleiodoxa conferta
75	PF75	12776	Unidentified	Nenga pumila
76	PF76	12777	Unidentified	Nenga pumila
77	PF77	12739	Denimasporium sp.	Nenga pumila
78	PF78	12740	Denimasporium sp.	Nenga pumila
79	PF79	12741	Didymosphaeria sp.	Nenga pumila
80	PF80	12742	Didymosphaeria sp.	Nenga pumila
81	PF81	12743	Helicoon sp.	Nenga pumila
82	PF82	12744	Helicoon sp.	Nenga pumila
83	PF83	12913	Phaeoisaria sp.	Eleiodoxa conferta
84	PF84	12914	Solhemia sp.	Eleiodoxa conferta
85	PF85	12915	Astrosphaeriella sp.	Eleiodoxa conferta
86	PF86	12916	Astrosphaeriella sp.	Eleiodoxa conferta
87	PF87	12917	Spadicoides sp.	Nenga pumila
88	PF88	12918	Spadicoides sp.	Nenga pumila
89	PF89	12919	Thozetella sp.	Eleiodoxa conferta
90	PF90 .	12920	Thozetella sp.	Eleiodoxa conferta
91	PF91	12921	Sporideamium sp.	Nenga pumila
92	PF92	12922	Ascominuta lignicola	Nenga pumila
93	PF93	12923	Unidentified	Nenga pumila
94	PF94	12924	Unidentified	Nenga pumila
95	PF95	12925	Unidentified	Nenga pumila
96	PF96	12926	Unidentified	Nenga pumila
97	PF97	12927	Phaeoisaria sp.	Eleiodoxa conferta

98	PF98	12928	Phaeoisaria sp.	Eleiodoxa conferta
99	PF99	12929	Unidentified	Nenga pumila
100	PF100	12930	Unidentified	Nenga pumila
101	PF101	12931	Unidentified	Nenga pumila
102	PF102	12932	Unidentified	Nenga pumila
103	PF103	12874	Astrocystis sp.	Eleiodoxa conferta
104	PF104	12875	Astrocystis sp.	Eleiodoxa conferta
105	PF105	12876	Astrocystis sp.	Eleiodoxa conferta
106	PF106	12877	Astrocystis sp.	Eleiodoxa conferta
107	PF107	12878	Sporidesmium sp.	Nenga pumila
108	PF108	12879	Sporidesmium sp.	Nenga pumila
109	PF109	12880	Lophiostoma sp.	Nenga pumila
110	PF110	12881	Sporidesmium sp.	Nenga pumila
111	PF111	13367	Flammispora bioteca	Licuala longecalycata
112	PF112	13368	Flammispora bioteca	Licuala longecalycata
113	PF113	13369	Flammispora bioteca	Licuala longecalycata
114	PF114	13370	Flammispora bioteca	Licuala longecalycata
115	PF115	13371	Flammispora bioteca	Licuala longecalycata
116	PF116	13372	Dactylaria flammulicornuta	Licuala longecalycata
117	PF117	13373	Dactylaria flammulicornuta	Licuala longecalycata
118	PF118	13374	Ascominuta sp.	Licuala longecalycata
119	PF119	13375	Solheimia sp.	Licuala longecalycata
120	PF120	13376	Solheimia sp.	Licuala longecalycata
121	PF121	15495	Astrosphaeriella sp.	Eleiodoxa conferta
122	PF122	15496	Phaeoisaria clematidis	Eleiodoxa conferta
123	PF123	14154	Submersisphaeria sp.	Eleiodoxa conferta
124	PF124	15497	Dactylaria sp.	Eleiodoxa conferta
125	PF125	14265	Stictis sp.	Eleiodoxa conferta
126	PF126	14264	Sporidesmium sp.	Nenga pumila
127	PF127	14263	Didymosphaeria sp.	Nenga pumila

128	PF128	14155	Unidentified	Nenga pumila
129	PF129	14262	Submersisphaeria sp.	Eleiodoxa conferta
130	PF130	15498	Astrosphaeriella aquatica-like	Eleiodoxa conferta
131	PF131	14156	Monotosporella rhizoidea	Eleiodoxa conferta
132	PF132	14157	Ascominuta lignicola	Nenga pumila
133	PF133	14158	Microtyrium sp.	Eleiodoxa conferta
134	PF134	14159	Stictis sp.	Eleiodoxa conferta
135	PF135	14162	Tubeufia2	Nenga pumila
136	PF136	14161	Vanakripa minutellipsoidea	Eleiodoxa conferta
137	PF137	14162	Unidentified	Nenga pumila
138	PF138	14163	Monotosporella rhizoidea	Eleiodoxa conferta
139	PF139	14164	Tubeufia1	Nenga pumila
140	PF140	14165	Tubeufia2	Nenga pumila
141	PF141	14166	Helicobeusia coronata	Eleiodoxa conferta
142	PF142	14167	Sporidesmium sp.	Nenga pumila
143	PF143	14168	Berkleasmium typhae	Eleiodoxa conferta
144	PF144	14169	Tubeufia sp.	Nenga pumila
145	PF145	14170	Custingophora undutistipes	Eleiodoxa conferta
146	PF146	14171	Coleodictyospora micronesica	Eleiodoxa conferta
147	PF147	14172	Chlamydospore	Eleiodoxa conferta
148	PF148	14173	Trichoderma sp.	Nenga pumila
149	PF149	14175	Dactylaria sp.	Nenga pumila
150	PF150		Unidentified	Licuala longecalycata
151	PF151		Helicoma sp	Licuala longecalycata
152	PF152		Zalerion sp.	Licuala longecalycata
153	PF153		Unidentified	Licuala longecalycata
154	PF154		Massarina sp.	Metroxylon sagus
155	PF155		Phaeodothis sp.	Licuala longecalycata
156	PF156		Tetraploa aristata	Metroxylon sagus
157	PF157		Unidentified	Licuala longecalycata
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	AOM			
186	00012.03	16084	Unidentified	Eleiodoxa conferta
107	AOM	1.600.5	TT.::44:6:-4	F1 - i - J
187	00052.02	16085	Unidentified	Eleiodoxa conferta
188	AOM 00201.01	15543	Stictis sp.	Caryota urens
100	AOM	13343	энсиз эр.	Caryota arens
189	00201.02	15544	Stictis sp.	Caryota urens
167	AOM	13344	Sucus sp.	Caryota arens
190	00202.01	15565	Ellisembia sp.	Caryota urens
170	AOM		инэстоги эр.	Caryota arens
191	00215.01	15946	Coleodictyospora micronisica	Caryota urens
	AOM			
192	00217.01	15944	Astrosphaeriella sp.	Caryota urens
	AOM		•	
193	00217.02	15945	Astrosphaeriella sp.	Caryota urens
	AOM			
194	00219.01	15947	Unidentified	Caryota urens
	AOM	-		
195	00222.01	15943	Monotosporella sp.	Caryota urens
	AOM			
196	00230.01	15969	Dictyosporium sp.	Caryota urens
	AOM		·	
197	00230.02	15970	Dictyosporium sp.	Caryota urens
	AOM			
198	00230.03	15971	Dictyosporium sp.	Caryota urens
	AOM			
199	00231.01	15972	Diplococcium sp.	Caryota urens
	AOM			
200	00231.02	15973	Diplococcium sp.	Caryota urens
	AOM			
201	00234.01	16052	Unidentified	Calamus sp.
200	AOM	1.051	*****	
202	00234.02	16051	Unidentified	Calamus sp.
202	AOM	1,0050	TT 11 .10 1	
203	00235.01	16050	Unidentified	Calamus sp.
204	AOM 00235.01	16050	Unidentified	Calarres
204	00233.01	10030	Omdenined	Calamus sp.

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December 2015   Columns   Columns	205	AOM 00237.03	16045	Unidentified	Calamus sp.
AOM		AOM			•
AOM	206		16088	Unidentified	Calamus sp.
AOM		AOM			
AOM	207		16149	Unidentified	Calamus sp.
AOM		1			
AOM	208	00238.03	16150	Unidentified	Calamus sp.
AOM		AOM			
AOM	209	00238.04	16151	Unidentified	Calamus sp.
AOM 211 00242.03 16046 Unidentified Calamus sp.  AOM 212 00244.01 16152 Astrosphaeriella sp. Eleiodoxa conferta  AOM 213 00244.02 16153 Astrosphaeriella sp. Eleiodoxa conferta  AOM 214 00244.03 16154 Astrosphaeriella sp. Eleiodoxa conferta  AOM 215 00244.04 16155 Astrosphaeriella sp. Eleiodoxa conferta  216 EP1 13146 Unidentified Licuala longecalycata 217 EP2 13147 Xylariaceous Licuala longecalycata 218 EP3 13148 Xylariaceous Licuala longecalycata 219 EP4 13149 Xylariaceous Licuala longecalycata 220 EP5 13150 Xylariaceous Licuala longecalycata 221 EP6 13151 Unidentified Licuala longecalycata 222 EP7 13152 Xylariaceous Licuala longecalycata 223 EP8 13153 Xylariaceous Licuala longecalycata 224 EP9 13154 Xylariaceous Licuala longecalycata 225 EP10 13155 Xylariaceous Licuala longecalycata 226 EP11 13156 Unidentified Licuala longecalycata 227 EP12 13157 Unidentified Licuala longecalycata		AOM			
211   00242.03   16046   Unidentified   Calamus sp.	210	00242.01	16047	Unidentified	Calamus sp.
AOM 212 00244.01 16152		AOM			
AOM 213 00244.02 16153	211	00242.03	16046	Unidentified	Calamus sp.
AOM 213 00244.02 16153		AOM			
AOM 214 00244.03 16154	212	00244.01	16152	Astrosphaeriella sp.	Eleiodoxa conferta
AOM 214 00244.03 16154		AOM			
AOM 215 00244.04 16155	213	00244.02	16153	Astrosphaeriella sp.	Eleiodoxa conferta
AOM 215 00244.04 16155		AOM			
21500244.0416155Astrosphaeriella sp.Eleiodoxa conferta216EP113146UnidentifiedLicuala longecalycata217EP213147XylariaceousLicuala longecalycata218EP313148XylariaceousLicuala longecalycata219EP413149XylariaceousLicuala longecalycata220EP513150XylariaceousLicuala longecalycata221EP613151UnidentifiedLicuala longecalycata222EP713152XylariaceousLicuala longecalycata223EP813153XylariaceousLicuala longecalycata224EP913154XylariaceousLicuala longecalycata225EP1013155XylariaceousLicuala longecalycata226EP1113156UnidentifiedLicuala longecalycata227EP1213157UnidentifiedLicuala longecalycata	214	00244.03	16154	Astrosphaeriella sp.	Eleiodoxa conferta
216 EP1 13146 Unidentified Licuala longecalycata 217 EP2 13147 Xylariaceous Licuala longecalycata 218 EP3 13148 Xylariaceous Licuala longecalycata 219 EP4 13149 Xylariaceous Licuala longecalycata 220 EP5 13150 Xylariaceous Licuala longecalycata 221 EP6 13151 Unidentified Licuala longecalycata 222 EP7 13152 Xylariaceous Licuala longecalycata 223 EP8 13153 Xylariaceous Licuala longecalycata 224 EP9 13154 Xylariaceous Licuala longecalycata 225 EP10 13155 Xylariaceous Licuala longecalycata 226 EP11 13156 Unidentified Licuala longecalycata 227 EP12 13157 Unidentified Licuala longecalycata		AOM			
217 EP2 13147 Xylariaceous Licuala longecalycata 218 EP3 13148 Xylariaceous Licuala longecalycata 219 EP4 13149 Xylariaceous. Licuala longecalycata 220 EP5 13150 Xylariaceous Licuala longecalycata 221 EP6 13151 Unidentified Licuala longecalycata 222 EP7 13152 Xylariaceous Licuala longecalycata 223 EP8 13153 Xylariaceous Licuala longecalycata 224 EP9 13154 Xylariaceous Licuala longecalycata 225 EP10 13155 Xylariaceous Licuala longecalycata 226 EP11 13156 Unidentified Licuala longecalycata 227 EP12 13157 Unidentified Licuala longecalycata	215	00244.04	16155	Astrosphaeriella sp.	Eleiodoxa conferta
218 EP3 13148 Xylariaceous Licuala longecalycata 219 EP4 13149 Xylariaceous. Licuala longecalycata 220 EP5 13150 Xylariaceous Licuala longecalycata 221 EP6 13151 Unidentified Licuala longecalycata 222 EP7 13152 Xylariaceous Licuala longecalycata 223 EP8 13153 Xylariaceous Licuala longecalycata 224 EP9 13154 Xylariaceous Licuala longecalycata 225 EP10 13155 Xylariaceous Licuala longecalycata 226 EP11 13156 Unidentified Licuala longecalycata 227 EP12 13157 Unidentified Licuala longecalycata	216	EP1	13146	Unidentified	Licuala longecalycata
EP4 13149 Xylariaceous. Licuala longecalycata  220 EP5 13150 Xylariaceous Licuala longecalycata  221 EP6 13151 Unidentified Licuala longecalycata  222 EP7 13152 Xylariaceous Licuala longecalycata  223 EP8 13153 Xylariaceous Licuala longecalycata  224 EP9 13154 Xylariaceous Licuala longecalycata  225 EP10 13155 Xylariaceous Licuala longecalycata  226 EP11 13156 Unidentified Licuala longecalycata  227 EP12 13157 Unidentified Licuala longecalycata	217	EP2	13147	Xylariaceous	Licuala longecalycata
220 EP5 13150 Xylariaceous Licuala longecalycata 221 EP6 13151 Unidentified Licuala longecalycata 222 EP7 13152 Xylariaceous Licuala longecalycata 223 EP8 13153 Xylariaceous Licuala longecalycata 224 EP9 13154 Xylariaceous Licuala longecalycata 225 EP10 13155 Xylariaceous Licuala longecalycata 226 EP11 13156 Unidentified Licuala longecalycata 227 EP12 13157 Unidentified Licuala longecalycata	218	EP3	13148	Xylariaceous	Licuala longecalycata
EP6 13151 Unidentified Licuala longecalycata  222 EP7 13152 Xylariaceous Licuala longecalycata  223 EP8 13153 Xylariaceous Licuala longecalycata  224 EP9 13154 Xylariaceous Licuala longecalycata  225 EP10 13155 Xylariaceous Licuala longecalycata  226 EP11 13156 Unidentified Licuala longecalycata  227 EP12 13157 Unidentified Licuala longecalycata	219	EP4	13149	Xylariaceous.	Licuala longecalycata
EP7 13152 Xylariaceous Licuala longecalycata  223 EP8 13153 Xylariaceous Licuala longecalycata  224 EP9 13154 Xylariaceous Licuala longecalycata  225 EP10 13155 Xylariaceous Licuala longecalycata  226 EP11 13156 Unidentified Licuala longecalycata  227 EP12 13157 Unidentified Licuala longecalycata	220	EP5	13150	Xylariaceous	Licuala longecalycata
223 EP8 13153 Xylariaceous Licuala longecalycata  224 EP9 13154 Xylariaceous Licuala longecalycata  225 EP10 13155 Xylariaceous Licuala longecalycata  226 EP11 13156 Unidentified Licuala longecalycata  227 EP12 13157 Unidentified Licuala longecalycata	221	EP6	13151	Unidentified	Licuala longecalycata
224 EP9 13154 Xylariaceous Licuala longecalycata 225 EP10 13155 Xylariaceous Licuala longecalycata 226 EP11 13156 Unidentified Licuala longecalycata 227 EP12 13157 Unidentified Licuala longecalycata	222	EP7	13152	Xylariaceous	Licuala longecalycata
225 EP10 13155 Xylariaceous Licuala longecalycata 226 EP11 13156 Unidentified Licuala longecalycata 227 EP12 13157 Unidentified Licuala longecalycata	223	EP8	13153	Xylariaceous	Licuala longecalycata
226 EP11 13156 Unidentified Licuala longecalycata  227 EP12 13157 Unidentified Licuala longecalycata	224	EP9	13154	Xylariaceous	Licuala longecalycata
227 EP12 13157 Unidentified Licuala longecalycata	225	EP10	13155	Xylariaceous	Licuala longecalycata
Dienan iongecarycan	226	EP11	13156	Unidentified	Licuala longecalycata
228 EP13 13158 Xylariaceous Licuala longecalycata	227	EP12	13157	Unidentified	Licuala longecalycata
	228	EP13	13158	Xylariaceous	Licuala longecalycata

229	EP14	13159	Unidentified	Licuala longecalycata
230	EP15	13160	Xylariaceous	Licuala longecalycata
231	EP16	13161	Xylariaceous	Licuala longecalycata
232	EP17	13162	Unidentified	Licuala longecalycata
233	EP18	13163	Xylariaceous	Licuala longecalycata
234	EP19	13164	Unidentified	Licuala longecalycata
235	EP20	13165	Xylariaceous	Licuala longecalycata
236	EP21	13166	Xylariaceous	Licuala longecalycata
237	EP22	13167	Unidentified	Licuala longecalycata
238	EP23	13168	Xylariaceous	Licuala longecalycata
239	EP24	13169	Unidentified	Licuala longecalycata
240	EP25	13170	Xylariaceous	Licuala longecalycata
241	EP26	13171	Unidentified	Licuala longecalycata
242	EP27	13172	Unidentified	Licuala longecalycata
243	EP28	13173	Unidentified	Licuala longecalycata
244	EP29	13174	Unidentified	Licuala longecalycata
245	EP30	13175	Unidentified	Licuala longecalycata
246	EP31	13176	Unidentified	Licuala longecalycata
247	EP32	13177	Unidentified	Licuala longecalycata
248	EP33	13178	Unidentified	Licuala longecalycata
249	EP34	13179	Xylariaceous	Licuala longecalycata
250	EP35	13180	Unidentified	Licuala longecalycata
251	EP36	13181	Unidentified	Licuala longecalycata
252	EP37	13182	Unidentified	Licuala longecalycata
253	EP38	13183	Unidentified	Licuala longecalycata
254	EP39	13184	Unidentified	Licuala longecalycata
255	EP40	13185	Unidentified	Licuala longecalycata
256	EP41	14140	Unidentified	Licuala longecalycata
257	EP42	14141	Unidentified	Licuala longecalycata
258	EP43	14142	Unidentified	Licuala longecalycata

259	EP44	14143	Unidentified	Licuala longecalycata
260	EP45		Unidentified	Licuala longecalycata
261	EP46		Unidentified	Licuala longecalycata
262	EP47		Xylariaceous	Licuala longecalycata
263	EP48		Unidentified	Licuala longecalycata
264	EP49		Unidentified	Licuala longecalycata
265	E50		Unidentified	Licuala longecalycata
266	EP50	-	Unidentified	Licuala longecalycata
267	EP52		Xylariaceous	Licuala longecalycata
268	EP53		Unidentified	Licuala longecalycata
269	EP54		Unidentified	Licuala longecalycata
270	EP55		Unidentified	Licuala longecalycata
271	EP56		Unidentified	Licuala longecalycata
272	EP57		Unidentified	Licuala longecalycata
273	EP58	14144	Unidentified	Licuala longecalycata
274	EP59	14145	Unidentified	Licuala longecalycata
275	EP60	14146	Unidentified	Licuala longecalycata
276	EP61	14147	Unidentified	Licuala longecalycata
277	EP62	14148	Unidentified	Licuala longecalycata
278	EP63	14149	Unidentified	Licuala longecalycata
279	EP64	14150	Unidentified	Licuala longecalycata
280	EP65	14151	Unidentified	Licuala longecalycata
281	EP66	14152	Unidentified	Licuala longecalycata
282	EP67		Unidentified	Licuala longecalycata
283	EP68	14153	Unidentified	Licuala longecalycata
284	EP69		Unidentified	Licuala longecalycata
285	EP70		Unidentified	Licuala longecalycata
286	EP71		Unidentified	Licuala longecalycata
287	EP72		Unidentified	Licuala longecalycata
288	EP73		Unidentified	Licuala longecalycata

289	EP94	14734	Unidentified	Licuala longecalycata
290	EP95	14735	Unidentified	Licuala longecalycata
291	EP96	14736	Unidentified	Licuala longecalycata
292	EP97	14737	Unidentified	Licuala longecalycata
293	EP98	14738	Unidentified	Licuala longecalycata
294	EP99	14739	Unidentified	Licuala longecalycata
295	EP100		Unidentified	Licuala longecalycata
296	EP101		Unidentified	Licuala longecalycata
297	EP102		Unidentified	Licuala longecalycata
298	EP103		Unidentified	Licuala longecalycata
299	EP104		Unidentified	Licuala longecalycata
300	EP105		Unidentified	Licuala longecalycata
301	EP106		Unidentified	Licuala longecalycata
302	EP107	«،	Unidentified	Licuala longecalycata
303	EP108		Unidentified	Licuala longecalycata
304	EP109		Unidentified	Licuala longecalycata
305	EP110		Unidentified	Licuala longecalycata
306	EP111		Unidentified	Licuala longecalycata
307	EP112		Unidentified	Licuala longecalycata
308	EP113		Unidentified	Licuala longecalycata
309	EP114		Unidentified	Licuala longecalycata
310	EP115		Unidentified	Licuala longecalycata
311	EP116		Unidentified	Licuala longecalycata
312	EP117		Unidentified	Licuala longecalycata
313	EP118		Unidentified	Licuala longecalycata
314	EP119		Unidentified	Licuala longecalycata
315	EP120		Unidentified	Licuala longecalycata
316	EP121	1	Unidentified	Licuala longecalycata
317	EP122		Unidentified	Licuala longecalycata
318	EP123		Unidentified	Licuala longecalycata

319	EP124	Unidentified	Licuala longecalycata
320	EP125	Unidentified	Licuala longecalycata
321	EP126	Unidentified	Licuala longecalycata
322	EP127	Unidentified	Licuala longecalycata
323	EP128	Unidentified	Licuala longecalycata
324	EP129	Unidentified	Licuala longecalycata
325	EP130	Unidentified	Licuala longecalycata
326	EP131	Unidentified	Licuala longecalycata
327	EP132	Unidentified	Licuala longecalycata
328	EP133	Unidentified	Licuala longecalycata
329	EP134	Unidentified	Licuala longecalycata
330	EP135	Unidentified	Licuala longecalycata
331	EP136	Unidentified	Licuala longecalycata
332	EP137	Unidentified	Licuala longecalycata
333	EP137.1	Unidentified	Licuala longecalycata
334	EP138	Unidentified	Licuala longecalycata
335	EP138.1	Unidentified	Licuala longecalycata
336	EP139	Unidentified	Licuala longecalycata
337	EP140	Unidentified	Licuala longecalycata
338	EP141	Unidentified	Licuala longecalycata
339	EP142	Unidentified	Licuala longecalycata
340	EP143	Unidentified	Licuala longecalycata
341	EP144	Unidentified	Licuala longecalycata
342	EP145	Unidentified	Licuala longecalycata
343	EP146	Unidentified	Licuala longecalycata
344	EP147	Unidentified	Licuala longecalycata

## 5. DELIVERABLES:

## 5.1. Published papers

- McKenzie, E.H.C., A. Pinnoi, M.K.M. Wong, K.D. Hyde & E.B.G. Jones. 2002. Two new hyaline *Chalara* and key to species described since 1975. Fungal Diversity 11: 129-139.
- 2. Hyde, K.D., Yanna, A. Pinnoi & E.B.G. Jones. 2002. *Goidanichiella fusiforma* sp. nov. from palm fronds in Brunei and Thailand. Fungal Diversity 11: 119-122.
- 3. Pinnoi, A., E.H.C. McKenzie, E.B.G. Jones & K.D. Hyde. 2003. Palm fungi from Thailand: *Custingophora undulatistipes* sp. nov. and *Vanakripa minutellipsoidea* sp. nov. Nova Hedwigia. 77: 213-219.
- 4. Pinnoi, A., E.H.C. McKenzie, E.B.G. Jones & K.D. Hyde. 2003. Aquatic fungi from peat swamp palms: *Unisetosphaeria penguinoides* gen. et sp. nov., and three new *Dactylaria* species. Mycoscience 44: 377-382.
- 5. Pinnoi, A., U. Pinruan, K.D. Hyde & S. Lumyong. 2004. Submersisphaeria palmae sp. nov. and key to the genus and notes on Helicoubisia. Sydowia 56: 72-78.
- 6. Pinruan, U., E. B. G. Jones & K. D. Hyde. 2002. Aquatic fungi from peat swamp palms: *Jahnula appendiculata* sp. nov. Sydowia 54: 242-247.
- 7. Pinruan, U., S. Lumyong, E.H.C. McKenzie, E.B.G. Jones & K.D. Hyde. 2004. Three new species of *Craspedodidymum* from a palm in Thailand. Mycoscience 45: 177-180.
- 8. Pinruan, U., J. Sakayaroj, E.B.G. Jones & K.D. Hyde. 2004. Aquatic fungi from peat swamp palms: *Phruensis brunniespora* gen. et sp. nov. and its hyphomycete anamorph. Mycologia 96: 1163-1170.
- 9. Pinruan, U., E.H.C.McKenzie, E.B.G.Jones & K.D.Hyde. 2004. Two new species of *Stachybotrys*, and a key to the genus. Fungal Diversity. In pressaccepted.
- 10. Pinruan, U., J. Sakayaroj, E.B.G. Jones & K.D. Hyde. *Flammispora* gen. nov., a new freshwater ascomycete from decaying palm leaves. Studies in Mycology. In Press-accepted.
- 11. Pinnoi, A., U. Pinruan, K.D. Hyde, S. Lumyong & E.B.G. Jones. 2004. Palm Fungi. In: Thai Fungal Fiversity (eds. E.B.G. Jones, M. Tanticharoen & K.D. Hyde). BIOTEC, Thailand: 181-187.

