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ISOLATION AND COLLECTION OF ENDOPHYTIC AND SAPROBIC FUNGI FROM TWO SPECIES OF PALMS IN DOI SUTHEP-PUI NATIONAL PARK

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MASTER OF SCIENCE
IN BIOLOGY

GRADUATE SCHOOL
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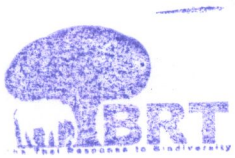

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A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY

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THIS THESIS HAS BEEN APPROVED
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5 October 2001

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Warin Techa

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Thesis Title Isolation and Collection of Endophytic and Saprobic Fungi from Two Species of Palms in Doi Suthep-Pui National Park

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Abstract

Fungal endophytes associated with the palms, *Calamus kerrianus* Becc. (rattan) and *Wallichia caryotoides* Roxb. (taorang) were investigated at two sites within Doi Suthep-Pui National Park. Fungi were isolated from different tissue types (petiole, leaf, lamina and leaf veins) during three periods of the year; rainy season (July - October, 1999), cold season (November 1999 - February 2000) and hot season (March - May 2000). Endophytic fungi isolated included Xylariaceous taxa (20 morphotypes), sterile mycelia (11 morphotypes), eight unidentified hyphomycetes and twelve identified taxa including *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora* like sp., *Fusarium* sp., *Guignardia cocoicola*, *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*- like sp., *Phomopsis* sp., *Phyllosticta* sp., and *Sarcopodium* sp. The endophyte

species, their relative frequency, isolate rate and diversity did not differ significantly between host species, tissue types and study-site except in seasons.

Dead petiole of palms, *C. kerrianus* and *W. caryotoides* were collected and examined for saprobic fungi from the two sites of Doi Suthep-Pui National park in the rainy season (July-October 2000). Thirty-three taxa of saprobic fungi were found; 28 Ascomycetes, four Hyphomycetes and one Coelomycete. The similarlity index between the two sites was 0.6 for *C. kerrianus* and 0.28 for *W. caryotoides*. Eight fungi were found exclusively on *C. kerrianus*; *Astrosphaeriella fissurisstoma*, *Chaetosphaerulina* sp., *Didymella* sp., *Lophiostoma* sp. 2, *Morenoina* sp., *Orbilia* sp., *Ornatispora* sp., and *Stylbohypoxylon rehmii* and 15 fungi only on *W. caryotoides*; *Byssosphaeria schneidermayiana*, *Canalisporium sp.*, *Diaporthe sp.*, *Dictyosporium sp.*, *Iodosphaeria hongkongensis*, *Kostermansinda minima*, *Lepteutypa* sp., *Lophiostoma* sp. 1, *Massarina* sp., 1, *Massarina* sp. 2, *Ophioceras* sp. 2, *Stachylidium bicolor*, *Stictis* sp., *Tubeufia* sp. and unidentified coelomycete. Fungi found on both palms were *Anthostomella ludoviciana*, *Bionectria* sp., *Capsulospora* sp., *Chaetosphaeria* sp., *Massarina* sp. 3, *Nectria* sp., *Nectriopsis* sp., *Ophioceras* sp. 1, *Oxydothis* sp. 1 and *Oxydothis* sp. 2.

ชื่อเรื่องวิทยานิพนธ์	การแยกและเก็บรวบรวมเชื้อราเอนโดไฟต์และแซพโปรไฟต์ของปาล์มสองชนิดในอุทยานแห่งชาติดอยสุเทพ-ปุย
ชื่อผู้เขียน	นางสาววารินทร์ เตจ๊ะ
วิทยาศาสตรมหาบัณฑิต	สาขาวิชาวิชาชีววิทยา
คณะกรรมการสอบวิทยานิพนธ์	รศ. ดร. สายสมร ลำยอง ประธานกรรมการ อ. ดร. อุราภรณ์ สอาดสุด กรรมการ อ. พิภพ ลำยอง กรรมการ