## 5.2. Papers in preparation

- 1. Anthostomella lunispora sp. nov. and Astrocystis eleiodoxae sp. nov. new Ascomycota on palm material from a peat swamp forest
- 2. Baipadsphaeria gen. nov., a new freshwater ascomycete from decaying palm leaves
- 3. Diaporthe setulae sp. nov. and its anamorph on palms
- 4. Biodiversity of fungi on the palm *Eleiodoxa conferta* in Sirindhorn Peat Swamp Forest, Narathiwat, Thailand
- 5. Diversity of microfungi on the palm *Licuala longecalycata* in a peat swamp forest
- 6. Factors affecting biodiversity of selected palms in a peat swamp

## 5.3. Oral presentations

- 1. Jones, E.B.G., U. Pinruan, A. Pinnoi, S. Lumyong & K.D. Hyde. 2002. Biodiversity of fungi on palms in an acidic environment. In: The III Asia-Pacific Mycological Congress on Biodiversity and Biotechnology. 4-8 November 2002, Kunming, Yunnan, China.
- 2. Pinnoi, A., U. Pinruan, K.D. Hyde, S. Lumyong & E.B.G. Jone. 2004. Factors affecting fungal diversity on peat swamp palm material. In: The IV Asia Pacific Mycological Congress and The IX International Marine and Freshwater Mycology Symposium. Chiang Mai, Thailand
- 3. Pinruan, U., A. Pinnoi, K.D. Hyde, S. Lumyong & E.B.G. Jone. 2004. Fungal diversity of selected palms in a peat swamp forest. In: The IV Asia Pacific Mycological Congress and The IX International Marine and Freshwater Mycology Symposium. 15-19 November 2004, Chiang Mai, Thailand.

## 5.4. Poster Presentations

1. Pinnoi, A., U. Pinruan, K.D. Hyde & E.B.G. Jones. 2001. Biodiversity on palms in Sirindhorn Peat Swamp Forest, Narathiwat, Thailand. In: BioThailand 2001. 7-10 November 2001, Queen Sirikit national convention center, Bangkok, Thailand.

- 2. Pinnoi, A., S. Lumyong, & K.D. Hyde. 2002. Biodiversity of fungi on the palm *Eleiodoxa conferta* in Sirindhorn Peat Swamp Forest, Narathiwat, Thailand. In: The III Asia-Pacific Mycological Congress on Biodiversity and Biotechnology. 4-8 November 2002, Kunming, Yunnan, China.
- 3. Pinruan, U., S. Lumyong, & K.D. Hyde. 2002. Fungal Diversity on the Palm *Licuala longecalycata* in Sirindhorn Peat Swamp Forest, Narathiwat, Thailand. In: The III Asia-Pacific Mycological Congress on Biodiversity and Biotechnology. 4-8 November 2002, Kunming, Yunnan, China.

## 7. ACKNOWLEDGMENTS:

We thank Prof. Morakot Tanticharoen and Dr. Ruud Valyasevi for their continued interest and support and to BRT grant BRT R\_145008. We also thank Manetr Boonyanant, Director of Sirindhorn Peat Swamp Forest for allowing us to study the fungi at this site. Especial thanks to Dr. Eric McKenzie for assistance in the identification of anamorphic fungi. Particular thanks for our colleagues for help with the field-werk.

## **APPENDIX 3**

# Aquatic fungi from peat swamp palms: *Phruensis brunneispora* gen. et sp. nov. and its hyphomycete anamorph

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Abstract: Phruensis brunneispora is a new genus and species occurring on decaying trunks of the palm Licuala longecalycata in Sirindhorn Peat Swamp Forest, Thailand. We compare the genus with other aquatic ascomycetes with falcate septate ascospores: Pseudohalonectria and Ophioceras. Ascospores differ from species in these genera in being brown with lighter end cells. Also, the ascus pore is subapical, with a channel leading to the apex. Lollipopaia minuta differs from Phruensis brunneispora in that the ascomata are borne in a stroma, asci have an apical pore and the ascospores are hyaline. No genus was found to accommodate the new species. Molecular analysis of rDNA ribosomal 18S confirmed the exclusion of the new species from Pseudohalonectria, and Ophioceras and Lollipopaia minuta formed a sister group with it. Phruensis brunneispora and Lollipopaia minuta grouped in the Diaporthales with 100% bootstrap support. Therefore, both morphological and molecular evidence supports erecting a new genus to accommodate this taxon. A hyaline Phialophora-like anamorph was formed when single ascospores were plated out on agar. The taxon is described and illustrated with light micrographs.

Key words: freshwater ascomycete, palm, peat swamp, taxonomy

Accepted for publication March 17, 2004.

#### INTRODUCTION

While investigating decaying palm material in Sirindhorn Peat Swamp Forest, Thailand (Hyde et al 2002, McKenzie et al 2002, Pinruan et al 2002), we collected a Pseudohalonectria-like ascomycete with brown versicolorous ascospores and isolated it into axenic culture. The isolate resembles species in the genera Pseudohalonectria, Ophioceras (Sordariales incertae sedis) and Lollipopaia (Diaporthales). Pseudohalonectria was described from balsawood submerged in a freshwater lake in Japan (Minoura and Muroi 1978) with P. lignicola as the type species. Eight additional species subsequently have been described (Shearer 1989, Hyde et al 1999, Cai et al 2002). Pseudohalonectria is characterized by bright yellow to brown ascomata with erumpent, cylindrical, periphysate necks and a 3-layered peridium; asci are unitunicate, cylindrical to clavate, with a nonamyloid, thimble-shaped, refractive apical apparatus, and ascospores are cylindrical, smooth, hyaline to slightly colored and usually multiseptate (Shearer 1989). Ophioceras is similar to Pseudohalonectria, and both are referred to Sordariales incertae sedis based on phylogenetic analysis of rDNA restriction and sequence data (Chen et al 1995). These molecular data confirmed that the two genera are distinct and well supported by 80-86% bootstrap values.

The new ascomycete differs from Pseudohalonectria in that it lacks the characteristic yellow pigmentation of the ascomata in nature and in culture the peridium is 2-layered, the ascospores are versicolored and produce an anamorph with hyaline falcate phialidic conidia. Lollipopaia minuta shows similarities to the new taxon, especially in the falcate, septate ascospores. Phruensis brunneispora differs in that L. minuta has a weakly developed stroma, the ascus pore is apical, the ascospores are hyaline and no anamorph has been reported. In this study we use phylogenetic analyses to investigate whether this palm ascomycete is congeneric with Pseudohalonectria, Ophioceras or Lollipopaia, genera from freshwater habitats with similar septate cylindrical ascospores.

#### MATERIALS AND METHODS

Collection and isolation.—During May 2001, submerged palm material was collected from Sirindhorn Peat Swamp

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TABLE I. Sequences from this study and those obtained from GenBank

Classification	Taxon	GenBank accession numbers
Order Diaporthales	Apioplagiostoma aceriferum (Cooke) M.E. Barr	AF277111
	Apiosporopsis carpinea (Fr.) Traverso	AF277110
A compared to the state of the	Cryphonectria cubensis (Bruner) Hodges	L42439
	Cryphonectria parasitica (Murrill) M.E. Barr	U78541
	Endothia gyrosa (Schwein.) Fr.	U78540
	Gnomonia padicola (Lib.) Kleb.	AF277112
	Leucostoma persoonii (Nitschke) Höhn.	M83259
	Lollipopaia minuta Inderbitzin	AF301534
	Phruensis brunneispora strain 1 (BCC11169)	AY580160
	Phruensis brunneispora strain 2 (BCC14138)	AY581944
	Valsa ambiens Nitschke	AF277120
Order Sordariales Incertae Sedis	Ophioceras tenuisporum Shearer, J.L. Crane & W. Chen, CS 652-1	AF050475
	Pseudohalonectria falcata Shearer, CS 617-2C	AF050477
	Pseudohalonectria lignicola Minoura & T. Muroi, J13-21	AF050478
Order Ophiostomatales	Ophiostoma pilliferum (Fr.) Syd. & P. Syd., CBS 129.32	AJ243295
	Ophiostoma ulmi (Buisman) Nannf.	M83261
Order Halosphaeriales	Halosphaeria appendiculata Linder	U46872
	Nais inornata Kohlm.	AF050482
	Nimbospora effusa Jørg. Koch	U46877
Order Microascales	Microascus cirrosus Curzi	M89994
	Petriella setigera (Alf. Schmidt) Curzi	U43908
Order Hypocreales	Hypocrella sp. GJS 89-104	U32409
	Hypomyces polyporinus Peck	U32410
	Nectria haematococca Berk. & Broome	U32413
Order Phyllachorales	Collectotrichum gloeosporioides (Penz.) Penz. & Sacc.	U76339
	Glomerella septospora Sivan. & W.H. Hsieh	U78779
order Sordariales	Chaetomium globosum Kunze	U20379
	Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	X69851
order Xylariales	Xylaria carpophila (Pers.) Fr.	Z49785
The state of the s	Xylaria sp. PF 1022	
Order Pezizales	Helvella terrestris (Velen.) Landvik	AB014042
The state of the s	Morchella esculenta (L.) Pers.	AF046216 U42642

Forest, Narathiwat, Thailand. The material was returned to the laboratory, incubated in plastic boxes on damp tissue paper and examined within 4 wk. Single spore isolations were made on cornmeal agar (CMA), with added antibiotics to suppress bacterial growth, following the method of Choi et al (1999). Two strains of the fungus from different collections (Wah 113.1, Wah 113.2) were used for the molecular study. All observations, including photographic documentation and measurements of the fungus growing on wood, were of material mounted in water and examined with a differential interference microscope. Other collections subsequently have been made and treated in the same manner.

Growth of fungi, DNA extraction, amplification and sequencing.—Stock cultures of the fungus were maintained on CMA at 25 C. The fungus was grown in liquid GYP (glucose, yeast extract, peptone; Abdel-Wahab et al 2001) broth on a rotary shaker at 200 rpm at 25 C. Fungal biomass was harvested by vacuum filtration and washed with sterile distilled water. The biomass was frozen in liquid nitrogen and

ground with a mortar and pestle. DNA was extracted using a NucleoSpin® Plant DNA extraction kit (MACHEREY-NA-GEL, Catalogue No. 740 590. 50). The small subunit ribosomal DNA (rDNA) was amplified using FINNZYMES, DyNAzyme® II DNA Polymerase Kit (MACHEREY-NAGEL, product code F-551S), in a Perkin Elmer thermal cycler. Primers NS1, NS4, NS5 and NS6 were used to amplify the small subunit rRNA (White et al 1990). The amplification cycles were performed following White et al (1990). The PCR product was purified using a NucleoSpin® Plant DNA purification kit (MACHEREY-NAGEL, Catalogue No. 740 570. 50), then sequenced automatically by the BIOTEC Service Unit (BSU) laboratory using primers NS1, NS3, NS5 and NS6 (White et al 1990).

Phylogenetic analysis.—Sequences of Ph. brunneispora (Accession numbers: AY580160, AY581944) were analyzed with other sequences obtained from the GenBank database (TABLE I). Morchella esculenta and Helvella terrestris served as outgroups. Sequences were aligned in Clustal W 1.6 program (Thompson et al 1994) and refined visually in Bioedit

TABLE II. Results of Kishino-Hasegawa maximum likelihood tests on alternative topologies

Analysis	No. of trees	Tree length (steps)	Consist- ency indices (CI)	Retention indices (RI)	Rescaled indices (RC)	−ln likelihood	Difference -ln L	<i>P</i> -value
Unweighted parsimony	2	869	0.666	0.730	0.485	7430.47546	2.00008	0.000*
Weighted parsimony-ti:tv	1	1014.2	0.671	0.736	0.496	7429.15521	0.67983	0.000*
Maximum likelihood	1	_	_	_	_	7428.47538	(best)	0.000

<sup>\*</sup> A significant difference at P < 0.05.

version 5.0.6 (Hall 2001) and Se-Al v1.Oa1 (Rambaut 1996). The alignment was entered into PAUP\* 4.0b10 (Swofford 2002) and MacClade 3.08 (Maddison and Maddison 2001). Phylogenetic trees were generated using unweighted parsimony, weighted parsimony and maximum likelihood criteria on 32 taxa. For the unweighted maximum parsimony analysis, we used a heuristic search with a stepwise starting tree, a random stepwise addition of 100 replicates and treebisection-reconnection branch-swapping algorithm, with gaps treated as missing data. Weighted parsimony analysis was performed using a stepwise matrix to weight nucleotide transformations based on the transition: transversion (ti:tv) ratio, estimated from the dataset using maximum likelihood score in PAUP\* (Swofford 2002). Maximum likelihood parameters: ti:tv ratio, proportion of invariable sites, gamma distribution shape parameter and base frequency, also were estimated from the dataset using maximum likelihood score in PAUP\*. For the maximum likelihood heuristic searches, we used these settings: stepwise addition of sequence, as-is stepwise addition sequence and TBR branch-swapping algorithm. Tree topologies from unweighted parsimony, weighted parsimony and maximum likelihood analyses were tested with the Kishino-Hasegawa (K-H) maximum likelihood test (Kishino and Hasegawa 1989) to find the most likely tree for the dataset. Bootstrap analysis (Felsenstein 1985) based on unweighted parsimony was performed with full heuristic searches on 1000 replicates, stepwise addition of sequence, 10 replicates of random addition of taxa, treebisection-reconnection branch swapping algorithm. Alignments were deposited in TreeBase: accession no. 51059, matrix accession number = M1806.

#### RESULTS

Phylogenetic analyses.—Maximum parsimony analysis of unweighted characters yielded two most parsimonious trees. Of the 1832 characters, 180 were variable (9.82%) and 279 parsimony informative (15.2%). The two trees differed by the position of taxa within the Hypocreales. The K-H test was applied to these trees, which resulted in the best tree for unweighted characters. The weighted parsimony analysis, with the assignment of weight 1.4 to transversion and 1 to transition based on the estimation of ti:tv ratio (ti:tv ratio = 1:1.47441), resulted in one tree with the same topology as the best tree of unweighted parsimony

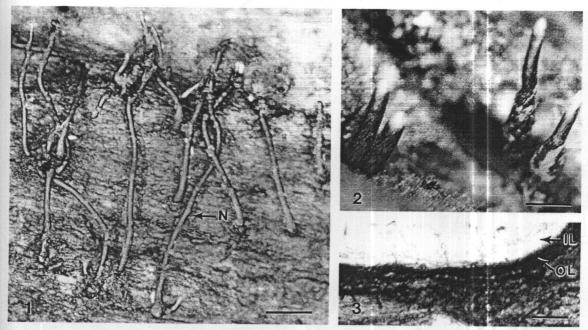
analysis. The result of the K-H test for all trees generated from unweighted, weighted parsimony and maximum likelihood analysis, and the tree generated by the latter method gave the best phylogenetic hypothesis for the dataset (TABLE II). The trees produced by all analyses were significantly different from the most likely tree (P < 0.05). However, the tree topology was the same. The maximum likelihood tree (Fig. 17) has given the -ln likelihood score of 7428.47538. The two strains of Ph. brunneispora sequenced are monophyletic and supported by 100% bootstrap value. Phylogenetic analyses grouped taxa into three clades. Xylaria species (Xylariales, subclass Xylariomycetidae) formed a basal clade to the subclass Sordariomycetidae. Clade 2 comprises the orders Halosphaeriales, Microascales, Phyllachorales, Hypocreales and Sordariales with high bootstrap support. Clade 3 comprised the Diaporthales, Ophiostomatales and Sordariales incertae sedis, the latter containing various Pseudohalonectria and Ophioceras species. Phruensis brunneispora is well supported in the Diaporthales and groups with Lollipopaia minuta (Fig. 17) with which it shares some morphological characteristics.

#### TAXONOMY

Phruensis Pinruan Ascomata immersa, subglobosa, coriacea, nigra, ostiolata, collo longis cylindrica. Peridium compositum ex 2 cellis laminae. Asci cylindrici vel fusiformes, unitunicati, pedecellati, J-, apparatu subapicale praediti. Ascosporae cylindricae, laevis vel curvae, verricolosae, brunneae, 9–11-septatae. Anamorph similis *Phialophora*.

Etymology. from the Thai Phru, meaning peat swamp and Latin -ensis meaning "pertaining to."

Ascomata immersed, subglobose, black, coriaceous, ostiolate, with long central cylindrical neck. Peridium composed of 2 layers, outer layer parenchymatous, intensely brown and merging with the host cells, inner layer, cells elongate and hyaline. Paraphyses hyaline, broad, septate and attached at the base of the centrum. Asci cylindrical to fusiform, unitunicate, apedicellate, apically rounded, with a re-



Figs. 1–3. Light micrographs of *Phruensis brunneispora* (from holotype). 1, 2. Ascomata embedded in the substratum with long necks, tips bright orange. 3. Horizontal section of ascoma with outer perithecial wall (arrow OL) dark brown to black, inner layer (arrow IL) of hyaline elongate cells. Scale bars: 1 = 10 mm; 2 = 5 mm;  $3 = 30 \mu \text{m}$ .

fractive, J-, subapical ring. Ascospores cylindrical, straight or curved, versicolorous, brown with hyaline or pale brown end cells, transseptate. Anamorph similar to *Phialophora*.

Typus species. Phruensis brunneispora Pinruan.

Phruensis brunneispora Pinruan, sp. nov. Figs. 1–16 Ascomata 950–980  $\mu$ m alta, 1.6–1.7 mm diametro, immersa, subglobosa, nigra, coriacea, ostiolata. Collum usque ad 7500  $\mu$ m longum, 500  $\mu$ m diametro, cylindricum, nigrum. Peridium usque ad 250  $\mu$ m crassum, compositum ex 2 cellis laminae. Paraphyses usque ad 12.5–15  $\mu$ m lata ad basim, Asci 260–275  $\times$  45–50  $\mu$ m, 8-spori, cylindrici vel fusiformes, unitunicati, pedecellati, J-, apparatu subapicale 5–6.3  $\mu$ m alta, 3.75–4.3  $\mu$ m diametro praediti. Ascosporae 115–120  $\times$  7.5–8.8  $\mu$ m, 4-seriatae vel fasciculatae, cylindricae, laevae vel curvae, brunneae, 9–11-septatae. Anamorph: cf. *Phialophora* sp.

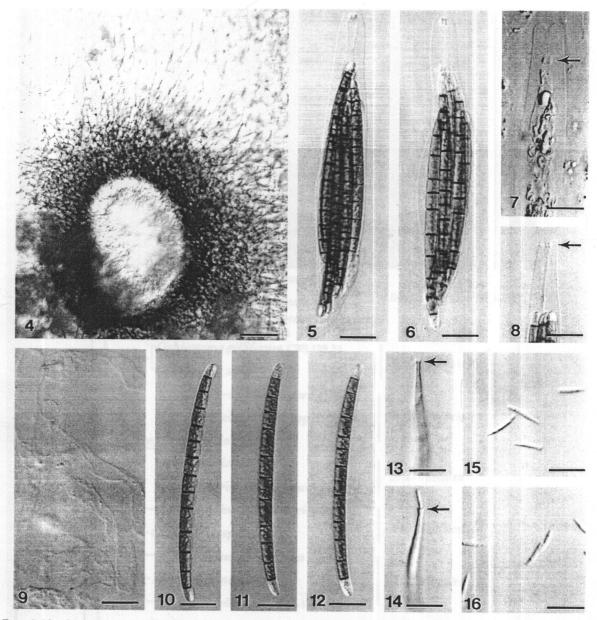
Holotypus. THAILAND. NARATHIWAT: Sirindhorn Peat Swamp Forest, on dead trunk of Licuala longecalycata, 12 May 2001, U. Pinruan (Wah 113.1) in BIOTEC Bangkok Herbarium (BBH). Culture of type isolate: BCC 11169.

Entymology. From brunneispora, in reference to the brown versicolorous ascospores.