บทคัดย่อ

ได้สำรวจเชื้อราเอนโดไฟต์ที่เกี่ยวข้องกับปาล์มสองชนิด *Calamus kerriaus* Becc. (หวาย) และ *Wallichia caryotoides* Roxb. (เต่าร้าง) จากสถานที่สองแห่ง ซึ่งตั้งอยู่ในอุทยานแห่งชาติดอยสุเทพ-ปุย เชื้อราเอนโดไฟต์ได้ถูกแยกออกมาจากส่วนต่างๆ ของพืช (ส่วนของก้านใบ ส่วนของใบ ส่วนที่อยู่ระหว่างเส้นใบ และส่วนของเส้นใบ) ในช่วงฤดูกาลต่างๆ เป็นระยะเวลา 1 ปี คือฤดูฝน (มิถุนายน - ตุลาคม พ.ศ. 2542) ฤดูหนาว (พฤษภาคม พ.ศ. 2542 - ตุลาคม พ.ศ. 2543) และฤดูร้อน (มีนาคม - พฤษภาคม พ.ศ. 2543) เชื้อราเอนโดไฟต์ที่แยกได้มีดังต่อไปนี้ เชื้อราในกลุ่ม Xylaria (จำแนกตามความแตกต่างของโคโลนี 20 กลุ่ม) เชื้อราเอนโดไฟต์ที่ไม่สร้างโครงสร้างสืบพันธุ์ 11 ชนิด เชื้อรา Hyphomycetes ที่ไม่สามารถบ่งชนิดได้ 8 ชนิด และเชื้อราเอนโดไฟต์อื่นๆ ได้แก่ *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp., *Fusarium* sp., *Guignardia cocoicola*, *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phomopsis* sp., *Phyllosticta* sp., และ *Sarcopodium* sp. ชนิดของเชื้อราเอนโดไฟต์ ค่าความถี่สัมพัทธ์ ค่า isolation rate และ ความหลากหลายของชนิดของเชื้อรา แตกต่างกันอย่างไม่มีนัยสำคัญทางสถิติระหว่างชนิดของพืชอาศัย ชนิดเนื้อเยื่อของพืชอาศัย และสถานที่เก็บตัวอย่าง ยกเว้นฤดูกาลเท่านั้นที่มีผลทำให้ค่าดังกล่าวเบื้องต้นมีความแตกต่างกันอย่างมีนัยสำคัยทางสถิติ

ก้านใบที่ตายแล้วของ *C. kerrianus* และ *W. caryotoides* ได้ถูกเก็บรวบรวมจากสถานที่สองแห่งในอุทยานแห่งชาติดอยสุเทพ-ปุย ในฤดูฝน (กรกฎาคม-ตุลาคม 2543) เพื่อสำรวจเชื้อราแซพโปรไฟต์ พบเชื้อรา 33 ชนิด จัดอยู่ใน Class Ascomycetes 28 ชนิด Hyphomycetes 8 ชนิด และ Coelomycetes 1 ชนิด ดัชนีบ่งความเหมือนกันของชนิดของเชื้อราระหว่างสถานที่สองแห่งคือ 0.6 ใน *C. kerrianus* และ 0.28 ใน *W. caryotoides* มีเชื้อรา 8 ชนิดที่พบเฉพาะบน *C. kerrianus* ได้แก่ *Astrosphaeriella fissurisstoma*, *Chaetosphaerulina* sp., *Didymella* sp., *Lophiostoma* sp. 2, *Morenoina* sp., *Orbilia* sp., *Ornatispora* sp., และ *Stylbohypoxylon rehmii* และ 15 ชนิดพบเฉพาะบน *W. caryotoides* ได้แก่ *Byssosphaeria schneidermayiana*, *Canalisporium sp.*, *Diaporthe sp.*, *Dictyosporium sp.*, *Iodosphaeria hongkongensis*, *Kostermansinda minima*, *Lepteutypa* sp., *Lophiostoma* sp. 1, *Massarina* sp., 1, *Massarina* sp. 2, *Ophioceras* sp. 2, *Stachylidium bicolor*, *Stictis* sp., *Tubeufia* sp. และ unidentified coelomycete. นอกจากนี้ยังมีเชื้อราแซพโปรไฟต์อีก 10 ชนิดซึ่งพบในปาล์มทั้งสองชนิด ได้แก่ *Anthostomella ludoviciana*, *Bionectria* sp., *Capsulospora* sp., *Chaetosphaeria* sp., *Massarina* sp. 3, *Nectria* sp., *Nectriopsis* sp., *Ophioceras* sp. 1, *Oxydothis* sp. 1, และ *Oxydothis* sp. 2.