Ascomata 950–980 µm high, 1.6–1.7 mm diam, deeply immersed, subglobose, black, coriaceous, ostiolate, scattered (Fig. 1). Neck up to 7.5 mm long, 0.5 mm diam., central, cylindrical, black, orange at

apex (Figs. 1, 2). Peridium up to 250 µm thick, comprising 2 layers, outer layer (40-45 µm) parenchymatous, intensely brown and merging with the host cells, inner layer, cells elongate and hyaline (25-30 μm) (Figs. 3, 4). Paraphyses up to 11.2-15 μm wide at the base, hypha-like, tapering distally, not embedded in a gelatinous matrix (Fig. 9). Asci 260-275 × 45-50  $\mu$ m ( $\bar{x} = 265 \times 48 \mu$ m, n = 25), 8-spored, cylindro-clavate to fusiform, unitunicate, apedicellate, apically rounded, with a refractive, J-, cuboid subapical ring, 5-6.2 µm high, 3.7-4.2 µm diam, with a faint channel leading to the apex (Figs. 5-8). Ascospores  $115-120 \times 7.5-8.7 \ \mu \text{m} \ (\bar{x} = 116.5 \times 7.8 \ \mu \text{m},$ n = 25), 4-seriate to fasciculate, cylindrical, straight or curved, versicolored, brown with hyaline to pale brown end cells, 9-11-septate, smooth-walled, with minute ephemeral mucilaginous material at the ends (Figs. 10-12).

Phialophora-like anamorph: Colonies (BCC 11169) on PDA reaching 2 cm diam in 7 d at room temperature (22–24 C), effuse, brown mycelium party immersed, nonstromatic. Conidiophores up to 5  $\mu$ m wide at the base, semimacronematous, mononematous, branched, straight or slightly flexuous, pale brown to brown, smooth. Conidiogenous cells monophialidic, determinate, with small collarettes (Figs. 13, 14). Conidia 11.5–14  $\times$  1.5  $\mu$ m ( $\bar{x}$  = 12  $\times$  1.5  $\mu$ m, n = 25), aggregated in slimy heads, semi-endog-



Figs. 4–16. Light micrographs of *Phruensis brunneispora* (from holotype) and *Phialophora*-like anamorph. 4. Cross section of neck with fine paraphyses. 5, 6. Asci cylindrical-clavate, ascospores 4-seriate. 7, 8. Cuboid subapical ring (arrows). 9. Hyphalike paraphyses. 10–12. Ascospores with 12–13-septae, central cells brown, end cells hyaline to pale brown. 13, 14. Conidiophores with small collarettes (arrows). 15, 16. Conidia. Scale bars:  $4 = 40 \mu m$ ;  $5-12 = 20 \mu m$ ;  $13-16 = 10 \mu m$ .

enous, straight or curved, oblong, colorless, smooth, 0-septate (Figs. 15, 16). Ascomata not formed in culture.

Other collections: THAILAND. NARATHIWAT: Sirindhorn Peat Swamp Forest, on dead trunk of *Licuala longecalycata*, 2 April 2003, U. Pinruan (Wah 113.2) in BBH. Culture from this collection deposited in BCC 14138.

#### DISCUSSION

Phylogenetic analyses showed that *Phruensis brun*neispora belongs in the order Diaporthales. This is supported by various characters such as saprobic on decaying plant material, bilayered ascomata, long and periphysate necks, paraphysate, and unitunicate asci with a refractive, apical J-ring (Barr 1991). Ph.

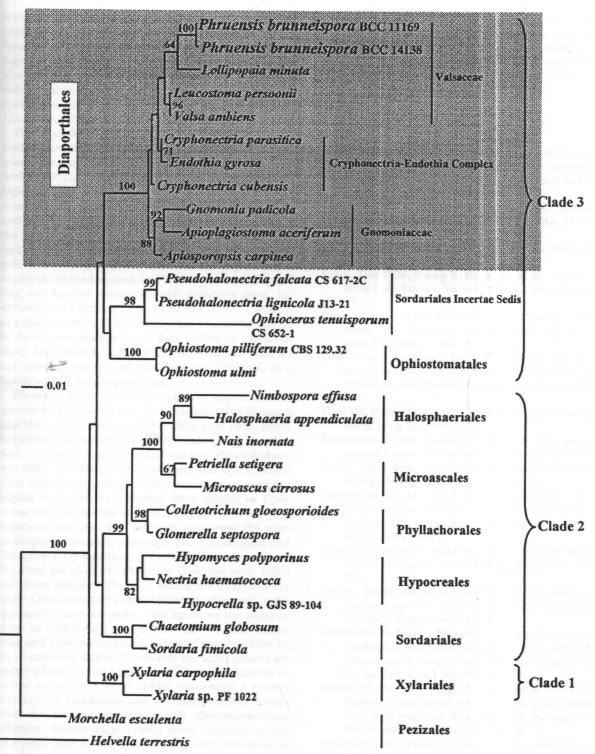


Fig. 17. A single most likely tree inferred from maximum likelihood analysis (-In likelihood score = 7428.47538), from rtial 18S rDNA sequences. Bootstrap values higher than 50% from maximum parsimony analysis are given on the branches, ale bar indicates one base substitution per 100 nucleotides.

brunneispora differs from other members of the Diaporthales in a number of respects: ascomata are not formed in a stroma, although they may be grouped together in the substratum; paraphyses are wide, hypha-like and persistent; and ascospores are falcate with numerous septa. Other genera in the Diaporthales also may have hyaline, falcate ascospores (e.g., Linospora, Sillia).

Phruensis brunneispora most closely resembles Lollipopaia minuta, which also differs morphologically from other taxa of the Diaporthales (Inderbitzin and Berbee 2001). Phruensis brunneispora differs morphologically from L. minuta in that its ascomata are not stromatic, the peridial walls comprise two tissues types and asci have a cuboid subapical, J-ring. In addition, the ascospores of Ph. brunneispora are wider, longer, versicolorous with obvious septa and have hyaline to pale brown end cells. In contrast, Lollipopaia has a weakly developed stroma, asci with a noncuboid apical ring and hyaline ascospores with indistinct septa. Inderbitzin and Berbee (2001) did not refer L. minuta to a family, merely commenting that it could be placed in the Gnomoniaceae sensu Barr (1990) or Valsaceae sensu Hawksworth et al (1995). Similarly, our data do not allow assignment of Phruensis and Lollipopia to a family within the Diaporthales.

Phruensis brunneispora is similar in appearance to species of Ophioceras and Pseudohalonectria (Minoura and Muroi 1978, Shearer 1989, Chen et al 1995, 1999) but differs in having versicolored ascospores. In addition, the ascal rings in Ophioceras and Pseudohalonectria differ in being thimble-shaped and apical rather than cuboid and subapical. Because of their morphological similarity, we included three species of Ophioceras and two species of Pseudohalonectria in our phylogenetic analyses. The analyses supported the morphological data that, although Ph. brunneispora and the genera Ophioceras and Pseudohalonectria are similar, they are distinct. Phruensis clustered with 100% bootstrap support (Fig. 17) within the Diaporthales, while Ophioceras and Pseudohalonectria clustered in the Sordariales incertae sedis. This confirms the observations of Inderbitzin and Berbee (2001) that Lollipopaia and Ophioceras/Pseudohalonectria species have little in common and indicates that the latter genera are better placed in the Magnaporthaceae, Sordariales incertae sedis. Chen et al (1999) sequenced a greater number of Ophioceras and Pseudohalonectria species and showed they grouped with Gaeumannomyces, whereas Shearer et al (1999) drew attention to the morphological similarity of these genera with those of the Magnaporthaceae.

#### ACKNOWLEDGMENTS

This project is supported by research grant BRT R\_145008 and BRTR\_245002. We are grateful to Graduate School,

Chiang Mai University, Saisamorn Lumyong, Ruud Valyasevi and Morakot Tanticharoen for continued support, to Ittichai Chatmala and Aom Pinnoi for field assistance and to Manetr Boonyanant and his staff for research facilities at the Sirindhorn Field and Nature Study Center, Narathiwat.

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#### L PAPER

ava Pinruan • Saisamorn Lumyong H.C. McKenzie • Evan B. Gareth Jones D. Hyde

## ee new species of Craspedodidymum from palm in Thailand

ed: July 16, 2003 / Accepted: December 16, 2003

act Three new species of Craspedodidymum, C. use, C. licualae, and C. microsporum, are described lustrated based on specimens collected on decaying and sheaths of the palm, Licuala longecalycata, in horn Peat Swamp Forest, Narathiwat, Southern Thai-They are compared with similar species, and a key to nus is provided.

ords Anamorphic fungi · Palm fungi · Peat swamp · Tropical fungi

#### uction

horn Peat Swamp Forest, Thailand offers an unusual t for tropical palms with its acidic water and humid ions. A study is in progress to document the fungal ity of selected palms in this unique habitat (Pinruan 2002). In this article, we describe three new species aspedodidymum Hol.-Jech. from decaying parts of palm Licuala longecalycata Furt. The genus edodidymum was erected by Holubová-Jechová for a dematiaceous anamorphic fungus producing macronematous conidiophores and apically swollen ogenous cells, with a large and distinct funnel-shaped al collarette. To date, eight species have been ac-

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for Research in Fungal Diversity, Department of Ecology diversity, The University of Hong Kong, Hong Kong cepted in the genus. Craspedodidymum was reviewed by Yanna et al. (2000), and they provided a key and synoptic table to the genus.

#### Materials and methods

Decaying parts of Licuala longecalycata were collected from Sirindhorn Peat Swamp Forest, Narathiwat, Thailand, and returned to the laboratory in sterile plastic bags. Samples were incubated in plastic boxes with moistened tissue paper. The samples were examined over a period of 4 weeks, and the developing fungi were identified. Single spore isolations of all species were made on corn meal agar (CMA) with added antibiotic (penicillin G, 0.5 g/l; streptomycin sulfate, 0.5 g/l) to suppress bacterial growth. Microscopic measurements were taken from specimens mounted in water except for C. licualae sp. nov., which was mounted in lactophenol.

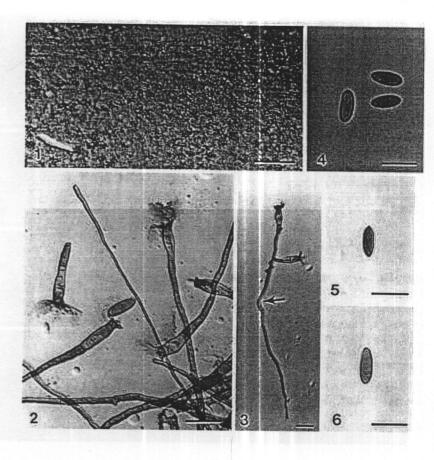
#### Species descriptions

Craspedodidymum siamense Pinruan, sp. nov. Figs. 1-6 Etymology: Siam, in reference to an earlier name for Thailand.

Coloniae in substrato naturali effusae, nigrae. Mycelium superficiale. Conidiophora macronematosa, mononematosa, erecta, brunnea, ad apicem pallida, recta vel leviter flexuosa, aliquando ramosa, laevia, 95–235 µm alta, ad basim 2.5–5 µm et ad apicem 6.2–8.7 µm lata. Cellulae conidiogenae integratae, terminales, 20–22.5 × 6.2–7.5 µm, enteroblasticae, monophialidicae, ampulliformes, collaretti distincto praeditae; collarettia infundibularia, ad apicem 12.5–15 µm diametro. Conidia 15–20 × 6.2–7.5 µm, ellipsoidea, mediocriter brunnea, aseptata.

Colonies on natural substrata effuse, black. Mycelium superficial. Conidiophores macronematous, mononematous, erect, brown, paler toward the apex, straight or slightly

Figs. 1-6. Light micrographs of Craspedodidymum samense (from holotype). 1 Colonies on substratum. 23 Conidiophores with with a large and distinct through terminal collarette. Note percurrent proliferation (arrow). 4-6 Conidia. Bars 1 100 µm; 2-6 20 µm



lexuous, sometimes branched, smooth, 95–235  $\mu$ m ( $\bar{x}=176\mu$ m, n=17), 2.5–5  $\mu$ m wide at the base ( $\bar{x}=4.1\,\mu$ m, n=10), 6.2–8.7  $\mu$ m wide at the apex ( $\bar{x}=7.5\,\mu$ m, n=20). Conidiogenous cells integrated, terminal, 20–22.5  $\times$  6.2–15  $\mu$ m ( $\bar{x}=22\times7.5\,\mu$ m, n=20), enteroblastic, monophialidic, with a large and distinct collarette; collarette funnel shaped, 12.5–15  $\mu$ m diameter at the opening ( $\bar{x}=13\,\mu$ m, n=20). Conidia 15–20  $\times$  6.2–7.5  $\mu$ m ( $\bar{x}=18\times7\,\mu$ m, n=25), ellipsoid, thick walled, mid brown, 0-sptate.

Holotype: On decaying sheath of Licuala longecalycata, hailand, Narathiwat, Sirindhorn Peat Swamp Forest. May 1001, U. Pinruan (Wah 31), in BIOTEC Bangkok Hertarium (BBH).

Distribution and habitat: Thailand, saprobic on decaying heaths of *Licuala longecalycata*.

Teleomorph: Unknown.

Note: Craspedodidymum siamense is unique in having dipsoid conidia that are rounded at the base. The conidia of C. siamense are most similar to those of C. proliferans V. have de Hoog and C. elatum Hol.-Jech. However, they look the papillate base of C. elatum, and are narrower and paler in color (Yanna et al. 2000; Ellis 1976). Craspedodidymum proliferans has shorter conidia, which are ovoid or trapezoid in shape (Rao and de Hoog 1989).

Inspedodidymum licualae Pinruan, sp. nov. Figs. 7-14 hymology: licualae in reference to the host, Licuala.

Coloniae in substrato naturali effusae, atrae. Mycelium perficiale. Conidiophora macronematosa, mononema-

tosa, erecta, brunnea, ad apicem pallida, recta vel flexuosa, laevia, exasperatus ad apicem, usque ad 95  $\mu m$  longa, ad basim 2.5  $\mu m$  et ad apicem 5  $\mu m$  lata. Cellulae conidiogenae integratae, terminales, 20–27.5  $\times$  6.2–7.5  $\mu m$ , enteroblasticae, monophialidicae, ampulliformes, collaretti distincto praeditae; collarettia infundibularia, ad apicem  $10\,\mu m$  diametro. Conidia  $13.7–17.5\times7.5–10\,\mu m$ , cylindrica, obovoidea vel ellipsoidea, brunnea, basi papillata, aseptata.

Colonies on natural substratum effuse, black. Mycelium superficial. Conidiophores macronematous, mononematous, erect, brown and paler toward the apex, straight or flexuous, smooth, but rough at the apex. Conidiogenous cells integrated, terminal,  $20-27.5 \times 6.2-7.5 \mu m$  ( $\bar{x}=24 \times 6.5 \mu m$ , n=20), enteroblastic, monophialidic, with a large and distinct collarette; collarette funnel shaped,  $10 \mu m$  diameter at the opening. Conidia  $13.7-17.5 \times 7.5-10 \mu m$  ( $\bar{x}=15 \times 9 \mu m$ , n=20), cylindrical, obovoid or ellipsoid, broadly rounded at both ends, brown, papillate at the basal end, 0-septate.

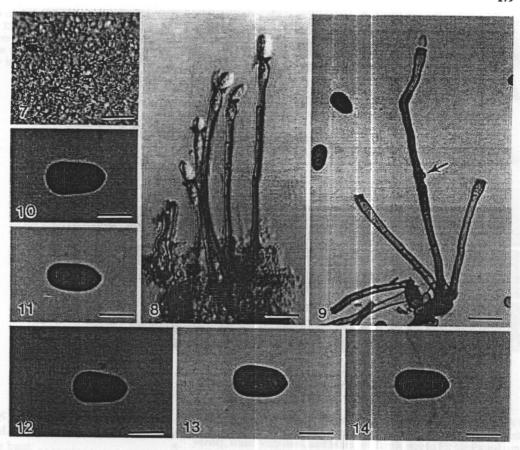
Holotype: On decaying trunk of Licuala longecalycata, Thailand, Narathiwat, Sirindhorn Peat Swamp Forest. Sept. 2001, U. Pinruan (Wah 136), in BBH.

Distribution and habitat: Thailand, saprobic on decaying trunks of *Licuala longecalycata*.

Teleomorph: Unknown.

Note: Craspedodidymum licualae can be distinguished from the other three species of Craspedodidymum, which have conidia with a papillate base, by the size and shape of the conidia. The conidia of the following species are much

7-14. Light micrographs of redodidymum licualae holotype). 7 Colonies on ratum. 8,9 Conidiophores are paler toward the apex. percurrent proliferation v). 10-14 Conidia. Bars 7 n; 8,9 20 µm; 10-14 10 µm



er  $(5-6.2 \times 3.5-4\mu m)$ , whereas the conidia of C. mense Lunghini & Onofri are spherical or obovoid, asse of C. elatum are broadly ellipsoid. In addition, C, is the only Craspedodidymum species to have hed conidiophores (Yanna et al. 2000).

edodidymum microsporum Pinruan, sp. nov.

Figs. 15-21

ology: In reference to the relatively small size of the a.

loniae in substrato naturali effusae, atrae. Mycelium ficiale. Conidiophora macronematosa, mononematerecta, pallide, brunnea, ad apicem hyalina, recta vel flexuosa, usque ad 85 µm longa, ad basim 2 µm et ad n 5 µm lata. Cellulae conidiogenae integratae, teres, 16.2–21.2 × 3.7–5 µm, enteroblasticae, monophiam ampulliformes, collaretti distincto praeditae; ettia infundibularia, ad apicem 7.5 µm diametro. ia 5–6.2 × 3.5–4 µm, obovoidea, sphaerica vel late bidea, basi papillata, aseptata.

donies on natural substratum effuse, black. Mycelium ficial. Conidiophores macronematous, mononematrect, pale brown, hyaline toward the apex, straight or y flexuous, smooth, up to  $85 \,\mu\text{m}$  long,  $2 \,\mu\text{m}$  wide at the  $5 \,\mu\text{m}$  wide at the apex. Conidiogenous cells integrated, and,  $16.2-21.2 \times 3.7-5 \,\mu\text{m}$  ( $\bar{x}=18 \times 4.5 \,\mu\text{m}$ , n=20), oblastic, monophialidic, with a large and distinct ette; collarette funnel-shaped,  $7.5 \,\mu\text{m}$  diameter at the

opening. Conidia 5-6.2  $\times$  3.5-4  $\mu$ m ( $\bar{x} = 5.8 \times 3.5 \mu$ m, n = 25), obovoid, spherical or broadly ellipsoid, pale brown, papillate at the basal end, 0-septate.

Holotype: On decaying trunk of *Licuala longecalycata*, Thailand, Narathiwat, Sirindhorn Peat Swamp Forest. Sept. 2001, U. Pinruan (Wah 142), in BBH.

Distribution and habitat: Thailand, saprobic on decaying trunks of Licuala longecalycata.

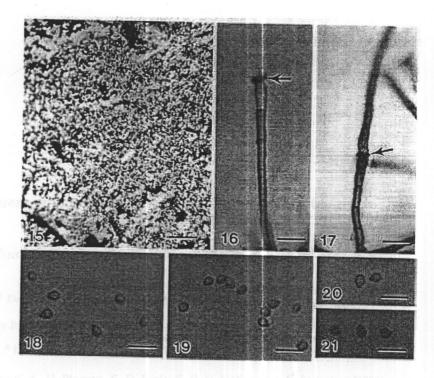
Teleomorh: Unknown.

Note: Four species of *Craspedodidymum* have conidia with a papillate base. The conidia of *C. microsporum* are distinct, being considerably smaller than those of the other three species (Yanna et al. 2000).

Currently, 11 Craspedodidymum species are recognized (8 and 3 described in this article), while C. pulneyensis Sub. & Bhat has been placed in synonymy with C. proliferans V. Rao & de Hoog (Bhat and Kendrick 1993).

Key to Craspedodidymum species (based on Yanna et al. 2000)

igs. 15-21. Light micrographs of Craspedodidymum icrosporum (from holotype). 15 Colonies on bstratum. 16,17 Conidiophores with a large and stinct funnel-shaped collarette (arrow in 16). Note ecurrent proliferation (arrow in 17). 18-21 Conidia. ars 15 100 µm; 16-21 10 µm



474
Conidia brown, 11–18 × (6–)7–9 µm
C. keniense (P.M. Kirk) Bhat & W.B. Kendrich
Conidia papillate at the base
Conidia truncate or rounded at the base
Conidia 5–6.2 $\times$ 3.5–4 $\mu$ m, pale brown
Conidia larger than 6.2 × 4 µm
Conidia broadly allipsoid 15 10 × 0 12
Conidia broadly ellipsoid, 15–19 × 9–12 µm; conidio
phores branched
Conidia cylindrical, spherical, or obovoid; conidio
phores unbranched
Conidia cylindrical or obovoid, $12.5-17.5 \times 7.5-10 \mu m$
conidiophores unbranched
Conidia spherical or obovoid, 13.5-14.5 × 14.5-16.5 µm
Conidia 18-24 µm diameter, globose, surrounded by a
sheath of fibrillar appendages
Conidia of other shapes, lacking a sheath of fibrillar
appendages9
Conidia ellipsoid, 15-20 × 6.2-7.5 µm
····· C. siamense sp. nov
Conidia of other shapes, shorter10
Conidia obovoid to pyriform, $11.5-15 \times 10.5-13 \mu m$
Conidia obovoid or trapezoid, 10-14 × 8-11 µm
The system with the de de 1100g

Acknowledgments This project is supported by research grant BRT R-145008. We are grateful to the Graduate School, Chiang Mai University, Ruud Valyasevi, and Morakot Tanticharoen for continued support, to Prasert Srikitikulchai for field assistance, and to Manatr Boonyanant and his staff for research facilities at the Sirindhorn Field and Nature Study Center, Narathiwat.

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Mycological Institute, Kew, UK
Holubová-Jechová V (1972) Craspedodidymum, new genus of phialosporus hyphomycetes. Česká Mykol 26:70–73
Pinruan U, Jones EBG, Hyde KD (2002) Aquatic fungi from peat

swamp palms: Jahnula appendiculata sp. nov. Sydowia 54(2):242-247 Rao V, de Hoog GS (1986) New or critical hyphomycetes from India. Stud Mycol 28:1-84

Yanna, Ho WH, Goh TK, Hyde KD (2000) Craspedodidymum nigroseptatum sp. nov., a new hyphomycete on palms from Brunei Darussalam. Mycol Res 104:1146-1151

## Submersisphaeria palmae sp. nov. with a key to species, and notes on Helicoubisia

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Pinnoi, A., U. Pinruan, K. D. Hyde, E. H. C. McKenzie & S. Lumyong (2004). Submersisphaeria palmae sp. nov. with a key to species, and notes on Helicoubisia. – Sydowia 56 (1): 72–78.

Submersisphaeria palmae sp. nov. is described and illustrated from petioles, rachides and trunks of palms at Sirindhorn Peat Swamp Forest, Narathiwat, in southern Thailand. This species has much smaller ascospores than most previously described species. A key to the five accepted species is given and S. palmae is compared with the most similar taxa. Helicoubisia coronata was collected from the palm, Eleiodoxa conferta, also in the Peat Swamp Forest. Helicoubisia is characterised by erect conidiophores bearing discrete, polyblastic conidiogenous cells at the apex and coiled, pale brown conidia. Helicoubisia and Moorella are discussed and M. monocephala is relegated to synonymy of H. coronata.

Keywords: Fungal diversity, Moorella, palm fungi, synonymy.

We are studying the fungi on submerged and terrestrial decaying parts of palms that grow in the acidic waters of Sirindhorn Peat Swamp Forest, in southern Thailand (Hyde & al., 2002; McKenzie & al., 2002). In this paper we describe a new species of Submersisphaeria collected on the submerged petioles, rachides and trunks of the palms Eleiodoxa conferta Griff., Nenga pumila H. Wendl. and Licuala longecalycata Furt. Helicoubisia coronata Lunghini & Rambelli was collected on a terrestrial sample of Eleiodoxa conferta and recorded for the first time in Thailand. The taxonomy and nomenclature of this species is clarified.

#### Materials and methods

Collected palm material was returned to the laboratory and incubated in plastic boxes on damp tissue paper. Samples were observed by stereomicroscopy and fungi were mounted in water for measurement and photography. Attempts were made to isolate both fungi from single spores and then grow them on potato dextrose agar

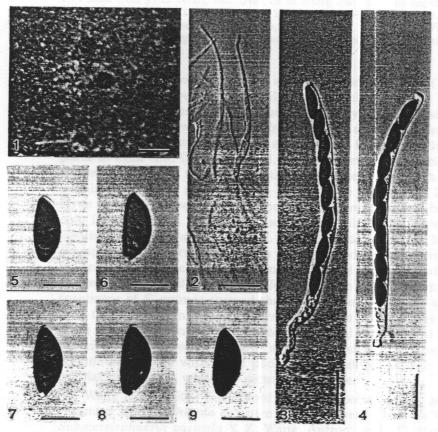
(PDA) (Choi & al., 1999). Type material is deposited in the BIOTEC Bangkok Herbarium (BBH) and axenic cultures are stored in the BIOTEC Culture Collection (BCC).

#### **Taxonomy**

Submersisphaeria palmae A. Pinnoi sp. nov. - Figs. 1-9.

Ascomata in substrato immersa, 200–300 µm diam, globosa vel subglobosa, solitaria. Asci  $100-127.5\times6.25-8.75$  µm, octospori, cylindrici, leptodermi, longe pedicellati, apparato apicali praediti. Ascosporae  $17.5-22.5\times5-7.5$  µm, uniseriatae, unicellulares, brunneae, ellipsoideae, appendiculatae.

Etymology. - palmae - in reference to the host family.



Figs. 1–9. Submersisphaeria palmae (from holotype). – 1. Ascomata on natural substratum. – 2. Paraphyses. – 3, 4. Cylindrical asci with refractive apical rings. – 5–9. Ascospores with paler area at end of spores. – Bars:  $1 = 400 \mu m$ ;  $2 = 25 \mu m$ ;  $3-9 = 20 \mu m$ .

As comata 200–300 µm diam, globose or subglobose, dark brown, coriaceous, solitary, immersed in substrata, visible as blackened dots on the host surface. Ostiole central. – Paraphyses 2.5–3.75 µm diam, hypha-like, filamentous, septate, tapering, numerous. – Asci 100–127.5 × 6.25–8.75 µm ( $\bar{x}$  = 111 × 7.15 µm, n = 25), 8-spored, unitunicate, cylindrical, thin-walled, with a long pedicel, and a relatively large, refractive, non-amyloid apical ring, 4–5 µm diam, 1–2 µm high ( $\bar{x}$  = 4.1 × 1.7 µm, n = 5). – Ascospores 17.5–22.5 µm × 5–7.5 µm ( $\bar{x}$  = 18.2 × 5.9 µm, n = 25), uniseriate, unicellular, ellipsoidal, smooth, olivaceous-brown, with small mucilage pads at each end.

Holotype. - THAILAND: Narathiwat, Sirindhorn Peat Swamp Forest, on submerged rachis of *Eleiodoxa conferta*, 13 Feb. 2002, A. Pinnoi (Aom 152 in BBH).

Other material examined. - THAILAND: Narathiwat, Sirindhorn Peat Swamp Forest, on submerged petiole of *Eleiodoxa conferta*, 12 May 2001, A. Pinnoi (Aom 42 in BBH); *ibid.*, on submerged rachis of *Nenga pumila*, 12 Feb. 2002, A. Pinnoi (Nen 27 in BBH); *ibid.* (Nen 28 in BBH); *ibid.*, on submerged trunk of *Licuala longecalycata*, 22 Jun. 2001, U. Pinraun (Wah 71 in BBH); *ibid.*, on submerged petiole of *Licuala longecalycata*, 22 Jun. 2001, U. Pinraun (Wah 101 in BBH); *ibid.*, on submerged trunk of *Licuala longecalycata*, 26 Sep. 2001, U. Pinraun (Wah 125 in BBH).

The genus Submersisphaeria was introduced by Hyde (1996) to accommodate S. aquatica K. D. Hyde and is characterised by subglobose, immersed ascomata and unitunicate, cylindrical, pedicellate asci, with a relatively large, refractive, non-amyloid apical ring. Ascospores are brown, one-celled and reported to have hyaline germ pores at each end. Campbell & al. (2003) reported S. aquatica from submerged wood in the USA and illustrated ascospores with bipolar mucilaginous pads and no germ pores. Hyde (1996) and Zhou & Hyde (2000) described the ascospores of S. aquatica and S. bambusicola D. Q. Zhou & K. D. Hyde as having polar germ pores. Submersisphaeria rattanicola J. Fröhl. & K. D. Hyde which has fusiform ascospores with narrow ends was also reported to have germ pores by Fröhlich & Hyde (2000). Re-examination of the type material of S. bambusicola and S. rattanicola, and close examination of the specimens of S. palmae showed that the ends of the spores are hyaline, thus having only the appearance of germ pores. These lighter areas at the spore ends are, probably, an optical artifact. The generic description of Submersisphaeria should, therefore, be amended to include ascospores with polar pad-like appendages as well as unicellular ascospores, while the germ pore is absent.

Wang & al. (2004) introduced a fourth species to the genus in renaming Amphisphaeria aquatica (Ellis & Everh.) Berl. & Vogl. as Submersisphaeria vasicola Y. Z. Wang, Aptroot & K. D. Hyde. The ascospores of S. palmae are similar in size to those of S. vasicola

(Wang & al., 2004). However, ascospores of *S. palmae* are unicellular, fusiform-ellipsoidal, olivaceous and flattened on one side, while those of *S. vasicola* are bicellular, ellipsoidal, brown and symmetrical. The only other species with unicellular ascospores is *S. bambusicola*, but this fungus has much larger ascospores.

#### Key to Submersisphaeria species

1. Ascospores unicellular
1*. Ascospores bicellular
2. Ascospores $28-36 \times 6-8 \ \mu m \dots S. \ bambusicola$
2*. Ascospores $17.5-22.5 \times 5-7.5 \mu m \dots S. palmae$
3. Ascospores $23-27 \times 7.5-10 \mu m$ , ellipsoidal with rounded ends
S. aquatica
3*. Ascospores less than 23 μm long
4. Ascospores $14.3-20.8 \times 5-6.8 \mu m$ , fusiform with narrowing ends
S. rattanicola
4*. Ascospores $16-22 \times 6-7$ µm, ellipsoidal with rounded ends

*Helicoubisia coronata* Lunghini & Rambelli, Mic. Ital. 1: 21 (1979). – Figs 10–19.

= Moorella monocephala Matsush., Matsushima Mycological Memoirs 7: 58. 1993.

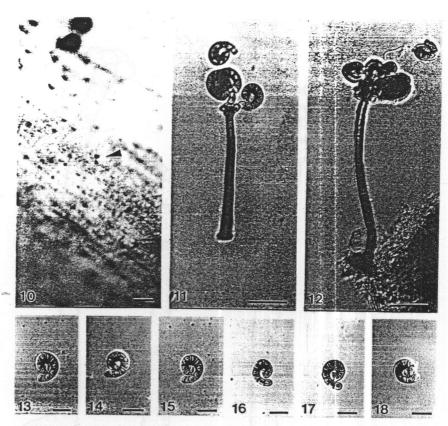
Conidiophores on dead pinnae, brown, unbranched, straight, erect, smooth-walled, 4–7-septate, macronematous, mononematous, 73–98 × 5–7  $\mu m.$  – Conidiogenous cells polyblastic, discrete, pale brown, 3–4  $\mu m$  long, 4–6  $\mu m$  wide, terminal. – Conidia helicoid, 1.5 times coiled, pale brown, smooth-walled, 10–12.5  $\mu m$  diam., filament 8–10-septate, 3.5–4  $\mu m$  diam. (x̄ = 3.9  $\mu m,$  n = 10), rounded at apical cell, truncate at base. – Colonies on PDA effuse, slow growing, reaching 1 cm diam. after 60 days at ~25°C, with superficial black mycelia, sterile.

Habitat. – Saprobic on dead pinnae of *Eleiodoxa conferta*, on decaying palm petioles (Matsushima, 1993) and unidentified dead leaves (Lunghini & Rambelli, 1979).

Known distribution. – Ecuador, Ivory Coast, Peru. Thailand.

Specimen examined. - THAILAND: Narathiwat, Sirindhorn Peat Swamp Forest, terrestrial pinnae of *Eleiodoxa conferta*, 12 May 2001. A. Pinnoi. Aom35 (living culture in BCC 9880).

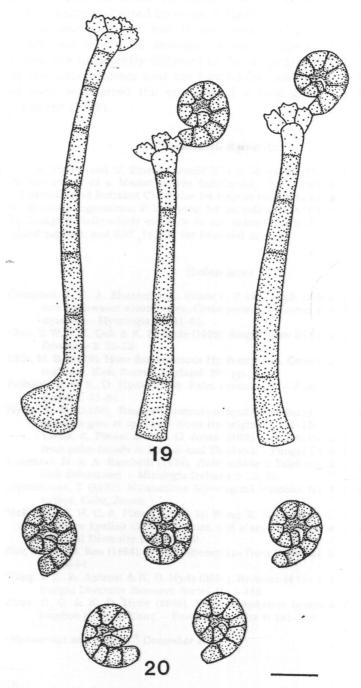
Moorella was introduced by Rao & Rao (1964) to accommodate M. speciosa P. R. Rao & D. Rao, an anamorphic fungus with macronematous, mononematous, septate, dark brown conidiophores with



Figs. 10–18. Helicoubisia coronata. – 10. Colonies on natural substratum. – 11, 12. Conidiophores, conidiogenous cells, and conidia. – 13–18. Conidia. – Bars: 10 = 200  $\mu$ m; 11–12 = 20  $\mu$ m; 13–18 = 10  $\mu$ m.

numerous short, septate, brown branches formed in verticils at intervals along the stipe. Conidiogenous cells were described as polyblastic, denticulate and terminal on the stipe and branches. Conidia were thin-walled, hyaline or subhyaline, 4–7-septate and 1–1.5 times coiled (Rao & Rao, 1964; Ellis, 1976). Moorella remained monotypic until Matsushima (1993) described M. monocephala Matsushima. Moorella monocephala was described with brown, unbranched, septate conidiophores, with an inflated apex bearing 2–6 discrete conidiogenous cells. Each conidiogenous cell was described as cuniform, pale brown and bearing 1–3 denticles. The conidia were described as solitary, helicoid, 1.5 times coiled. dry. smooth, 9–12  $\mu m$  in diam., filaments 4–6  $\mu m$  wide, 6–7-septate, and moderate to pale brown (Matsushima, 1993).

The monotypic genus *Helicoubisia* was introduced by Lunghini & Rambelli (1979) to accommodate *H. coronata*. *Helicoubisia* cor-



Figs. 19–20.  $Helicoubisia\ coronata\ (line\ drawing).$  – 19. Conidiophores and conidiogenous cells. 20. Conidia. – Bars: 19–20 = 10  $\mu m$ .

onata is identical to Moorella monocephala as described and illustrated by Matsushima (1993) and therefore, the first name has priority over the latter. The features of *H. coronata* are very similar to the fungus collected by us on *Eleiodoxa conferta*, and we consider the current specimen and *H. coronata* identical. The discrete conidiogenous cells of *H. coronata*, borne on the apex of the conidiophore, are sufficiently different to the integrated conidiogenous cells of Moorella speciosa that are formed on short verticillate branches, to have warranted the erection of a new genus by Lunghini & Rambelli (1979).

#### Acknowledgments

A. Pinnoi and U. Pinraun would like to thank BIOTEC, Bangkok, Thailand for the award of a Master degree Scholarship; P. Srikitikulchai, J. Sakayaroj, I. Chatmala and Rattaket Choeyklin for help in collecting samples; S. Somrithipol for drawing suggestions; P. Lumyong for providing laboratory facilities; W. Gams for bringing *Helicoubisia coronata* to our attention and E. B. G. Jones for continued support; and BRT\_145008 for financial support.

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(Manuscript accepted 28th December 2003)

# Aquatic fungi from peat swamp palms: Jahnula appendiculata sp. nov.

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Pinruan, U., E. B. G. Jones & K. D. Hyde, (2002). Aquatic fungi from peat swamp palms: *Jahnula appendiculata* sp. nov. – Sydowia 54(2): 242–247.

A new species of *Jahnula* is described based on a specimen from a submerged palm trunk in a peat swamp forest in Thailand. *Jahnula appendiculata* sp. nov. is unique in the genus in having ascospores that are surrounded by a sheath and in having bipolar cellular appendages. The taxon is illustrated with light micrographs and a brief discussion of appendages in freshwater ascomycetes is provided.

Keywords: freshwater ascomycete, palm, peat swamp, taxonomy.

We are investigating the fungi occurring on palms in Sirindhorn Peat Swamp Forest, Narathiwat, in southern Thailand. Fronds from the palms may fall into the acidic waters of the peat swamp (pH 5.8–6.2), while others may remain attached to the trees or become lodged in plants and litter above the water. This latter can be regarded as terrestrial. The fungi that colonise the palm fronds in the terrestrial milieu are typical palm fungi (e.g. Fröhlich & Hyde, 1999; Yanna & al., 2001), while those colonizing submerged fronds can be regarded as aquatic species (e.g. Goh & Hyde, 1999). The latter habitat (submerged palm material) has not been studied previously and is an interesting source of novel fungi. In this paper we report on a new species of Jahnula Kirschst. with fascinating appendages.

Jahnula has been reviewed by Hyde & Wong (1999) and includes eight species. It is an aquatic genus and is unusual in having large cells in the peridium and ascomata that are attached to the substratum by often quite long stalks, e.g. up to 300  $\mu$ m length, 40–55  $\mu$ m width in Jahnula siamensiae S. Sivichai & E. B. G. Jones (Pang & al., 2002). Based on these characters and supportive molecular data, the order Jahnulaes has been erected for Jahnula and related genera (Pang & al., 2002). Hyde & Wong (1999) originally thought that the thick stalks might be algal associations, but this is unlikely. Ascos-

pores are brown and often surrounded by a sheath or have mucilaginous pad-like appendages. We collected a unique species of Jahnula in which the ascospores are surrounded by a sheath and also have bipolar cellular appendages. It is therefore described as a new species.

#### Material and methods

Submerged palm material was collected from Sirindhorn Peat Swamp Forest, Narathiwat, southern Thailand during May 2001. The material was returned to the laboratory, incubated in plastic boxes on damp tissue paper and examined within 4 weeks. Type material has been deposited in the BIOTEC Herbarium (BBH) and a culture in the BIOTEC Culture Collection (BCC). Single spore isolations were made on corn meal agar with added antibiotics to suppress bacterial growth. All observations, including photographic documentation, were of material mounted in water, using differential interference microscope. The range between minimum and maximum values for microscopic measurements is given, mean values are in brackets, n being the number of items measured.

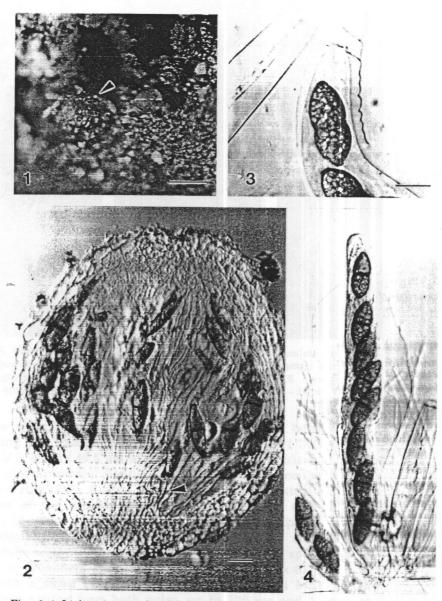
#### **Taxonomy**

Jahnula appendiculata Pinruan, K. D. Hyde & E. B. G. Jones, sp. nov. – Figs. 1–14.

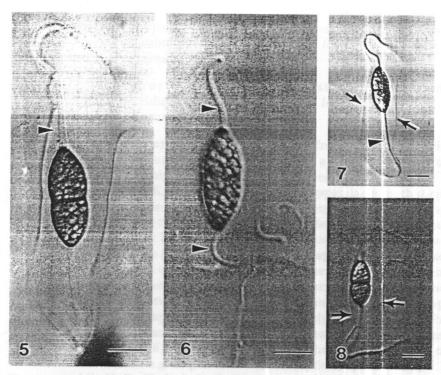
Ascomata 305–325 µm diametro, semi-immersa, erumpentia, globosa vel subglobosa, hyalina vel pallide straminea, membranacea, ostiolata, breviter papillata, solitaria, setis hyalinis usque ad 80 µm longis praedita, stipite hyalino. Filamenta interascalia pseudoparaphyses. Asci 360–410  $\times$  41–43 µm, octospori, cylindrici vel cylindrico-clavati, bitunicati, fissitunicati, camera oculari et annulo tenui instructi. Ascosporae 47.5–55  $\times$  23.5–26.5 µm, 1–2–seriatae, ellipsoideo-fusiformes, brunneae, guttulatae, 1–septatae, ad septum constrictae, paries ornamento punctiformi, tunica gelatinosa circumdatae et duobus appendicibus cellularibus terminalibus longis hyalinis praeditae.

Etymology of species epithet. – In reference to the appendaged ascospores.

Ascomata 305–325  $\mu$ m diam, semi-immersed, becoming erumpent, but with the base remaining immersed, globose to subglobose, hyaline to pale straw-coloured, membraneous, ostiolate. short papillate, easily detaching from wood, solitary, covered with short hyaline setae up to 80  $\mu$ m long, with a hyaline stalk-like strand attached to the base, 37.5  $\mu$ m length, 35  $\mu$ m width. (Figs. 1, 2). – Peridium ca. 36  $\mu$ m wide, comprising 4–6 rows of large angular cells with hyaline walls (Fig. 2). – Pseudoparaphyses up to



Figs. 1-4. Light micrographs of Jahnula appendiculata (from holotype, mounted in water). - 1. Erumpent ascomata (arrowhead; mature ascoma). - 2. Section of ascoma. Note the peridium and the cushion-like structure from which the asci arise (arrowhead). - 3. Apical region of a fissitunicate ascus. - 4. Asci and pseudoparaphyses. - Bars: 1 = 160 µm, 2-4 = 20 µm.



Figs. 5–8. Light micrographs of ascospores of Jahnula appendiculata (from holotype, mounted in water). – 5, 7, 8. Ascospores with sheath (small arrows) and appendages (arrowhead), the latter still within the sheath. – 6. Ascospore with polar appendages (arrowheads), the sheath having dissolved. – Bars =  $20~\mu m$ .

1.2 µm wide, hypha-like, septate, unbranching between asci, branching and anastomosing above (Fig. 4). – Asci 360–410 × 41–43 µm ( $\bar{x}=368.7\times42.5$  µm, n = 20), 8-spored, cylindrical to cylindric-clavate, bitunicate, fissitunicate, with a shallow ocular chamber and faint ring (Figs. 3, 4). – Asci forming from a central cushion at the base of the ascoma (Fig. 2). – Ascospores 47.5–55 × 23.5–26.5 µm ( $\bar{x}=51.3\times24.8$  µm, n = 25), 1–2-seriate, ellipsoid-fusiform, ends pointed, brown, guttulate, 1-septate, slightly constricted at the septum, wall ornamentation minutely verrucose, spore surrounded by a prominent mucilaginous sheath, ca 160 × 40 µm, which ends with a small subapical hood-like rim, and a long, cellular appendage arising from both poles, up to 120 µm and 4 µm diam. (Figs. 5–8).

Colonies on CMA effuse, reaching 2.5 cm in diam. in 11 days at room temperature (22–24°C), mycelium 12.5–17.5  $\mu m$  wide, brown, a small amount of aerial mycelium present, hyphae smooth-walled and loose, no anamorph observed.

Holotypus. - Thailand, Narathiwat, Sirindhorn Peat Swamp Forest, on submerged trunk of *Licuala longecalycata* Furt., 12 May 2001, U. Pinruan (Pinruan 96 in BBH).

Isotypus. – Thailand, Narathiwat, Sirindhorn Peat Swamp Forest, on submerged trunk of *Licuala longecalycata* Furt., 12 May 2001, U. Pinruan (Pinruan 96 in PDD).

Jahnula appendiculata is distinct from all other species in the genus by the ascospores that have both a sheath and bipolar appendages. The only other species with appendages is Jahnula bipolaris (K. D. Hyde) K. D. Hyde, however, the appendages in that species are cap-like (Hyde & Wong, 1999). When J. appendiculata ascospores are released from the asci they are surrounded by a mucilaginous sheath with an outer layer (membrane). Long cellular appendages are visible within the sheath at this stage. The appendages are attached to a hood-like rim at each end of the ascospores and are curled inside the ends of the sheath (Figs. 5, 7-8). In water, and with time, the ends of the sheath break or dissolve and release the curled appendages which then expand and become less defined (Figs. 5-8). The sheath eventually dissolves, or spreads, and only the appendages remain visible (Figs. 6). Other Jahnula species have spores with a mucilaginous sheath e.g. J. granulosa K. D. Hyde & S. W. Wong, J. potamophila K. D. Hyde & S. W. Wong, J. seychellensis K. D. Hyde & S. W. Wong, and J. systyla K. D. Hyde & S. W. Wong, the latter species being similar to the one in J. appendiculata.