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Symbols and Abbreviations

°C	=	Degree Celsius
cm	=	Centimeter
CR	=	Colonization rate
IR	=	Isolation rate
IV	=	Inter vein
m	=	Meter
ml	=	Milliliter
MEA	=	Malt extract agar
NaOCl	=	Sodium hypochlorite
nUV	=	Near Ultraviolet
P	=	Petiole
%	=	Percent
RF	=	Relative frequency
V	=	Vein
μm	=	Micronmeter

Chapter 1

Introduction

1.1 Importance of study fungi

Fungi are microorganisms which play an important role in the environment. They have the ability to decompose dead plant tissues because their hyphae are well adapted to growing through bulky fibrous materials, such as wood, and as they produce extracellular enzymes (Hudson, 1986). Fungi produce enzymes that can degrade many kinds of organic and some of inorganic substrates (Edward, 1988). They are also have mutualistic, symbiotic and antagonistic relationships with animals and plants and may be antagonist to other fungi (Cooke,1977).

The knowledge and interest in microfungi in tropical regions is increasing, but tropical microfungi still represent an unexplored universe of biodiversity (Rossman, 1997). There are a large numbers of new fungal species that remain to be discovered in this region and with the massive habitat lose, that is presently occurring, many will be lost. This will include many species able to produce important and novel chemical molecules (Wildman, 1997). Novel fungi are therefore potentially important organisms awaiting discovery. The study of tropical fungi is therefore urgently needed because many countries in the tropics are developing and it is probable that habitats might be disturbed or destroyed and fungi lost. In this study, the isolation and collection of endophytic and saprobic fungi from two species of palms in Doi Suthep-Pui National

Park will be an advancement towards collecting and investigating the fungal diversity in Thailand.

1.2 Biodiversity of fungi

At present vascular plant species are being described more quickly than fungi, but it is expected that fungi may eventually prove to be one of the most species rich of all groups (Hammond, 1992). Approximately 72,000 species of fungi have been described and Hawksworth (1991) estimated that there are as many as 1.5 million fungal species in this world. This estimate was based on a comparison between the number of fungi known in all habitats in a single geographical region and the number of native and naturalized plant species in the same area. The results from a ratio of six fungi per plant species was extrapolated using a conservative estimate of 270,000 global vascular plants with certain allowances, yielded a global total of 1.5 million species of fungi. This figure is now generally accepted (Hammond, 1992; Hawksworth and Hyde, 1997 Rossman, 1994).

1.3 Palms

Palms belong to the family Arecaceae (Palmae). There are some similar plants such as Cyclanthaceae and Pandanaceae often included with them by inexperienced botanists (Jones, 1994). They are widely distributed in this world where there is an adequate water supply, with 2,800 species in 200 genera (Krempin, 1993). They are very common in the tropics, but are absent or rare in temperate regions (Jones, 1994). Fungi associated with palms also probably require moisture and warm conditions to develop.

Hyde *et al.* (1997) categorized palms into three groups by using substrate types. There are small palms which develop small amounts of woody tissue, it is difficult to find associated fungi. The larger palms produce larger quantities of woody tissue and there are numerously associated fungi. Petioles and rachides tissue have abundant microfungi but few number of them are reported and record from trunks.

1.4 Palms of Thailand

The climate of Thailand affects the distribution of palms, especialy in the south Peninsula. Species richness is greatest from about 250-1000 meters elevation. The area of moist to wet, evergreen mountain forest in the west and north near Myanmar and mountain tops scattered in the east, contain fewer species than the southern Peninsula which has the highest rainfall throughout the year. The most common and widespread palms in Thailand are *Areca catechu, Borassus flabellifer*, *Cocos nucifera* and *Salacca wallichina*. *Caryota mitis* and *Licuala spinosa* are also rather common. *Calamus* and *Wallichia* species are numerous in the evergreen mountain forests (Hodel and Vatcharakorn, 1998).