The ascospores of J. appendiculata are unique, with appendages at both poles and surrounded by a sheath, and differ from those of all other Jahnula species. In many other freshwater ascomycetes, ascospores are surrounded by various types of sheaths, e.g. Annulatascus velatisporus K. D. Hyde (Hyde, 1992). In Pseudoproboscispora aquatica S. W. Wong & K. D. Hyde appendages are cellular and appear to be similar once they are released from the ascus, but they are curled up in a proboscis-like manner within the ascus (Wong & Hyde, 1999). Several species (e.g. Aniptodera Shearer & M. A. Mill., Diluviocola K. D. Hyde, S. W. Wong & E. B. G. Jones, Halosarpheia Kohlm. & E. Kohlm., Phaeonectriella R. A. Eaton & E. B. G. Jones) have thread-like, bipolar, unfuring, filamentous appendages (Hyde & al., 1998, 1999). In Fluminicola bipolaris S. W. Wong, K. D. Hyde & E. B. G. Jones the appendages are bipolar, initially flattened and become cup-like when released in water (Wong & al., 1999). In all of the above species asci are unitunicate. It is rare to observe ascospore appendages in bitunicate ascomycetes, even amongst marine fungi (Hyde & al., 2000). Appendages, however, only occur in a small number of species e.g. Massarina bipolaris K. D. Hyde (Hyde, 1995).

#### Acknowledgments

This project is supported by research grant BRT R-1444012. We are grateful to Pipob and Saisamorn Lumyong, Ruud Valyasevi, Morakot Tanticharoen for continued support, to Prasert Srikitikulchai for field assistance and to Manot Boonyanant, and his staff, for research facilities at the Sirindhorn Field and Nature Study Center, Narathiwat.

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(Manuscript accepted 28th June 2002)

# Goidanichiella fusiforma sp. nov. from palm fronds in Brunei and Thailand

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Hyde, K.D., Yanna, Pinnoi, A. and Jones, E.B.G. (2002). Goidanichiella fusiforma sp. nov. from palm fronds in Brunei and Thailand. Fungal Diversity 11: 119-122.

Goidanichiella fusiforma sp. nov. was identified from collections of decaying palm fronds in tropical rainforests in Brunei and Thailand. The new taxon is described and illustrated, and compared with similar taxa.

Key words: anamorphic fungi, palm fungi, systematics, taxonomy.

#### Introduction

We are studying the fungi occurring on tropical palm species and have described several species new to science (Yanna et al., 1998a,b, 1999; Goh et al., 1999). Collections of fungi on fronds of palms in tropical rainforests yielded a new species of Goidanichiella and this taxon is described and illustrated in this paper.

#### Taxonomy

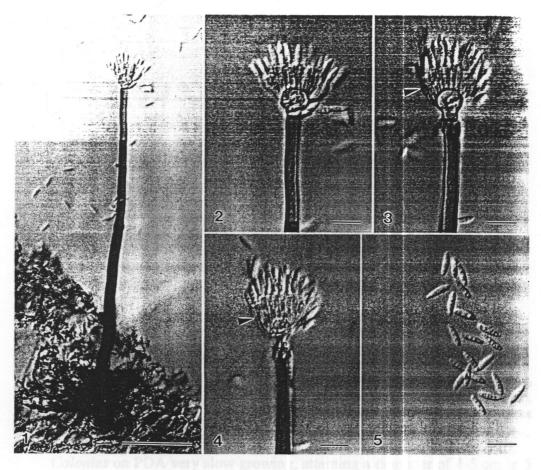
Goidanichiella fusiforma K.D. Hyde, Yanna, Pinnoi & E.B.G. Jones, sp. nov. (Figs. 1-7)

Etymology: referring to the fusiform conidia.

Mycelium immersa et superficialia. Conidiophora macronemata, mononemata, solitaria, erecta, recta vel paulo flexuosa, simplicia, laevia, brunnea, 240-300 × 6-9  $\mu$ m, apicem subhyalina, apicem inflata 8-12  $\mu$ m. Cellulae conidiogenae monoblasticae, determinatae, discretae, cylindricae, hyalinae vel pallid brunnae, 11-23 × 2-3  $\mu$ m. Conidia acrogena, aggregata, hyalina, fusiformes, aseptata, laevia, 9-11 × 2.5-3  $\mu$ m.

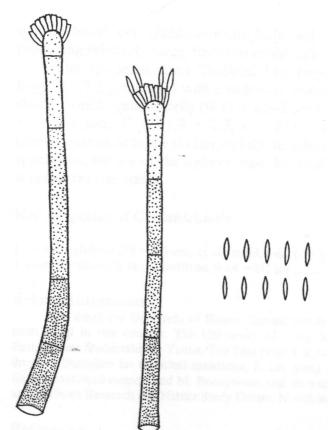
Colonies scattered, sparse, brown. Mycelium immersed or superficial, composed of brown, septate, smooth, thin-walled, branched hyphae. Stoma absent. Setae and hyphopodia absent. Conidiophores macronematous,

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Figs. 1-5. Goldanichiella fusiforma (from holotype). 1. A conidiophore bearing conidia on natural substratum. 2-4. Close-up of apical region of conidiophores. Note penicillate branches which bear conidiogenous cells (arrowheads) with developing conidia. 5. Hyaline, fusiform conidia. Bars:  $1 = 50 \mu m$ ;  $2-5 = 10 \mu m$ .

mononematous, solitary, erect, branched at the apex forming stipe and head; stipe straight or flexuous, swollen at the apex, smooth, cylindrical, brown to dark brown, paler towards the apex,  $240\text{-}300 \times 6\text{-}9 \text{ }\mu\text{m}$  ( $\bar{x} = 255 \times 8 \text{ }\mu\text{m}$ , n = 25); apex forming a swollen head,  $8\text{-}12 \text{ }\mu\text{m}$  ( $\bar{x} = 10 \text{ }\mu\text{m}$ , n = 25), bears primary branches which themselves bear secondary branches arranged penicillately (Figs. 1, 6). *Conidiogenous cells* monoblastic, determinate, terminal, discrete, cylindrical, hyaline to pale brown, borne at the ends of secondary branches,  $11\text{-}23 \times 2\text{-}3 \text{ }\mu\text{m}$  ( $\bar{x} = 12.5 \times 2.5 \text{ }\mu\text{m}$ , n = 25) (Figs. 2-4, 6). *Conidia* enteroblastic, acrogenous, solitary, aggregated in slimy heads, hyaline, fusiform, aseptate, smooth,  $9\text{-}11 \times 2.5\text{-}3 \text{ }\mu\text{m}$  ( $\bar{x} = 9.5 \times 2.8 \text{ }\mu\text{m}$ , n = 25) (Figs. 2-5, 7). *Conidial secession* schizolytic.



Figs. 6, 7. Goldanichiella fusiforma (from Herb. BIOTEC, AOM 0008). 6. Conidiophore and conidiogenous cells. 7. Hyaline, fusiform conidia.

Colonies on PDA very slow growing, attaining a diameter of 4-5 cm in 5 months at 25 C, pale brown, texture silky, flat, colouring agar pale brown; reverse colour unchanged.

Material examined: BRUNEI, Temburong, Batu Apoi Forest Reserve, The University of Brunei Darussalam Kuala Belalong Field Studies Centre (KBFSC), Baki Tributary, on decaying rachis of Oncosperma horridum, Feb. 1999, YAN 60 Ar [HKU(M) 13225, holotype designated here] - living culture in HKUCC 4666, 4667; on decaying rachis of Salacca affinis, Feb. 1999, YAN 60 Ar [HKU(M) 13256]; THAILAND, Narathiwat, Sirindhorn Peat Swamp Forest, on submerged dead petiole of Eleiodoxa conferta, 25 Sep. 2001, A. Pinnoi (Herb. BIOTEC, AOM 0008).

Habit: Saprobic on fronds of Eleiodoxa conferta (petiole), Oncosperma horridum (rachis) and Salacca affinis (rachis).

Known distribution: South East Asia (Brunei and Thailand).

Notes: Goidanichiella was reviewed by Gams et al. (1990) and a single species G. barronii W. Gams, Steiman & Seigle-Murandi was accepted. Goidanichiella sphaerospora Matsush. had been invalidly published and the

type material lost. Goidanichiella fusiforma is distinct from G. barronii in producing relatively large, fusiform conidia (9-11  $\times$  2.5-3  $\mu$ m).

The specimen from Thailand had larger conidiophores (205-520  $\mu$ m long, 10-17.5  $\mu$ m diam.) with a narrower swollen head (5-7.5  $\mu$ m) and slightly shorter conidiogenous cells (9-12 × 2.5-3  $\mu$ m) and slightly longer conidia (11-14 × 2-3  $\mu$ m,  $\bar{x}=11.9\times2.7$ , n=25). As the width of the conidia and conidiogenous cells are similar and the lengths overlap with those of the Brunei specimens, we are of the opinion that the Thailand specimen does not require separate species status.

## Key to species of Goidanichiella

### Acknowledgements

We thank the Universiti of Brunei Darussalam for organising the permission to study palm fungi in this country. The University of Hong Kong is thanked for the award of a Postgraduate Studentship to Yanna. The Thai project is supported by BRT grant R-144012. We thank B. Bussaban for technical assistance, P. Lumyong for laboratory facilities, S. Lumyong for her continued support and M. Boonyanant and his staff for their assistance with field work at Sirindhorn Research and Nature Study Centre, Narathiwat.

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(Received 18 February 2002; accepted 6 July 2002)

Two new hyaline *Chalara* species, and a key to species described since 1975

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McKenzie, E.H.C., Pinnoi, A., Wong, M.K.M., Hyde, K.D. and Jones, E.B.G. (2002). Two new hyaline *Chalara* species, and a key to species described since 1975. Fungal Diversity 11: 129-139.

Chalara siamense sp. nov. is described from dead petioles of Eleiodoxa conferta (Arecaceae) collected in Thailand, while a second hyaline species, C. schoenoplecti sp. nov., is described from senescent culms of Schoenoplectus litoralis (Cyperaceae) collected in Hong Kong. They are compared with similar species. Three species informally described by T. Matsushima are given Latin binomials and type specimens indicated, and a key to species described since 1975 is provided.

Key words: anamorphic fungi, hyphomycetes, Matsushima, new species.

#### Introduction

In this paper we report on two hyaline species of *Chalara*, which cannot be assigned to any previously described species. A study of fungi on palms in Sirindhorn Peat Swamp Forest, Narathiwat, Thailand, an environment suspected to support a high diversity of fungi, has yielded a new species of *Chalara*, while a similar new species has been found during a study of fungi on sedges in Hong Kong. Previously many fungal taxa have been described from tropical and subtropical palms (Hyde *et al.*, 1998; Fröhlich and Hyde, 1999; Yanna *et al.*, 2001).

Chalara species are characterized by having sessile or stalked phialides, conidiogenous cells with a basal venter and a long collarette, a deep-seated conidiogenous locus, and mainly hyaline conidia which are usually cylindrical, 1-2-celled, and extruded in long chains. The conidiophore and/or conidiogenous cell is usually pigmented. Nag Raj and Kendrick (1975)

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monographed the genus *Chalara* and provided a key to the accepted species. Since 1975 many new species of *Chalara* have been formally described, and several *Chalara*-like fungi have been described as the anamorphs of various ascomycetes. Matsushima (1971) recorded a species of *Chalara* on a rotten leaf of *Castanopsis* from Papua New Guinea as *Chalara montellica*. Later, he reported on two additional specimens of this fungus from Japan, on *Castanea crenata* and *Thujopsis dolabrata* (Matsushima, 1975), and referred to all three specimens as *Chalara* sp. Matsushima (1975) also described two other species, which he did not name. We formally name and describe Matsushima's three species, and provide a key to the new species described since 1975.

### **Taxonomy**

Chalara siamense Pinnoi, sp. nov.

(Figs. 1-15)

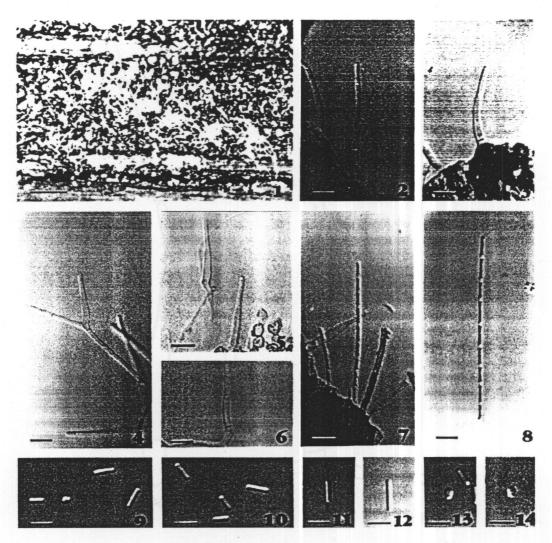
Etymology: in reference to the country, Siam (Thailand), in which this taxon was collected.

Coloniae albae, effusae. Conidiophora macronematosa, mononematosa, singula, sparsa, hyalina, 1-septata, interdum ramosa, laevia, 41-57  $\times$  5-6  $\mu$ m. Cellulae conidiogenae monophialidicae, in conidiophoris incorporatae, hyalina, laevia, 35-42  $\mu$ m longa, venter subcylindricus, 10-20  $\times$  4.5-6  $\mu$ m; collum cylindricum, 20-25  $\times$  2.5-3.5  $\mu$ m; transitio e ventre ad collum gradata. Conidia endogena, catenate extrusa, hyalina, cylindrica, laevia, eseptata, ambo extrema rotundata, 7-12  $\times$  2.5-3  $\mu$ m.

Colonies on natural substrate white, effuse, with long chains of conidia (Fig. 1). Conidiophores macronematous, mononematous, solitary, scattered, hyaline, ascending, arising laterally from aerial hyphae, 1-septate, sometimes branched, smooth, 41-57  $\times$  5-6  $\mu$ m (Figs. 2-6). Conidiogenous cells monophialidic, hyaline, smooth, 35-42 long, venter subcylindrical, 10-20  $\times$  4.5-6  $\mu$ m, collarette cylindrical, 20-25  $\times$  2.5-3.5  $\mu$ m, transition from venter to collarette gradual, subtending cell 6-15  $\times$  5-5.5  $\mu$ m (Figs. 2-6). Conidia endogenous, catenate, hyaline, cylindrical, smooth, aseptate, rounded at each end, 7-12  $\times$  2.5-3  $\mu$ m ( $\overline{x}$  = 9.3  $\times$  2.8  $\mu$ m, n = 25) (Figs. 7-12), first formed conidia ellipsoid, truncate at base, rounded at apex, 5-6  $\times$  2.5-3  $\mu$ m (Figs. 13, 14).

Colonies on PDA (potato dextrose agar) fast growing, reaching 1.4 cm diam. at 20 C, 2 cm diam. at 25 C and 1.1 cm diam. at 37 C in 4 days, hyphae effuse, woolly, white, colony edge crenulate, sporulating. Colonies on CMA (corn meal agar) fast growing, reaching 1 cm diam. at 20 C, 1.4 cm diam. at 25 C and 1 cm diam. at 37 C in 3 days, hyphae effuse, slender, white, colony edge crenulate, sporulating.

Holotype: THAILAND, Narathiwat, Sirindhorn Peat Swamp Forest, on submerged Eleiodoxa conferta, 20 June 2001, A. Pinnoi (AOM 0100 in BBH, living culture in BCC 9882, isotype PDD 75046).



Figs. 1-14. Chalara siamense (from holotype). 1. Colonies on substratum. 2-6. Conidiophores. 7-8. Chains of conidia. 9-12. Conidia. 13, 14. First formed conidia. Bars:  $1 = 150 \mu m$ ;  $2-14 = 10 \mu m$ .

Chalara schoenoplecti M.K.M. Wong, sp. nov.

(Figs. 16-21)

Etymology: in reference to the host, Schoenoplectus, from which this taxon was collected.

Coloniae albae, effusae. Conidiophora macronematosa, mononematosa, singula, sparsa, hyalina, (0-)1(-2) septata, interdum ramosa, laevia, 45-70  $\mu$ m. Cellulae conidiopenae monophialidicae. in conidiophoris incorporatae, hyalina, laevia, 40-65  $\mu$ m longa, venter subcylindricus, 12.5-25  $\times$  5-7.5  $\mu$ m; collum cylindricum, 20-32.5  $\times$  3-3.75  $\mu$ m; transitio e ventre ad collum gradata. Conidia endogena, catenate extrusa, hyalina, cylindrica, laevia, eseptata, ambo extrema truncata, (8-)12-17.5  $\times$  3-5  $\mu$ m.

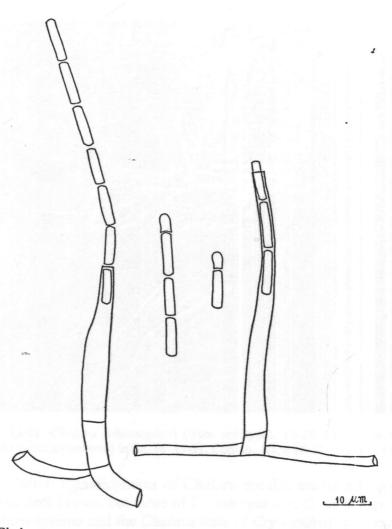
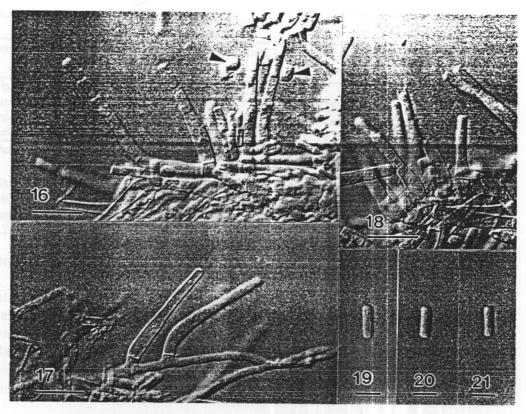


Fig. 15. Chalara siamense (line drawing). Conidiophore, conidia and first formed conidia.

Colonies on natural substrate white, effuse, with long chains of conidia. Conidiophores macronematous, mononematous, solitary, scattered, hyaline, ascending, arising laterally from aerial hyphae, (0-)1(-2) septate, sometimes branched, smooth, 45-70  $\mu$ m. Conidiogenous cells monophialidic, hyaline, smooth, 40-65  $\mu$ m long, venter subcylindrical, 12.5-25 × 5-7.5  $\mu$ m, collarette cylindrical, 20-32.5 × 3-3.75  $\mu$ m, transition from venter to collarette gradual, subtending cell 5-10 × 5-7.5  $\mu$ m (Figs. 16-18). Conidia endogenous, catenate, hyaline, cylindrical, smooth-walled, truncate at each end, aseptate, (8-)12-17.5 × 3-5  $\mu$ m ( $\overline{x}$  = 13.8 × 4.2  $\mu$ m, n = 25), first formed conidia subglobose or ellipsoid, 5-6.25 × 4.5-5  $\mu$ m.

Holotype: HONG KONG, New Territories, Mai Po Marshes, Gei Wai number 9, on standing, semi-submerged culms of Schoenoplectus litoralis, Sep. 1999, M.K.M. Wong MW305SL41 [HKU(M) 12654].



Figs. 16-21. Chalara schoenoplecti (from holotype). 16-18. Conidiophores. Note the first formed conidia (arrowed) in fig. 16. 19-21. Conidia. Bars:  $16-18=20 \mu m$ ;  $19-21=10 \mu m$ .

Notes: Conidiophores of Chalara species are usually pigmented, being pale to dark brown, but those of C. siamense and C. schoenoplecti are hyaline. Chalara hyalina and the Chalara state of Cryptendoxyla hypophloia also have similar hyaline conidiophores. There are differences between these four species both in conidial size and in the length of the conidiogenous cells (phialides) (Table 1), and they also occur on different substrata. Chalara hyalina is found on nematodes (Morgan-Jones et al., 1984), while the Chalara state of Cryptendoxyla hypophloia, which is constantly associated with its teleomorph, is found on wood of Acer and Betula in North America (Nag Raj and Kendrick, 1975). Chalara siamense and C. schoenoplecti are found as saprobes on the palm Eleiodoxa conferta and the sedge Schoenoplectus litoralis, respectively.

## Chalara kobensis McKenzie, sp. nov.

Etymology: in reference to the locality, Kobe City, where this taxon was collected. Coloniae parvae, albae. Conidiophora macronematosa, mononematosa, solitaria, sparsa, atro-brunnea, eseptata, laevia, (50-)60-80(-100) μm longa, basi inflata, 5-6.5 μm crassa.

Table 1. Some salient features of four hyaline species of Chalara.

Species	Conidial size (µm)	Phialide length (μm)	Venter size (μm)	Collarette size
C. hyalina	10-11 × 3-4	22-32		
C. schoenoplecti	$(8-)12-17.5 \times 3-5$	40-65	12.5-25 × 5-7.5	20-32.5 × 3-3.75
C. siamense	7-12 × 2.5-3	35-42	10-20 × 4.5-6	$20-25 \times 2.5-3.5$
Chalara sp.*	3.5-11 × 1.5-2.5	13-28	$7.5-14 \times 2-2.5$	5.5-11 × 1.5-2

\*Chalara state of Cryptendoxyla hypophloia.

Cellulae conidiogenae monophialidicae, in conidiophoris incorporatae, venter subcylindricus,  $25\text{-}40 \times 5~\mu\text{m}$ ; collum cylindricum,  $30\text{-}45 \times 3~\mu\text{m}$ ; transitio e ventre ad collum subito. Conidia endogena, catenate extrusa, hyalina, cylindrica, laevia, eseptata, ambo extrema truncata,  $10\text{-}15 \times 2.2\text{-}2.5~\mu\text{m}$ .

Holotype: JAPAN, Kobe City, Hyogo, on rotting leaves of Pasania edulis, Apr. 1969, T. Matsushima (MFC 2557).

Colonies small, white. Conidiophores macronematous, mononematous, solitary, scattered, dark brown, narrowly vasiform, 0-septate, reduced to phialide, smooth, (50-)60-80(-100)  $\mu$ m long, base inflated, 5-6.5  $\mu$ m thick. Conidiogenous cells monophialidic, venter subcylindrical, 25-40  $\times$  5  $\mu$ m, collarette cylindrical, 30-45  $\times$  3  $\mu$ m, transition from venter to collarette abrupt. Conidia endogenous, catenate, hyaline, cylindrical, smooth-walled, truncate at each end, aseptate, 10-15  $\times$  2.2-2.5  $\mu$ m.

## Chalara matsushimae McKenzie, sp. nov.

Etymology: in reference to T. Matsushima, collector and original describer of this taxon. Hyphae hyalinae-brunneae. Conidiophora macronematosa, mononematosa, solitaria, sparsa, pallide brunnea, 1-3 septata, laevia, 50-80 μm longa. Cellulae conidiogenae monophialidicae, in conidiophoris incorporatae, brunnea, apicem versus pallidiora, laevia, 37-45 μm longa, venter cylindricus, ca. 20 × 2 μm; collum cylindricum, 18-32 × 2-2.4 μm; transitio e ventre ad collum subito. Conidia endogena, catenate extrusa, hyalina, cylindrica, laevia, eseptata, ambo extrema truncata, 2.5-4.5 × 1.5-2 μm.

Holotype: JAPAN, Izumi-dake, Sendai City, Miyagi, forest soil, Sep. 1973, T. Matsushima (MFC 4802).

Hyphae hyaline to brown. Conidiophores macronematous, mononematous, solitary, scattered, brown, narrowly vasiform, 1-3 septate, smooth, 50-80 μm long, 3-3.5 μm wide above base. Conidiogenous cells monophialidic, brown, paler towards apex, 37-45 μm long, venter cylindrical, smooth-walled, ca. 20 × 2 μm, collarette cylindrical, 18-32 × 2-2.4 μm, transition from venter to collarette abrupt. Conidia endogenous, hyaline, cylindrical, smooth-walled, catenate, truncate at each end, aseptate, 2.5-4.5 × 1.5-2 μm.

### Chalara paramontellica McKenzie, sp. nov.

Etymology: in reference to this species apparent similarity to Chalara montellica.

Hyphae hyalinae vel pallide grisea-brunneae. Conidiophora macronematosa, mononematosa, solitaria, sparsa, brunnea, 1-7 septata, laevia,  $60-130 \times 5.5-7$  µm. Cellulae conidiogenae monophialidicae, in conidiophoris incorporatae, pallide brunnea, laevia, 50-65 µm longa, venter cylindricus,  $14-25 \times 5-7$  µm; collum cylindricum,  $28-40 \times 3-3.5$  µm, transitio e ventre ad collum subito. Conidia endogena, catenate extrusa, hyalina, cylindrica, laevia, 1-septata, ambo extrema truncata,  $10-17 \times 2.4-3$  µm.

Holotype: PAPUA NEW GUINEA, Bulolo, on rotten Castanopsis leaf, 27 Jan. 1970, T.

Matsushima (MFC 2704).

Hyphae hyaline to pale brownish-grey. Conidiophores macronematous, mononematous, solitary, scattered, brown, 1-7 septate, smooth, 60-130  $\times$  5.5-7  $\mu$ m. Conidiogenous cells monophialidic, pale brown, smooth, 50-65  $\mu$ m long, venter cylindrical, 14-25  $\times$  5-7  $\mu$ m, collarette cylindrical, 28-40  $\times$  3-3.5  $\mu$ m, transition from venter to collarette abrupt. Conidia endogenous, catenate, hyaline, cylindrical, smooth-walled, 1-septate, truncate at each end, 10-17  $\times$  2.4-3  $\mu$ m.

Matsushima (1975) reported on two additional specimens of this fungus, on Castanea crenata and Thujopsis dolabrata, both from Japan. At this time he gave conidial measurements of  $15\text{-}25 \times 2.2\text{-}2.5 \mu m$ , somewhat longer than those reported earlier (Matsushima, 1971). In addition, the conidiophores of the fungus described in 1975 appear to consist of a phialide plus 1-2 support cells, whereas the figure accompanying the earlier description depicted the conidiophores as up to 7 septate.

## Key to species of Chalara described since Nag Raj and Kendrick (1975)

This key contains those species of *Chalara* published after Nag Raj and Kendrick (1975). The key is arranged in three parts: 1) conidia usually 1-celled, 2) conidia 2-celled, and 3) conidia multicelled.

There have been several reports of *Chalara*-like anamorphs in the ascomycete orders Dothideales, Laboulbeniales, Leotiales, Microascales, Sordariales and Trichosphaeriales. These are summarised by Paulin and Harrington (2000). Where there is a published description of the *Chalara* anamorph, then these unnamed *Chalara* states have been incorporated into the key.

As a result of a phylogenetic and taxonomic evaluation of several *Chalara* spp., Paulin-Mahady *et al.* (2002) transferred several suspected plant parasitic species of *Chalara* to the genus *Thielaviopsis*, including four species of *Chalara* described since 1975. These four species are included in the key.

- A. Conidia usually 1-celled
- 1. Conidiophores rarely consisting of more than a single stalk cell and/or a phialide ......2

2. 2.	Conidia coloured
3. 3.	Conidia dark brown, (7-)10-14 × (5-)6-8 µm; on Cyathea
4. 4.	Conidia less than 6 µm long
5. 5.	Conidia $4-6\times0.8-1.2~\mu m$ ; collarettes $5.5-8\times1.4-1.6~\mu m$ ; on <i>Pinus</i>
6. 6.	Conidia usually less than 12 µm long
7.	cylindrical conidia $6.6-11.6(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8(-16.5) \times 2.8-4.4 \mu m$ ; conidia of other shapes $5-12.7 \times 3.3-8.8 \mu m$ .
7.	,
8. 8.	Conidiophores hyaline 9 Conidiophores hyaline to pale brown 10
9. 9.	Conidiogenous cells 35-42 µm long, arising from a single stalk cell; conidia (7-)9.3(-12) × (2.5-)2.8(-3) µm; on Eleiodoxa
	Conidiogenous cells 25-47 µm long, arising from a single stalk cell; conidia (9.4-)11.7(-12.6) × (3.7-)4(4.7) µm; on Nothofagus
11. 11.	Transition from venter to collarette gradual or imperceptible
	Conidia (8-)12-17.5 $\times$ 3-5 $\mu$ m, cylindrical; first formed conidia subglobose or ellipsoid 5-6.25 $\times$ 4.5-5 $\mu$ m; on Schoenoplectus
12.	Conidia 12.1-26.4 × 2.5-3.3 $\mu$ m, cylindrical; first formed conidia turbinate, 6.5-9.5 × 4.8-6 $\mu$ m; on Ficus
13.	Collarettes 65-115 × 5-6.5 μm; conidia (13-)14-17(-24) × 3.5-4.5 μm; on <i>Chionochloa</i>
13.	Collarettes 30-45 × 3 μm; conidia 10-15 × 2.2-2.5 μm; on <i>Pasania</i>
14. 14.	Conidia clavate, subclavate or wedge-shaped

15.	Conidia (8-)9-12.5 × 2.5-3.2 µm, apex rounded, base truncate with minute frill; on rotten wood
15.	Conidia less than 6 µm long
	Conidia 4-6 × 1-1.3 μm wide at truncate base, 1.3-2.3 μm wide at rounded apex; phragmospores 30-652 × 4.3-8.6 μm also produced; endophytic in <i>Vaccinium C. vacciniii</i> Conidia more than 1.3 μm wide
	I Committee and the second
	Conidia 3.5-5 × 1.3-2.5 µm; collarette funnel-shaped, 1-2.5 µm long; phialide sympodially proliferating; on <i>Pinus</i>
17.	Conidia (2.5-)3-5 × 1.5-2 μm; collarette cylindrical, 7-10.5 μm long; on Abies
18.	Conidiophores of 2 distinct sizes, 2.3-5.7 $\mu$ m wide or 5.2-7.4 $\mu$ m wide; conidia of 2 distinct sizes, 4-8.6 $\times$ 1.3-2.1 $\mu$ m and 4.3-10 $\times$ 2.4-4.5 $\mu$ m, respectively; on <i>Larix</i> and <i>Pinus</i>
18.	Conidiophores of 1 size
19.	Conidia dimorphic, cylindrical conidia abundant, 5-34.5 × 2.5-4 µm; rounded conidia not abundant, 7-11.5 µm diam; weakly parasitic on <i>Eucalyptus</i>
19.	Conidia of only 1 kind
20. 20.	Conidia ellipsoid, 3-4 × 2-2.1 µm; from soil
	Conidia mainly less than 6 µm long
22. 22.	Conidiogenous cells verruculose; conidia 4-6.5 × 1.75-2 µm; on Rubus C. verruculosa Conidiogenous cells smooth
23. 23.	Conidia 2.5-4 × 0.5-0.8 µm; on lichens
24. 24.	Conidia $2.5-4.5 \times 1.5-2  \mu m$ ; from soil
25.	Transition from venter to collarette abrupt; conidia 7-11.4 × 1.3-2.1 μm; on Nothofagus C. dualis
25.	Transition from venter to collarette usually gradual or imperceptible
26.	Conidia (3.7-)6.8(-10.5) × (1.5-)2(-2.3) µm; on Coffea and Psidium
26.	Conidia often longer than 10 µm
27.	Conidia (6-)15(-18.8) × (2.2-)3(-3.8) µm; parasitic on bark of <i>Populus</i> and <i>Salix</i>
27.	Conidia very variable in size, (2.5-)4.5-20(-26) × (1.5-)2-2.5(-3) µm; associated with Platypus tunnels in Nothofagus wood

B.	
1.	Conidiophores never consisting of more than a single stalk cell and/or a phialide2
1.	Conidiophores mostly consisting of one or more stalk cells and phialide4
2.	Setae present, surrounded by 2-4 conidiophores; conidia 16.5-19 × 2.5-3 µm; on Eucalyptus
2.	Setae absent
	The property of the second of the Court of the Court of the second of th
3.	Conidiophores 30-50 µm long; conidia (15.5-)18-20(-22.5) × 2-2.5 µm; on <i>Dracophyllum</i>
3.	Conidiophores 46-87 µm long; conidia 15-18 × 2-2.5 µm; on Quercus C. alabamensis
	destroy, M. C. Strategistical and sales and second of the C. C. S. C.
4.	Conidia mainly 1-celled, occasionally 1-septate
4.	Conidia always 2-celled5
5. 5.	Conidia 8-12 $\times$ 1.8-2.5 $\mu$ m; conidiophores up to 8 septate; on <i>Picea</i>
6.	Transition from venter to collarette abrupt; conidia 10-17 × 2.4-3 μm; on Castanopsis
0.	C. paramontellica
6.	Transition from venter to collarette gradual
	· · · · · · · · · · · · · · · · · · ·
7.	Conidia 12-17 × 2.5-3 µm; on dead wood
7.	Conidia (17-)18-22(-23) × (2.5-)3(-3.5) µm; on <i>Dracophyllum</i>
•	Could's model of the
1.	Conidia multicelled Conidia 22-42 × 4.8-6(-7) µm, (2-)3(-6) septate; on <i>Quercus</i>
1.	Conidia more than 7 µm wide
••	
2.	Conidia (25-)30-50(-70) × 10-12.5 µm, (2-)3(-7) septate; on palm
2.	Conidia 25-45(-56) × 7.5-10.5 μm, (3-)4 septate; on palm

Acknowledgements

A. Pinnoi thanks grant BRT R\_144012 for supporting this project. Boonsom Bussaban is thanked for technical help. K.D. Hyde thanks The Institute of Science and Technology Development of Chiang Mai University for funds to visit Chiang Mai University.

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(Received 16 February 2002; accepted 12 July 2002)

#### **PAPER**

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### tic fungi from peat swamp palms: *Unisetosphaeria penguinoides* gen. nov., and three new Dactylaria species

November 25, 2002 / Accepted: June 13, 2003

Unisetosphaeria penguinoides gen. et sp. nov. and w species of Dactylaria are described from dead and rhachides of the palms Eleiodoxa conferta nga pumila (Arecaceae) collected in Sirindhorn amp Forest, southern Thailand. Unisetosphaeria ycota) is compared with similar genera, and its nt at the family level is considered. The three new ia species (anamorphic fungi) are compared with pecies in the genus.

rds Hyphomycetes · Palm fungi · Peat swamp ropical fungi

collected in Sirindhorn Peat Swamp Forest, Narathiwat, in southern Thailand. Specimens consisting of dead palm fronds were returned to the laboratory and incubated under damp conditions in plastic boxes. The palm tissue was periodically examined for 3 weeks, and fungi appearing were isolated for identification. All measurements were made from materials mounted in water. Type material is deposited in the BIOTEC Bangkok Herbarium (BBH), Thailand. Single spore isolates were made on cornmeal agar (CMA) plates, with added antibiotics (penicillin G, 0.5 g/l and streptomycin 0.5 g/l) to suppress bacterial growth, and deposited in the BIOTEC Culture Collection (BCC).

#### tion

our study of the fungal diversity of palms in a peat orest, several new taxa have been encountered and d (Hyde et al. 2002; McKenzie et al. 2002; Pinruan 02). In this article, we describe Unisetosphaeria cota) and three new species of Dactylaria Sacc.

#### s and methods

i examined in this study were from decaying tissues doxa conferta Giff. or Nenga pumila H. Wendl.

Unisetosphaeria Pinnoi, E.B.G. Jones, McKenzie & K.D. Hyde, gen. nov.

**Species descriptions** 

Ascomata immersa, semi-immersa vel superficialia, pyriformia, hyalina vel pallide brunnea, ad apicem atrobrunnea, coriacea, ostiolata, papillata, sparsa. Papilla periphysata, pilis atris brevibus circumcincta, ad apicem seta singulari alta ex cellulis, multi-serialibus brunneis composita unilaterale enascenti. Peridium angulatibus, brunneis compositum. Paraphyses sparsae, indistinctae, ex cellulis ovoideis vel oblongis seriem brevem formantibus compositae. Asci 8-spori, clavati, unitunicati, breviter pedicellati, apice truncati, annulo apicali jodo non cyanescenti praediti. Ascosporae 2-seriales, hyalinae, septatae.

Type species: Unisetosphaeria penguinoides Pinnoi, E.B.G. Jones. McKenzie & K.D. Hyde.

Ascomata immersed, semiimmersed to superficial, pyriform, hyaline to light brown, dark brown near the apex. coriaceous. ostiolate, papillate, scattered. Papilla periphysate, surrounded by short dark hairs. A single long seta, made up several rows of brown cells, arises from the ostiolar region. Peridium composed of angular brownwalled cells. Paraphyses sparse, obscure, comprising short rows of ovoid to oblong cells. Asci 8-spored, clavate, unitunicate, short pedicellate, apically truncate, with a

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refractive, J-apical ring. Ascospores 2-seriate, hyaline, septate.

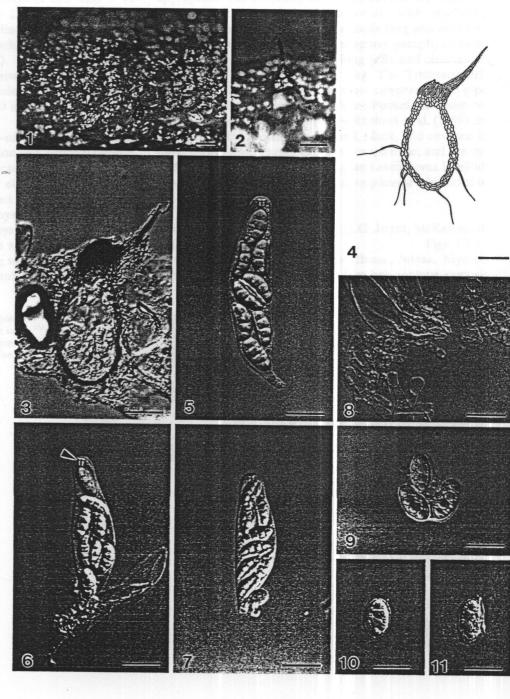
Etymology: from *uni* meaning one and *seta* meaning bristle, in reference to the single seta on the neck of the ascomata. It is also similar to the genus *Chaetosphaeria* Tul. & C. Tul.

Unisetosphaeria penguinoides Pinnoi, E.B.G. Jones, McKenzie & K.D. Hyde, sp. nov. Figs. 1-11

Ascomata 75-100 µm diam., 125-200 µm alta, immersa, semi-immersa vel superficialia, pyriformia, hyalina vel pallide brunnea, ad apicem atro-brunnea, coriacea, ostiolata, papillata, sparsa. Papilla periphysata, pilis brevibus

atris circumcincta, ad apicem seta singulalia alta (10 150 μm alta, 10–12.5 μm diam.) ex cellulis multi-serialib brunneis composita unilaterale enascenti. Peridiu usque ad 10 μm crassum, ex cellulis 2–4 stratosis angula brunneis compositum. Paraphyses sparsae, indistinctae, cellulis ovoideis vel oblongis ~12 × 7 μm seriuem brevie formantibus compositae. Asci 75–102 × 20–25 μm, 8-spo clavati, unitunicati, breviter pedicellati, apice trunca annulo apicali jodo non cyanescenti 4.5–5 μm alto, 4–5 μ diam. praediti. Ascosporae 18.5–22.5 × 10–14 μm, seriales, ovoideae vel fusoideae, rectae vel curvata hyalinae, 3-septatae, pariete laevi, tunica gelatino cicumdantes.

Figs. 1-11. Light micrographs and diagram of Unisetosphaeria penguinoides (from holotype). 1, 2 Ascomata on substratum. 3 Section of ascoma. Note the blackened neck and single long brown seta. 4 Diagrammatic representation of ascoma section. 5-7 Asci. Note the relatively large subapical ring (arrowhead in 6). 8 Paraphyses comprising short rows of ovoid to oblong cells. 9-11 Ascospores with thin mucilaginous sheath. Bars 1, 2 150 µm; 3, 4 50 µm; 5-11 20 µm



Holotypus: In petiolidibus submersis emortuisque iodoxae confertae 22:6:2001 A. Pinnoi (Aom103 in H).

Ascomata 75-100 µm diameter, 125-200 µm high, imrsed, semiimmersed or superficial, pyriform, hyaline to it brown, dark brown near the apex, coriaceous, ostioe, papillate, scattered (Figs. 1, 2). Papilla periphysate rounded by short dark hairs (Figs. 3, 4). A single seta, 1–150 μm long, 10–12.5 μm diameter, made up of several vs of brown cells arises from the ostiolar region (Figs. 3, Peridium up to 10 µm wide, composed of 2-4 layers angular, brown-walled cells (Fig. 4). Paraphyses sparse, scure, comprising short rows of ovoid to oblong cells, ~12  $7 \mu \text{m}$  (Fig. 8). Asci 75–102 × 20–25  $\mu \text{m}$  ( $\bar{x} = 87 \times 21.8 \mu \text{m}$ ), pored, clavate, unitunicate, short pedicellate, apically ncate, with a refractive, J-apical ring, 4.5-5 µm long, jum diameter (Figs. 5-7). Ascospores  $18.5-22.5 \times 10$ um ( $\bar{x} = 21.5 \times 11.8 \mu \text{m}$ , n = 25), 2-seriate, ovoid to oid, straight or curved, hyaline, 3-septate, smoothlled, with a large guttule in each cell, surrounded by a n layer of mucilage (Figs. 9-11).

Holotype: Thailand, Narathiwat, Sirindhorn Peat amp Forest, on submerged petiole of *Eleiodoxa conferta*, ne 22, 2001, A. Pinnoi (Aom103 in BBH).

Isotype: (PDD 76344).

Etymology: from penguin and -oides, in reference to the bilarity of the ascomata in section to a penguin outline. This taxon is somewhat characteristic of the Chaephaeriaceae (sensu Réblová et al. 1999) in having perficial ascomata with setae, asci with a refractive apical g, and transversely 3-septate, hyaline ascospores. In the aetosphaeriaceae, the taxon keys out to Chaetosphaeria. differs in having ascomata with a papilla surrounded by ort dark hairs and a single long seta made up of several we of brown cells, sparse paraphyses comprising short

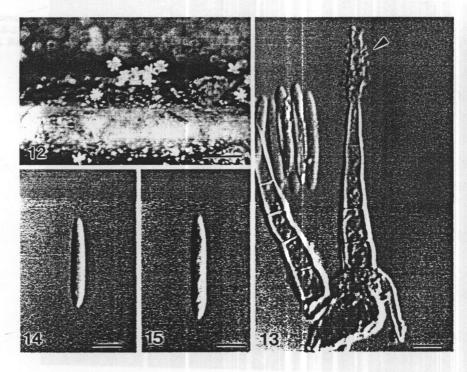
rows of ovoid to oblong cells, and clavate asci with a large refractive, J-apical ring. Another family to consider is the Trichosphaeriaceae (sensu Barr 1990). In the key provided by Réblová (1999b) this taxon is similar to Miyoshiella Kawam. or Rhamphoria Niessl. Unisetosphaeria differs from Miyoshiella and Rhamphoria in having a single long seta, near the ostiole of the ascoma, made up of several rows of brown cells and sparse paraphyses comprising short rows of ovoid to oblong cells. Miyoshiella has a Sporidesmium anamorph (Réblová 1999a), whereas Rhamphoria species have ascospores with longitudinal septa (Sivanesan 1976). There appears to be no existing genus that can accommodate Unisetosphaeria and, therefore, the introduction of a new genus is warranted.

The placement of Unisetosphaeria at the family level requires discussion. The Chaetosphaeriaceae may be appropriate but Unisetosphaeria has several incompatible characters; these include the ascomata with blackened necks with short dark hairs and a single long seta made up of several rows of brown cells, the sparse paraphyses comprising short rows of ovoid to oblong cells, and clavate asci with a large refractive, J-apical ring. The Trichosphaeriaceae (sensu Barr 1990) appears more congruent. The type species, Trichosphaeria pilosa (Pers.) Fuckel, has been recently discussed and illustrated by Réblová et al. (1999). In this species the ascomata are small, black, and covered in dark, short setae. Paraphyses are conspicuous, asci are cylindrical with a distinct apical ring, and ascospores 1-celled. It is, therefore, suggested that Unisetosphaeria is included in the Trichosphaeriaceae.

Dactylaria uliginicola Pinnoi, E.B.G. Jones, McKenzie, & K.D. Hyde, sp. nov. Figs. 12-15

Coloniae in substrato naturo effusae, luteae. Mycelia superficialia, ex hyphis hyalinis laevibus septatis ramosis

s. 12–15. Light micrographs of *Dactylaria* inicola (from holotype). 12 Colonies on stratum. 13 Conidiophores, conidiogenous (arrowhead), and conidia. 14, 15 Conidia. 12 100 μm; 13–15 5 μm



composita. Setae et hyphopodia absentes. Stromata non evolventia. Conidiophora erecta, solitaria, cylindrica, 60–90  $\times$  6.25–10  $\mu m$ , versus apicem attenuata (~4  $\mu m$  ad apicem), recta vel leviter flexuosa, laevia, 3–6-septata, hyalina. Cellulae conidiogenae integratae, 15–37.5  $\mu m$  altae, hyalinae, multidenticulatae; denticulae cylindricae. Conidia 21–28  $\times$  3–4.5  $\mu m$ , ad basim angustata, apice rotundata et centro aculeata, hyalina, laevia, fusiformia, basi leviter truncata, 0–1-septata; disjunctio conidiorum schizolytica.