Calamus is a large genus of palm with 375 species. They are widely distributed in tropical regions including Africa, India, South East Asia, Malasia, Indonisia, Fiji, New Guinea and Australia. Most species are climbing palms with narrow stems; others are shrubby with erect stems; some are stemless or have solitary trunks. These palm are known as rattans, their stems are used for furniture construction (Jones, 1994). *Calamus kerrianus* Becc. (rattan) is a high climbing palm (Fig. 1 and 2). The climbing stems is

about 2 cm diameter. Leaf sheaths are green with deciduous grayish indument. The petiole as long as 15 cm with spines (Hodel and Vatcharakorn, 1998). Several *Calamus* species have been studied for associated fungi (Table 1).

Table1. Some fungi associated with *Calamus* species in tropical regions. (Fröhlich and Hyde, 1996; Hyde, 1996; Hyde and Fröhlich, 1996).

Fungi	Palm	Country
Anthostomella calamicola K.D. Hyde.	*Calamus* sp.	Australia
A. francisiae K.D. Hyde.	*Calamus* sp.	Indonesia
A. minutoides K.D. Hyde.	*Calamus* sp.	Indonesia
A. pandani K.D. Hyde.	*Calamus* sp.	Australia
Arecomyces frondicola K.D. Hyde.	*Calamus* sp.	Brunei
Rachidicola palmae K. D. Hyde & J. Fröhlich	*Calamus* sp.	Hong Kong

The *Wallichia* genus occurs in the himalayan region of northern India to adjacent areas in Burma, southern China and Thailand. They can be found in forests at near sea level to mountains regions. *Wallichia caryotoides* Roxb. (Taorang) (Fig 3) is approximately 3 meters high. Leaflets are irregular in shape with bright green above and silvery beneath (Jones, 1994). There are no previous research of the fungi associate with this palm. *Calamus kerrianus* and *Wallichia caryotoides* were very common at two study sites, Huay Kog Ma and Medicinal Plant Garden in Doi Suthep-Pui National park.

1.5 Palm fungi

Petioles, rachides and leaves of palms support a greater diversity of fungi, with fewer recorded from influorescences and trunks. In 1994, there were 1,580 fungi from palms had been described as new species from palms including 650 ascomycetes, 270 basidiomycetes, 400 hyphomycetes and 260 coelomycetes. Seventy-five percent of fungi collected from palms have been reported as new to science in recent years. 26:1 was the ratio of fungi to palms which investigated in north Queensland, while the ratio of fungi to other plants given by Hawksworth (1991) was 6:1. Ascomycetes are very common on palms. The woody tissue of palms, warmth and humid climate in tropics are though to be a properties that support the diversity of fungi species. (Hyde *et al.* 1997).

For the study of palm endophytes in 1996, Rodrigues isolated fungal endophytes from three different ages of *Euterpe oleracae* which grow in Amazonian of Brazil. The mature and newly expanded leaves showed the greatest frequency of colonization and the older foliage host had alarge number of endophytes. Taylor (1999) investigated fungal endophytes of *Trachycarpus fortunei* in temperate region; Australia, China and Switzerland. There were 1,985 isolates recovered from a total 3,256 leaf discs. The colonization rate in each country was significant difference and in all cases, there were high colonization rate in palm tissues from China than from Australia or Switzerland.

Fig. 1 *Calamus kerrianus* Becc.