Holotypus: In rachidibus submersis emortuisque *Eleiodoxae confertae* 22:6:2001 A. Pinnoi (Aom113 in BBH).

Ex holotypo: Living culture BCC 9883.

Colonies on natural substrata effuse, yellow (Fig. 12). Mycelium superficial, comprising hyaline, smooth, septate, branched hyphae. Setae and hyphopodia absent: Stromata not developed. Conidiophores erect, solitary, arising from hyphae, cylindrical, 60–90 × 6.3–10 µm, tapering apically (to ~4 µm near the apex), straight or slightly flexuous, unbranched, smooth, 3–6-septate, septa more or less equidistantly spaced, hyaline (Fig. 13). Conidiogenous cells integrated, 15–37.5 µm long, hyaline, polydenticulate; denticles cylindrical. Conidia 21–28 × 3–4.5 µm, slightly narrow at the base, hyaline, smooth, fusiform, apex acutely rounded, base similar but slightly truncate, 0–1-septate (Figs. 14, 15). Conidial secession schizolytic.

Holotype: Thailand, Narathiwat, Sirindhorn Peat Swamp Forest, on submerged rachis of *Eleiodoxa conferta*, June 22, 2001, A. Pinnoi (Aom113 in BBH).

Isotype: (PDD 76345).

Ex-holotype: Living culture BCC 9883.

Etymology: From the Latin *uliginicola*, living in swamps. Colonies on potato dextrose agar (PDA), reaching 2cm

diameter in 25 days, woolly with central tuftlike growth. outwardly immersed, central area brown with paler to whitish tufts, outer immersed area pale grey, grey-brown from below, not staining agar, not sporulating within 1 month.

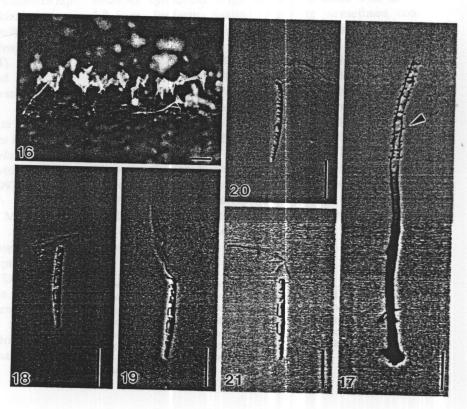
The conidia of *Dactylaria uliginicola* are similar to those of *D. chrysosperma* (Sacc.) G.C. Bhatt & W.B. Kendr., *D. fusifera* (Berk. & M.A. Curt.) de Hoog, and *D. candidula* (Höhn.) G.C. Bhatt & W.B. Kendr. The tapering hyaline conidiophores of *D. uliginicola* distinguish it from *D. chrysosperma*. The conidiophores of *D. uliginicola* are longer and broader than those of *D. fusifera* (60–90 × 6.25–10  $\mu$ m vs. 25–30 × 4–6  $\mu$ m), whereas the conidia are shorter (21–28 × 3–4.5  $\mu$ m vs. 30–40 × 3.8–4.6  $\mu$ m) (Hoog 1985). The conidiophores of *D. candidula* are shorter (60–90  $\mu$ m vs. 20–35(–50)  $\mu$ m), the conidia are smaller (21–28 × 3–4.5  $\mu$ m vs. 15–23 × 2.5–3.4(–4.2)  $\mu$ m) and constricted at the median septum.

Dactylaria flammulicornuta Pinnoi, E.B.G. Jones McKenzie & K.D. Hyde, sp. nov. Figs. 16-21

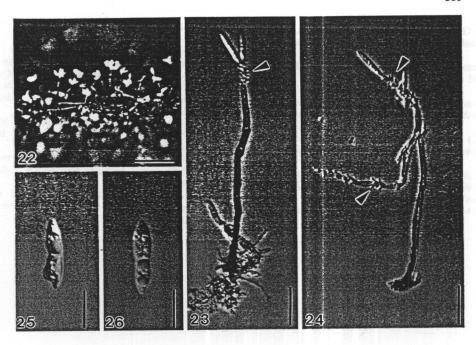
Coloniae in substrato naturo effusae, albinae. Mycelia superficialia, ex hyphis brunneis laevibus septatis ramosis composita. Setae et hyphopodia absentes. Stromata non evolventia. Conidiophora erecta, solitaria, cylindrica, 160–250 × 4.5–6.25 μm, recta vel flexuosa, laevia, 8–12-septata, brunnea, ad apicem pallide brunnea. Cellulae conidiogenae integratae, 27.5–90 × 5–6.5 μm, hyalinae, multidenticulatae; denticulae cylindricae. Conidia 42.5–62.5 × 4.5–5 μm, cylindrica, hyalina, 0–1-septata; disjunctio conidiorum schizolytica.

Holotypus: In petiolibus emortuis ad terram Nengae pumilae 12:2:2002 A. Pinnoi (Nen29 in BBH).

Figs. 16–21. Light micrographs of *Dactylaria flammulicornuta* (from holotype). 16
Colonies on substratum. 17 Conidiophores and conidiogenous cells (*arrowhead*). 18–21
Conidia with long flamelike appendage. *Bars* 16 200 μm; 17–21 25 μm



2-26. Light micrographs of daria palmae (from holotype). 22 lies on substratum. 23, 24 liophores and conidiogenous cells wheads). 25, 26 Conidia. Bars 22 lip; 23, 24 25 μm; 25, 26 12 μm



olonies on natural substratum effuse, white (Fig. 16). elium superficial, comprising brown, smooth, septate, ched hyphae. Setae and hyphopodia absent. Stromata developed. Conidiophores erect, solitary, arising from tae, cylindrical,  $160-250 \times 4.5-6.3 \mu m$ , straight or tly flexuous, occasionally branched, smooth, 8-12-ate, brown, pale brown at apex (Fig. 17). Conidiogencells integrated,  $27.5-90 \times 5-6.5 \mu m$ , hyaline, polydenate; denticles cylindrical (Fig. 17). Conidia  $42.5-62.5 \times 5 \mu m$  ( $\bar{x} = 56 \times 4.6 \mu m$ , n = 25), cylindrical, hyaline, 0-ptate, with an apical appendage with a flamelike aprance (Figs. 18-21). Conidial secession schizolytic.

Iolotype: Thailand, Narathiwat, Sirindhorn Peat mp Forest, on terrestrial petiole of *Nenga pumila*, ruary 12, 2002, A. Pinnoi (Nen29 in BBH).

tymology: from the Latin *flamma* and *cornula* = ned, in reference to flamelike appearance of the endage.

The conidia of Dactylaria flammulicornuta are unique in the genus Dactylaria. No other species is known to an apical appendage, although Dactylaria tunicata & K.D. Hyde has been described with a fragile, line, gelatinous sheath (Goh and Hyde 1997).

tylaria palmae Pinnoi, E.B.G. Jones. McKenzie & Figs. 22–26. Hyde, sp. nov. Figs. 22–26. Coloniae in substrato naturo effusae, luteae. Mycelia erficialia, ex hyphis brunneis laevibus septatis ramosis aposita. Setae et hyphopodia absentes. Stromata non liventia. Conidiophora erecta, solitaria, cylindrica, –150 × 3–4.5 μm, recta vel flexuosa. laevia. brunnea. lulae conidiogenae integratae, 25–60 × 3–3.8 μm, linae, multidenticulatae; denticulae cylindricae. Conidia 3–25 × 3.8–5 μm, fusiformia, hyalina. 1-septata: unctio conidiorum schizolytica.

Holotypus: In vaginis folii emortuis ad terram Nengae pumilae 12:2:2002 A. Pinnoi (Nen35 in BBH).

Colonies on natural substratum effuse, yellow (Fig. 22). Mycelium superficial, comprising brown, smooth, septate, branched hyphae. Setae and hyphopodia absent. Stromata not developed. Conidiophores erect, solitary, arising from hyphae, cylindrical,  $100-150 \times 3-4.5 \,\mu m$ , straight or slightly flexuous, sometimes branched, brown, pale brown toward the apex (Figs. 23, 24). Conidiogenous cells integrated,  $25-60 \times 3-3.8 \,\mu m$ , hyaline, polydenticulate; denticles cylindrical (Figs. 23, 24). Conidia  $23.8-25 \times 3.8-5 \,\mu m$ , fusiform, hyaline, 1-septate, sometimes constricted at septum (Figs. 25, 26). Conidial seccession schizolytic.

Holotype: Thailand, Narathiwat, Sirindhorn Peat Swamp Forest, on terrestrial sheath of *Nenga pumila*, February 12, 2002, A. Pinnoi (Nen35 in BBH).

Etymology: In reference to its association with palms.

Dactylaria palmae is similar to D. tunicata Goh & K.D. Hyde, D. candidula (Höhn) G.C. Bhatt & W.B. Kendr., D. cymbiformis Matsush., and D. mucronulata Ellis. & Langl. However, the conidiophores of D. palmae are branched and the conidia lack a sheath. The other three species have smaller conidia than those of D. palmae [D. candidula, 15–23 × 2.5–3.4(–4.2) μm; D. cymbiformis, 15–26.5 × 4–6(–8) μm; D. mucronulata, 8.5–11 × 2.5–3.6μm] (Matsushima 1980).

Acknowledgments This work is financed by BRT R\_145008. A. Pinnoi thanks Prasert Srikitikulchai, Jariya Sakayaroj, and Ittichai Chatmala for help in collecting material and technical assistance, and Saisamorn Lumyong for her continued support. Chavalit Niyomtum is thanked for identifying the palms. Manetr Boonyanant, Director of Sirindhorn Peat Swamp Centre, Narathiwat and his staff are thanked for permission and assistance in the collecting of material and for providing laboratory facilities.

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## **APPENDIX 4**

# Flammispora gen. nov., a new freshwater ascomycete from decaying palm leaves

Umpaya Pinruan<sup>1\*</sup>, Jariya Sakayaroj<sup>1</sup>, Emasan B. Gareth Jones<sup>1</sup> and Kevin D. Hyde<sup>2</sup>

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Abstract: Flammispora bioteca gen. et sp. nov., a freshwater ascomycete (Ascomycota incertae sedis) is characterised by black immersed ascomata, weakly persistent asci and 5-septate hyaline ascospores with a basal appendage. It is described from submerged decaying leaves of the peat swamp palm Licuala longeculycata. Although it is characteristic of the Halosphaeriales, sequencing data indicates it to be distantly related to this order. No genus can be found to accommodate this taxon and based on morphological and molecular evidence a new genus is justified. The genus is, however, compared with the Halosphaeriales and its taxonomic position discussed.

Taxonomic novelties: Flammispora U. Pinruan, J. Sakayaroj, K.D. Hyde & E.B.G. Jones gen. nov., Flammispora bioteca U. Pinruan, J. Sakayaroj, K.D. Hyde & E.B.G. Jones sp. nov.

Key words: Freshwater ascomycetes, palm, heat swamp, systematics, tropical fungi.

#### INTRODUCTION

Submerged leaves of the peat swamp palm Licuala longecalycata Furt, have yielded a number of new fungal taxa (Hyde et al. 2002, McKenzie et al. 2002, Pinruan et al. 2002), including the freshwater ascomycete Jahnula appendiculata (Jahnulales) (Pang et al. 2002). Several freshwater ascomycetes from tropical locations have also been reported (Cai et al. 2003. Tsui et al. 2003, Lou et al. 2004). Those with appendaged ascospores are usually members of the Annulatascaceae (Sordariomycetidae) or Halosphaeriales while other taxa have sheaths (e.g. Massarina velatospora (Hyde & Borse 1986) or lack appendages (e.g. Kirschsteiniothelia elaterascus, Shearer 1995). Currently, 44 genera and 136 species are assigned to the Halosphaeriales (Pang 2002), while other taxa continue to be described (Hyde 2002, Pang et al. 2004). Most are marine, while a few are known from brackish and freshwater habitats e.g. Ariptodera chesapeakensis, A. triseptata, Fluviatispora spp., Halosarpheia spp., Lignincola laevis, Nais inornata (Hyde et al. 1999, 2000, Hyde 2002, Fryar et al. 2004. Tsui et al. 2004). Hyde (1994) described Flaviatispora, with two species from material collected in freshwater habitats in Ecuador, and Papua New Guinea, while a new species has recently been described from Brunei (Fryar & Hyde 2004). The Annulatascaceae comprises 10 genera and 38 species and are characterised by thin-walled cylindrical asci with a relatively massive refractive J- apical ring and ascospores with polar

appendages as gelatinous sheaths (Wong et al. 1998, Ranghoo et al. 1999). The purpose of this paper is to describe a new genus, which typically like the *Halos-phaeriaceae*, has appendaged ascospores. We have used morphology and analysis of DNA sequences to establish whether the taxon should be included in the *Halosphaeriaceae*. The result and description of the new species are presented here.

#### MATERIALS AND METHODS

#### Isolates

Submerged material of the palm *Licuala longecaly-cata* was collected from Sirindhorn Peat Swamp Forest, Narathiwat, southern Thailand on February 2002. The material was returned to the laboratory, incubated in plastic boxes on damp tissue paper and examined within 4 wks. Type material has been deposited in the BIOTEC Bangkok Herbarium (BBH) and cultures deposited in the BIOTEC Culture Collection (BCC) and Centraalbureau voor Schimmelcultures (CBS). Single-spore isolations were made on cornmeal agar (CMA) with added antibiotics to suppress bacterial growth following the method of Choi *et al.* (1999). All observations, including photographic documentation, were of material mounted in water and examined with a differential interference microscope.

ble 1. SSU rDNA sequences obtained from the GenBank.

Classification (Orders)	Taxon	GenBank accession numbers
Halosphaeriales	Nohea umiumi Kohlm, & Volkm,-Kohlm.	U46878
auspnaeraies	Halosphaeria appendiculata Linder	U46872
	Halosarpheia retorquens Shearer & J.L. Crane	AF352086
	Lignincola laevis Höhnk	U46873
	Note the second of Making	AF050482
	Halosphaeriopsis mediosetigera (Cribb et J.W. Cribb) T.W. Johnson	U32420
	Nereiospora comata (Kohlm.) E.B.G. Jones, R.G. Johnson & S.T. Moss	AF050485
dicroascales	Pseudallescheria ellipsoidea (Arx & Fassat.) McGinnis, A.A. Padhve &	U43911
TR Touscures	AjelloPseudallescheria boydii (Shear) McGinnis, A.A. Padhye & Ajello	M89782
	Petriella setifera (J.C. Schmidt) Curzi	143908
	Microascus cirrosus Curzi	M89994
Incorpoles -	Mellanospora fallax Zukal	U47842
Melanospora	Kallichroma tethys (Kohlm, & E. Kohlm.) Kohlm, & VolkmKohlm, BCC13048	AY722099
	Nectria haematococca Berk, & Broome	AF141952
	Paecilomyces tenuipes (Peck) Samson	AB070372
	Sphaerostibella aureonitens (Tul. & C. Tul.) Seifert, Samuels & W. Gams	U32415
	Hypocrea lutea (Tode) Peck	AF543791
Phyllachorales	Glomerella septospora Sivan. & W.H. Hsieh	U78779
nymuc mortures	Colletotrichum gloeosporioides (Penz.) Penz. & Sacc.	AY083798
	Plectosphaerella cucumerina (Lindf.) W. Gams	AF176951
italics	Polystigma ochraceum (Wahlenb.) Sacc. Not italics	AF276299
ordariales	Guanomyces polythrix M.C. González, Hanlin & Ulloa	AF207683
chamaics	Chaetomium globosum Kunze	AB048285
	Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	X69851
	Ascovaginospora stellipala Fallah, Shearer & W.D. Chen Not italics	U85087
Ophiostomatales	Ophiostoma pilliferum (Fr.) Syd. & P. Syd.	AY281094
2.3	Ophiostoma ulmi (Buisman) Nannf.	M83261
Diaporthales	Cryphonectria havanensis (Bruner) M.E. Barr	1.42440
italics	Endothia gyrosa (Schwein.) Fr. Not italics	1.42443
(vlariales	Xylaria sp.	AB014042
y tuz tures	Xylaria carpophila (Pers.) Fr. Monosporascus ibericus J. Collado et al.	Z49785
	2. Maria Carpophia (1 Cl. 27) 1 1	AF340015
Dothideales	Dothidea insculpta Wallr Dothidea hippophaës (Pass.) Fuckel	U42474
zonnucure.	Southern the tipe of the transfer the transfer transfer tensor	U42475
Ascomycota	Flammispora bioteca U. Pinruan et al., sp. nov. BCC13367	AY722100
ncertae sedis	Flammispora bioteca U. Pinruan et al., sp. nov. BCC13368	AY722101
ne criac seurs	Transmispera project C. Finacar et al., spenov. De e 15.50	

A extraction, amplification and sequencing

ock cultures of *Flammispora bioteca* were mainned on potato dextrose agar (PDA) at 25 °C. Two ains of the fungus were grown in liquid GYP (Gluse Yeast Extract Peptone) (Abdel-Wahab *et al.* 01) broth on a rotary shaker at 200 rpm at 25 °C. Fungal biomass was harvested and washed with rile distilled water. The biomass was frozen in

rile distilled water. The biomass was frozen in uid nitrogen and ground with a mortar and pestle. NA was extracted using a NucleoSpin<sup>R</sup> Plant DNA traction kit (MACHEREY-NAGEL). Partial small punit (SSU) ribosomal DNA (rDNA) was amplified ing FINNZYMES. DyNAzyme<sup>TM</sup> II DNA Polygrase Kit (MACHEREY-NAGEL, Product code F-IS), in a Perkin-Elmer thermal cycler. Primers NS1, 34, NS5 and NS6 were used following White *et al.* 2000). The PCR product was purified using a NucleoSpin<sup>R</sup> Plant DNA purification kit (MACHEREY-MGEL), then sequenced automatically by the Bio rvice Unit (BSU) laboratory using the following mers: NS1, NS3, NS5 and NS6 (White *et al.* 1990).

Phylogenetic analysis

Partial SSU rDNA of Flammispora bioteca was analyzed along with other sequences obtained from the GenBank database (Table 1). Sequences were aligned in Clustal W 1.6 programme (Thompson et al. 1994) and refined visually in BioEdit version 6.0.7 (Hall 2004) and Se-Al v. 1.Oa1 (Rambaut 1999). Alignment was entered into PAUP v. 4.0b10 (Swofford 2002). Phylogenetic trees were generated using maximum parsimony, characters were equally weighted, followed by a heuristic search with a stepwise starting tree, a random stepwise addition of 100 replicates and tree-bisection-reconnection (TBR) branch-swapping algorithm, with gaps treated as missing data. Finally, bootstrap analysis (Felsenstein 1985) was performed using full heuristic searches on 1000 replicates, stepwise addition of sequence, 10 replicates of random addition of taxa and TBR branchswapping algorithm.

#### RESULTS

Phylogenetic analysis

Initially, 1807 characters from the SSU sequences were included in the analysis (17.6 % parsimony informative sites). Nine major orders were analysed (Halosphaeriales, Microascales, Hypocreales, Phyllachorales, Sordariales, Ophiostomatales, Diaporthales and Xylariales) including the Dothideales as outgroup. This yielded five most parsimonious trees (MPTs) with tree lengths, consistency indices (CI) and retention indices (RI) of 949 steps, 0.5933 and 0.7239, respectively. All five MPTs differ only with minor topological differences within the Phyllachorales (data not shown). The tree shown in Fig. 1 is the best

hypothesis for our SSU dataset, resulting from the Kishino-Hasegawa (K-H) maximum likelihood test (Kishino & Hasegawa 1989).

The phylogenetic tree shows a number of major clades: A: Halosphaeriales-Microascales clade. B: Hypocreales-Phyllachorales clade. C: Sordariales clade and D: Ophiostomatales-Diaporthales-Xylariales clade (Fig. 1). The two strains of Flammispora bioteca sequenced are monophyletic and supported by 100% bootstrap value. In every analysis, Flammispora bioteca formed a distinct clade, with a long branch length, between clades A and B, although with low bootstrap support (Fig. 1).

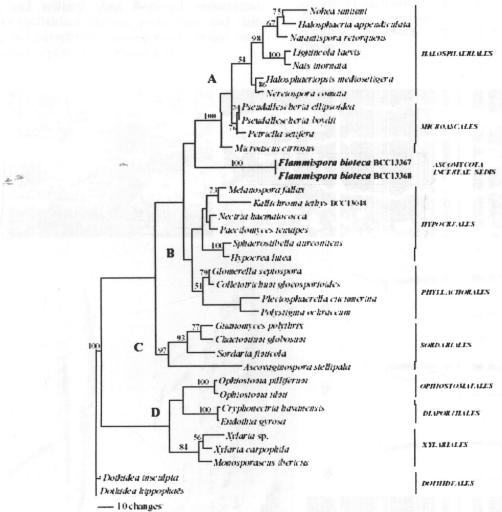


Fig. 1. One of the most parsimonious trees from partial SSU rDNA sequences. Bootstrap values higher than 50 % are given on the branches. Scale bar indicates ten character state changes.

#### l'axonomic descriptions

Flammispora U. Pinruan, J. Sakayaroj, K.D. Hyde & E.B.G. Jones. ▼gen. nov. MycoBank XXXXXX, Figs 2–9. & K.D. Hyde

Etymology: from Latin flamme - 'flame' meaning in reference to the flame-like basal appendage.

Ascomata immersa vel semi-immersa, coriacea, ostiolata, solitaria. Asci octospori, unitunicati, clavati vel cylindro-lavati, pedicellati, deliquescens, sine paraphyses. Ascosporae biseriatae, fusiforme, hyalinae, septatae, appendici.

Ascomata immersed, or semi-immersed, coriaceous, ostiolate and solitary. Asci 8-spored, unitunicate, clavate to cylindrical clavate, pedicellate and deliquescent. No paraphyses. Ascospores biseriate, fusiform, hyaline, septate, and appendaged.

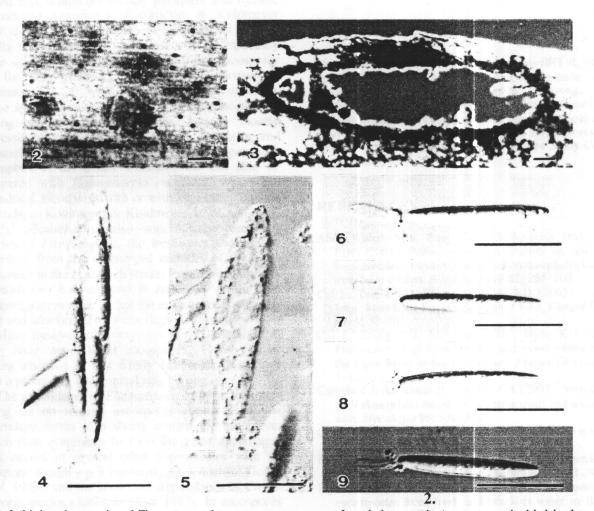
Typus generis: Flammispora bioteca U. Pinruan, J. Sa-kayaroj, E. B. G. Jones & K. D. Hyde, sp. nov.