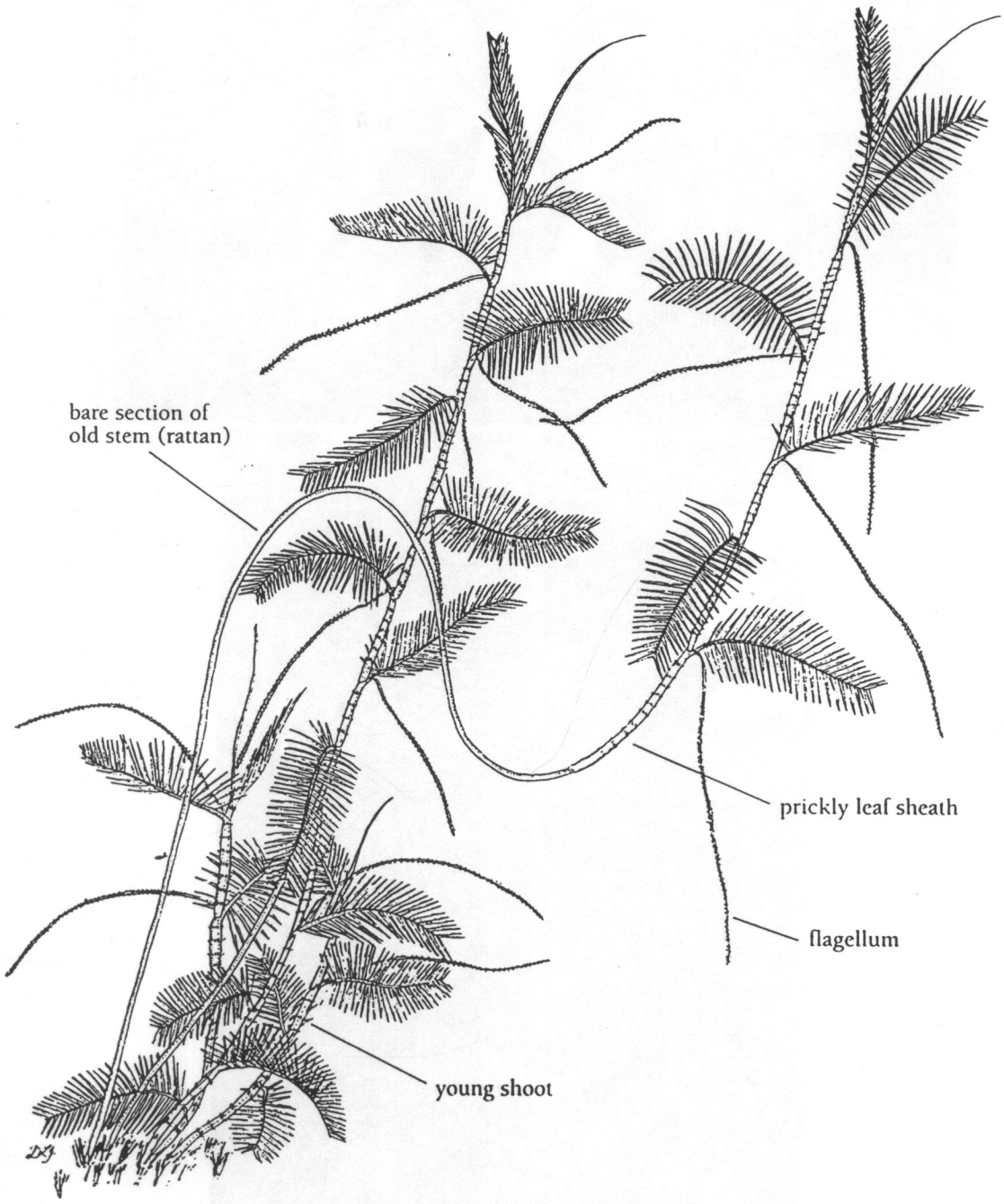


Fig. 2 *Calamus* sp.

Habit of a climbing palm

Fig. 3 *Wallichia caryotoides* Roxb.

Chapter 2

Endophytes of Palms

2.1 What are endophytes

Endophytes are organisms that live within a plant tissues. It considered to be non aggressive organisms and mutualism within their hosts by some researchers. Now the definition of the term 'endophyte' includes all organisms that live symtomlessly within plant tissues at sometime of their life cycle (Petrini, 1998). They can be pathogen or symbionts, but are asymtomatic and may also be described as mutualistic with both partners benefiting from the association (Clay, 1991; Ingold, 1993; Hudson,1993). Exclusively fungi are though to be endophyte, but a large number of bacteria are known to live endophytically (Petrini, 1998). Fungal endophyte works were most investigated in the last decades, since the demonstration of symptomless ascomycetes and dueteromycetes of European conifer needles were present. Although the symptomlessly endophytes other than grass have been known for more than 70 years. Fungal endophytes provide the protection to their host. For example, fungi in redwood may function as antagonists or stimulators to pathogens (Espinosa-García *et al.*, 1996). *Acremonium* species provide the increasing drought tolerance and chemicals to their host; tall fescue (*Festuca arumdinaceaew* Schreb.) and perennial ryegrass (*Lolium perenne* L.). The chemicals are ergot alkaloids, tremogenic neurotoxins and paramine (Bacon and Hill,

1996). However, Colonization or infection by endophytic organisms cannot be considered as causing disease, because a plant disease is an interaction between the host, parasite, vector and environment and symptoms are a result from the interaction (Rossman, 1997).