Cultura ex-typus: BCC13367 (CBSxxxx), BCC13368 (CBSxxxx).

Flammispora bioteca U. Pinruan, J. Sakayaroj, K.D. Hyde & E.B.G. Jones, sp. nov. MycoBank XXXXXX Figs 2 9.

Etymology: from Latin bioteca - named after BIO-TEC.—A Thai Research Institute.

Ascomata 225–275 μm diam, immersa et semi-immersa, subglobosa, nigra, coriacea, ostiolata, solitaria, sine paraphyses. Asci 82.5–87.5 × 16–21 μm, cylindrico-clavati vel clavati, unitunicati, pedicellati. Ascosporae 47.5–55 × 5–6.5 μm, 2–3-seriatae, fusiformes vel cylindrico, hyalinae, 5-septatae, guttulatae, appendicibus ad basim.



Figs 2–9. Light micrographs of Flammispora bioteca gen et sp. nov. (from holotype). T. Ascomata embedded in the substratum. 2. Vertical section of ascomata with perithecial wall dark brown to black.  $\frac{3}{4}$  4. Asci cylindrical-clavate, thin-walled, ascosperes 2–3-seriate, apical ring absent. 5–8. Ascospore with a single polar appendage, hyaline, and 5-septate. Scale bars: 1 = 100  $\mu$ m, 2–9 = 20  $\mu$ m. 6-9.

Cultural characteristics: Colonies on PDA (BCC13367 and BCC13368 used for the molecular study) cottony, reaching 1 cm in diam in 15 d at room temperature (22–24 °C), with grey-brown mycelium, hyphae smooth-walled. No ascomata formed in culture.

Holotype: Thailand, Narathiwat, Sirindhorn Peat Swamp Forest, on dead leaves of Licuala longecalycata, 13 Feb. 2002. U. Pinruan (WAH 134 in BBH holotype), cultures ex-type CBS xxxx and CBS xxxx.

#### DISCUSSION

Flammispora bioteca cannot be assigned to a family or order at this time, although morphologically it shares a number of features in common with members of the Halosphaeriales. This includes simple ascomata, a thin-walled peridium, lack of paraphyses, thinwalled asci, which are weakly persistent and hyaline appendaged ascospores (Kohlmeyer & Kohlmeyer 1979. Jones 1995). Flammispora resembles aquatic genera with polar appendaged ascospores, especially some species with cylindrical to filiform ascospores and the taxa: Ascosacculus aquaticus, Ascosalsum cincinnatulum, A. viscidulum and A. unicaudatum. These species, however, differ from Flammispora in having hamate polar appendages, initially closely adpressed to the ascospore wall, then separating and eventually unfurling to form long, narrow appendages (Campbell et al. 2003). Flammispora can also be compared with Halosphaeria cucullata, which has cylindrical ascospores with or without a mucilaginous appendage (Kohlmeyer & Kohlmeyer 1979, Cai et al. 2002). Flammispora also superficially resembles species of Fluviatispora, the freshwater ascomycete described from the submerged rachides of the palm Livistonia in the Bensbach River, Papua New Guinea. Fluviatispora tunicata and F. reticulate differ from Flammispora bioteca in that the ascospores are unicellular and surrounded by a mucilaginous sheath.

Many freshwater ascomycetes in the Armulatascaceae have appendaged ascospores. However, all genera assigned to this family have cylindrical asciwith a prominent large apical ring (Wong et al. 1998).

The appendages in *Flammispora bioteca* are interesting, as on release, ascospores appear as if the appendage forms as a sheath around the ascospore, which then evaginates to form the polar appendage. This occurs in several other ascomycetes with an exosporic sheath e.g. *Chaetosphaeria chaetosa* (Jones *et al.* 1983), and the secondary appendages of *Corollospora* species (Jones & Moss 1987). In ascospores observed within the asci, the appendages are apparent and this probably precludes this type of appendage formation. It therefore appears that the appendage is produced as an outgrowth of the cell wall at the basal

pole. This is similar to several other ascomycete taxa with appendaged ascospores e.g. *Torpedospora radiata*.

Our molecular data using two strains of Flammispora bioteca (isolated from the holotype on the same occasion) and analyses of the sequence data confirms they are monophyletic. Flammispora bioteca is distantly related to the Halosphaeriales, although it shares some morphological characteristics. It forms a distinct clade to other ascomycetes with unitunicate asci, between the Microascales and Hypocreales clades, although with weak support (Fig. 1). However, a long branch length was observed in the sequence of F. bioteca. This could indicate a high number of autapomorphic molecular characters, and a sequence difference with neighbouring clades, or may be due to the lack of other closely related taxa in the analyses. At the moment, no family or order is appropriate to accommodate this fungus, therefore a new genus with uncertain taxonomic position is proposed.

#### ACKNOWLEDGEMENTS

This project is supported by research grants BRT R\_145008 and BRT R\_245002. We are grateful to Graduate School Chiang Mai University, Saisamorn Lumyong, Ruud Valyasevi, and Morakot Tanticharoen for continued support, to Ittichai Chatmala and Aom Pinnoi for field assistance and to Manetr Boonyanant, and his staff, for research facilities at the Sirindhorn Field and Nature Study Center, Narathiwat.

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### Two new species of Stachybotrys, and a key to the genus

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Pinruan, U., McKenzie, E.H.C., Jones, E.B.G. and Hyde, K.D. (2004). Two new species of *Stachybotrys*, and a key to the genus. Fungal Diversity.

Stachybotrys palmae sp. nov. found on decaying petioles of Licuala longecalycata in Sirindhorn Peat Swamp Forest, Thailand, and S. cordylines sp. nov. found on decaying leaves of Cordyline banksii in New Zealand are described and illustrated. The new species are compared with other species in the genus, and a key is provided to all species of Stachybotrys.

Key words: anamorphic fungi, hyphomycetes, monocotyledonous plants, palm fungi.

#### Introduction

We are studying fungi occurring on monocotyledonous plants. In this paper we describe two new species of *Stachybotrys*. The first species was found on decaying petioles of *Licuala longecalycata* Furt., a tropical palm species from Sirindhorn Peat Swamp Forest, Narathiwat, Thailand. The second species occurred on *Cordyline banksii* Hook. f., a New Zealand species of Agavaceae. *Stachybotrys* species are saprobes, common in soil and on decaying plant material. They produce single-celled

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conidia aggregated in slimy heads. The genus is worldwide in distribution although some species are restricted to the tropics and subtropics. More than 50 species of *Stachybotrys* are accepted, including those species formerly included in the genus *Memnoniella*. The new species are described and compared to similar species.

#### Materials and methods

Decaying fronds of *Licuala longecalycata* were collected from Sirindhorn Peat Swamp Forest, Narathiwat, Thailand, and decaying leaves of *Cordyline banksii* were collected from the Waitakere Ranges, Auckland, New Zealand. The decaying tissues were returned to the laboratory and incubated in damp chambers. Single spore isolates of both fungi were obtained. Microscopic measurements for *S. palmae* were taken from specimens mounted in water while those for *S. cordylines* were taken from specimens mounted in lactophenol.

#### Taxonomy

Stachybotrys palmae Pinruan, sp. nov.

(Figs. 1-5)

Conidiophora macronematosa, mononematosa, solitaria vel fasciculata, eramosa, erecta, recta vel paulo flexuosa, laevia, 2–5 septata, brunnea, pallidae ad apicem, crassitunicata, (80–)110–230 µm longa, 6.3–10 µm crassa. Cellulae conidiogenae monophialidicae, discretae, 5–7 in verticillo dispositae, clavatae, 11–

 $12.5 \times 6-7.5$  µm, laevae, hyalinae. Conidia in massis globosis aggregata, ellipsoidea,

hyalina, verrucosa,  $10-15 \times 5-7.5 \mu m$ ; apice et basi truncata.

Etymology: In reference to its association with palms.

Conidiophores macronematous, mononematous, single or in groups,

unbranched, erect, straight or slightly flexuous, smooth, 2-5 septate, brown, apical

cell hyaline, thick-walled, (80-)110-230 µm long, 6.3-10 µm wide. Conidiogenous

cells monophialidic, discrete, determinate, clavate, smooth, hyaline, forming a whorl

of 5-7 at the apex of the conidiophores,  $11-12.5 \mu m$  long,  $6-7.5 \mu m$  thick in the

broadest part. Conidia aggregated in pale cream slimy heads, ellipsoidal or boat-

shaped, truncate at the base and apex, hyaline, verrucose,  $10-15 \times 5-7.5 \, \mu m$  ( $\overline{x} = 13$ 

 $\times$  7 µm, n = 20).

Material examined: THAILAND, Narathiwat, Sirindhorn Peat Swamp Forest,

on decaying rachis of Licuala longecalycata Furt., 12 May 2001, U. Pinruan, (Wah

35) [BIOTEC Bangkok Herbarium (BBH), holotype].

Habitat: Saprobic on Licuala longecalycata.

Distribution: Thailand.

Stachybotrys palmae is one of only five species of Stachybotrys that produce

hyaline conidia. Of these, S. palmae is the only species with rough-walled conidia.

Those of the other four species (S. bambusicola Rifai, S. bisbyi (Sriniv.) G.L. Barron,

3

S. guttulispora Muhsin and Al-Helfi, S. palmijunci Rifai) have conidia that are smooth-walled.

Stachybotrys cordylines McKenzie, sp. nov.

(Figs. 6-13)

Conidiophora macronematosa, mononematosa, solitaria, eramosa, erecta, recta vel paulo flexuosa, laevia, interdum granulis magnis tecta, 4–7 septata, hyalina, crassitunicata, 95–160  $\mu$ m longa, basi 5.8–8  $\mu$ m, prope apicem 3–4.2  $\mu$ m, apice inflata 3.5–5.1  $\mu$ m diam. Cellulae conidiogenae monophialidicae, discretae, 7–8 in verticillo dispositae, clavatae, 11–14(–16) x 3.8–5.4  $\mu$ m, laevae, hyalinae. Conidia in massis globosis aggreata, ellipsoidea vel obovoidea, olivacea, rugulosa, eseptata, 7.0–8.3 × 3.2–5.1  $\mu$ m.

Etymology: cordylines, referring to the host genus Cordyline.

Conidiophores macronematous, mononematous, single, unbranched, erect, straight or slightly flexuous, smooth or sometimes covered with a few large granules near the base, 4–7 septate, hyaline, thick-walled, 95–160  $\mu$ m long, 5.8–8  $\mu$ m thick near the base, tapering to 3–4.2  $\mu$ m near the apex, slightly enlarged at the apex to 3.5–5.1  $\mu$ m thick and bearing a whorl of 7–8 phialides. Conidiogenous cells monophialidic, discrete, clavate, 11–14(–16)  $\mu$ m long, 3.8–5.4  $\mu$ m thick in the broadest part, smooth, hyaline. Conidia aggregated in slimy, black, glistening heads, ellipsoid or obovoid, olive green, rugulose, non-septate, 7.0–8.3  $\times$  3.2–5.1  $\mu$ m ( $\overline{x}$  = 7.6  $\times$  4.5  $\mu$ m, n = 20).

Material examined:. NEW ZEALAND, Waitakere Ranges, near Spraggs Bush, on dead leaves of Cordyline banksii Hook. f., 4 Aug 2003, R.E. Beever [PDD 78085, holotype].

Habitat: Saprobic on Cordyline banksii.

Distribution: New Zealand.

The conidia of S. cordylines are similar in size and shape to those of S. albipes, however, the latter fungus has smooth conidia whereas those of S. cordylines are rugulose. It differs from S. chartarum by having hyaline conidiophores; those of S. chartarum are dark olivaceous towards the apex.

Note: Memnoniella and Stachybotrys have been considered distinct genera (e.g., Ellis 1971, 1976). However, the only difference between these two genera is that the conidia are in long, dry chains in Memnoniella while they are in slimy masses in Stachybotrys. Some authors have considered that this is not a valid generic distinction and have suggested that the two genera be combined under the older name of Stachybotrys. This is supported by the molecular results of Haugland et al. (2001). All valid species of Stachybotrys and Memnoniella are included in the following key. Some species of Memnoniella have not been transferred to Stachybotrys.

### Key to the accepted species of Stachybotrys

1.	Conidia when mature roughened, or surface ridged or banded, or with destriations	elicate
1.	Conidia when mature smooth	37
2.	Conidial surface ridged, banded, striate or rugulose	3
۷.	Conidia rough-walled	-

3.	Conidia cylindrical, with delicate, oblique striations, 11–16 × 4–6 μm
3.	C avlindrom or
4. 4.	
5. 5.	Conidia with a ridged or banded surface, $7-12 \times 4-6 \mu m$
6. 6.	Conidiophores synnematous
7. 7.	Conidia subglobose, 7–12 µm diam, apex black, base pale <i>Memnoniella leprosa</i> Conidia globose, 4–5.5 µm diam
8. 8.	Conidia produced in dry chains, sometimes also in slimy heads
9. 9.	Conidia of two kinds; catenate and globose, 5-5.5 µm diam; non-catenate and cylindrical, 7-10 × 3.5-5 µm
	. Conidia globose to subglobose, 3-5 µm diam; rarely producing cylindrical conidia in slimy heads, 7-9 x 3-5 µm
11.	Conidia globose, 5–7 µm diam
12.	Conidia mainly globose or subglobose
13.	Conidia more than 10 µm diam
14.	Conidia 11–12 µm diam
	Conidiophores regularly sympodially branched; conidia 4.5-8 µm diam
15.	Conidiophores not regularly sympodially branched
16. 16.	Conidia 5–6 µm diam
17.	Conidia 6–8 μm diam; conidiophores (40–)70–90 μm longS. ruwenzoriensis Conidia 7.5–10.5 × 7–10.5 μm; conidiophores 58–272 μm longS. kaniti

18. 18.	Conidia reniform or curved	.19
19. 19.	Conidia tightly reniform, (11–)13–15(–15.5) × (10.5–)12–14 $\mu$ m S. nephroconidia less than 10 $\mu$ m wide	des
20.	Conidiophores distinctly sinuous, branched; conidia 8–12 x 6–7 µm	••••
	Conidiophores not sinuous	ara
21.	Conidia strictly kidney-shaped, 10–13.5 × 6–9.5 µm; phialides 10–14 × 5–6 µ conidiophores smooth, 86–137 µm long	ım;
	Conidia more variable, not strictly kidney-shaped	
22.	Conidia reniform or comma-shaped, $8-12 \times 4-6(-7)$ µm; phialides smooth, $12 \times 5-6$ µm; conidiophores smooth or roughened, up to 400 µm long	••••
22.	Conidia ovoid to reniform, 9–12 $\times$ 4.5–8 $\mu$ m; phialides smooth or verruculo 10–21 $\times$ 4–7 $\mu$ m; conidiophores smooth, up to 190 $\mu$ m long	100
23. 23.	Conidia usually more than 10 µm long	.24 .30
24. 24.	Conidia more than 8 µm wide	25 26
	Conidia ellipsoid or obovoid, 10–15 × 9.5–11(–12.5) µm; conidiophores 80–2 µm long	ra
	75 μm long	4— ?re
26. 26.	Conidia cylindrical or ellipsoid; conidiophores sometimes branched	27 28
27.	Conidia cylindrical, (10–)11–13(–15) × (3.5–)4–4.5(–5.3) $\mu$ m; conidiophores to 320 $\mu$ m long	up
27.	to 320 µm long	ım
28.	Conidia hyaline, ellipsoid or navicular, 10–15 × 5–7.5 µm; conidiophor smooth, 80–230 µm long	res
28.	Conidia dark-coloured.	<i>ue</i> 29
29.	Conidia ellipsoid, 12–13 × 5–5.5 μm; conidiophores warted, up to 100 μm long  S. virga	
29.	Conidia ellipsoid, 10–15 × 6–8µm; conidiophores smooth, up to 180 µm long  S. kampalens	
30. 30.	Conidia more than 5 µm wide	
		~ /

	Conidia ellipsoid or ovoid, 7.5–10(–14) × 5–7 μm; conidiophores up to 270 μm long
31	Conidia ellipsoid, 7–9 × 6 μm; conidiophores 45–50 μm long
32. 32.	Conidia biguttulate, oblong, (5.5–)6–8 × 3–4 µm
	Conidiophores often branched, up to 130 µm long; conidia cylindrical or ellipsoid, 6.5-9.5 × 2-3.5 µm
<i>55</i> .	Conidiophores unbranched or rarely branched
	Conidiophores smooth, up to 200 µm long; conidia ellipsoid, 3-6 × 2.5-3.5 µm; phialides 7-11 × 3-4 µm
34.	Conidiophores less than 100 µm long
	Conidia ovoid or ellipsoid, 5-8 × 2.5-3.5 µm; phialides 5.5-11 × 2.5-3 µm; conidiophores verrucose, 63-95 µm long
35.	Conidia usually wider
36.	Conidia ellipsoid, 7–9 × 3.5–4.5 µm; phialides 9–11 × 4–5 µm; conidiophores smooth, 48–85 µm long
36.	Conidia ellipsoid or cylindrical, 7–9 x 3–6 µm; phialides 7.5–11 x 3 µm; conidiophores smooth or verrucose, 48–94 µm long
37.	Conidia produced in dry chains, subglobose, 4–6 µm diam
37.	Conidia aggregated in slimy heads
38. 38.	Conidia reniform or curved
39. 39.	Conidiophores proliferating or branched; conidia 4.5–7 $\times$ 3–4.5 $\mu m$
	S. renisporoides
40. 40	Conidiophores proliferating through apex; conidia 5.5–7 × 4–5 µmS. proliferata
70.	Conidiophores sympodially proliferating or branching; conidia 5.2-7 × 3.5-5.2 µm
41.	Conidia usually more than 14 µm in length
41.	Conidia usually less than 14 µm in length
42.	Conidia broadly ellipsoid, 16–28 × 12–17 µm, with a basal apiculus
42.	Conidia smaller, without an apical apiculus
43. 43.	Conidia cylindrical-clavate or ellipsoid, $14.5-17.5 \times 6.5-11 \mu m$

44. 44.	Conidia fusiform or limoniform
45.	Conidia limoniform or fusiform, hyaline, pink in mass, $8-14 \times 6-9 \mu m$ or $10-16 \times 3-6 \mu m$
45.	Conidia fusoid, cylindrical or ellipsoid, grey to dark grey, black in mass, 7–10.2 × 2.5–3.5 µm
46. 46.	Conidia mostly more than 6 µm wide
	Conidia subglobose or ellipsoid, dark brown, 7–9 × 6–7 µm; conidiophore sympodially branched
48. 48.	Conidia cylindrical, 8.8–12 × 2–2.4 µm
49. 49.	Conidia distinctly biguttulate, 9–12 × 3.5–5 µm
50.	Conidiophores often sympodially branched; conidia ellipsoid, $8.5-15.5 \times 3.5-5$ $\mu$ m; phialides $8.5-12 \times 5-7$ $\mu$ m; conidiophores up to $68$ $\mu$ m long
50.	Conidiophores not usually sympodially branched
(	Conidia ovoid, $(4-)7-9(-12) \times (3-)5-6(-7)$ µm; phialides $9-16 \times 3-5$ µm; conidiophores usually smooth, hyaline, up to 250 µm long
51.	Melanopsamma pomiformis (anamorph S. albipes) Conidia usually less than 5 μm wide
i	Conidia cylindrical or subcylindrical, occasionally rough-walled, 7–11 × (2.5–) 3.5–5 µm; phialides 8–13 × 3.2–5 µm; conidiophores grey to black, 70–120 µm long
(	Conidia navicular or ellipsoid, $6-9 \times 3-4 \mu m$ ; phialides $8-13 \times 3-4 \mu m$ ; conidiophores subhyaline to pale brown, up to 60 $\mu m$ long

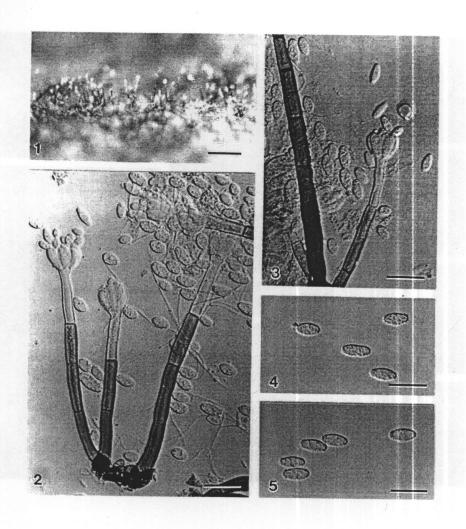
### Acknowledgements

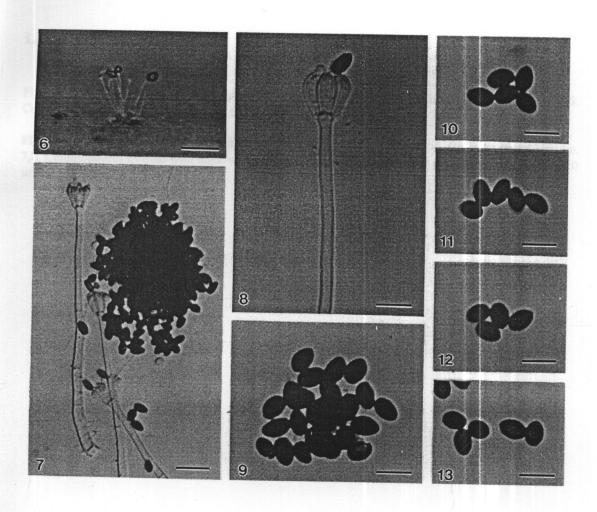
This project is supported by Thailand research grant BRT R\_145008, and New Zealand Foundation for Research and Technology. We are grateful to Graduate

School, Chiang Mai University, Saisamorn Lumyong, Ruud Valyasevi, and Morakot Tanticharoen for continued support, and to Manetr Boonyanant and his staff, for research facilities at the Sirindhorn Field and Nature Study Center, Narathiwat.

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#### **LEGENDS**

Figs. 1–5. Light micrographs of *Stachybotrys palmae* (from holotype). 1. Colonies on substratum. 2, 3. Conidiophores with pale apical cell. 4, 5. Conidia. Bars:  $1 = 200 \mu m$ ;  $2-5 = 20 \mu m$ .

Figs. 6-13. Light micrographs of *Stachybotrys cordylines* (from holotype). 6. Colonies on substratum. 7,8. Conidiophores hyaline with thick-walled. 9-13. Conidia. Bars:  $6 = 150 \mu m$ ;  $7 = 20 \mu m$ ;  $8-13 = 10 \mu m$ .