2.2 Studies on endophytes of various plants

Most fungal studies have been carried out in temperate regions (Rodrigues, 1996; Rodrigues and Petrini, 1997). The study of endophytic fungi, especially the study of their biodiversity has mostly been carried out in temperate regions. Johnston (1998) isolated endophytes from manuka leaves (*Leptospermum scoparium*) that grow in New Zealand. *Alternaria* sp. *Botryosphaeria* spp., *Chaetomella* sp., *Cladosporium* sp., 2 coelomycetes, *Diploceras leptospermi, Epicoccum nigrum, Glomerella* spp., *Nigrospora* sp., *Pestalotiopsis* sp., *Phomopsis* sp., *Pleospora* sp., *Phyllosticta* sp., mycelia sterilia and other fungi include *Xylaria* were discovered from this study. Bayman *et al.* (1998) studied distribution and dispersal of *Xylaria* endophytes in the Australian pine (*Casuarina equisetifolia*) and ausubo (*Manilkara bidentata*) in Peurto Rico. This study found that the distribution of *Xylaria* in *Casuarina* and *Manilkara* leaves is greater than in seeds except for *Casuarina equisetifolia* which sampled from beaches. The *Xylaria* species in *Manilkara bidentata* leaves from the forest sampling were highest as compare to highest the leaf samples from forest and greenhouse seedlings. Sieber *et al.* (1999) isolated endophytic fungi from healthy-looking needles of *Pinus mugo* that grow in Switzerland and Germany. *Cenangium ferruginosum, Cyclaneusma minus, Geniculosporum* sp., *Lophodermium pinastri* and mycelia sterilia were recovered from

this study. Shamoun and Sieber (2000) isolated fungal endophytes from leaves and twigs of *Rubus spectabilis* and *R. parviflorus* collected from Vancouver Island, British Columbia and Canada for the long term goal as biocontrol. In this study, *Acremonium* sp., *Anthostomella* sp., *Aposphaeria* sp., *Coniochaeta velutina, Didymella applanata, Gelasinospora* sp., *Geniculosporium* sp., *Gnomoniella rubicola, Leptosphaeria coniothyrium, Melanconis* sp., *Mollisia* sp., *Clavata sp., Monochaetia* sp., *Nodulisporium* sp., *Ophiobolus* sp. and *Phoma* spp. were isolated

2.3 Studies on tropical endophytes

Although the report of endophytic fungi studied in tropics were less in the recent years (Rodriguess, 1996; Hyde *et al.*, 1997) but it is increasing now. Because the knowledge and research of fungi are developing and plant species in these regions have a greater diversity than in the temperate (Ashton, 1990). Palms are also widely distributed in tropics (Jones, 1994) their endophytes have been studied by mycologists but were not much as the study of their saprobes. These are some of palms endophyte works in tropical regions; Rodrigues, 1994 studied fungal endophytes living in foliage of Euterpe oleraceae (Amazonian palm) that grows in the Brazillion Amazon estuary and in 1996 studied fungal endophgytes in *E. oleraceae* leaves that grow in amazonian floodplains. Fröhlich (1997) studied endophytic fungi in *Licuala* sp. from Brunei Darussalam and *L. ramsayi* from Queensland, Australia. Some endophyte works in other plants exclusive palms carried out in these regions; Mekkamol (1998) studied endophytic fungi in *Tectona grandis* (Teak) in Chiang Mai, Thongkanta (1999) isolated fungal endophytes from 13 native of Thai bamboo species which grew in Chiang Mai and studied their capable of

polysaccharide and hydrolyzing enzyme production. Permpoolsombat (2000) isolated endophytic fungi from herbal plants in Chiang Mai and selected some isolates which capable to produce L-Asparagine. Photita (2001) studied endophytic fungi in wild banana (*Musa acuminata*) in Chiang Mai.

2.4 Objectives of the study

Endophytic fungi were isolated from *Calamus kerrianus* and *Wallichia caryotoides* to determine the biodiversity of fungi within the 2 species of palms and to compare the difference in species of fungal communities between two sites at Doi Suthep-Pui National Park over three seasons; rainy season, cold season and hot season.

2.5 Materials and methods

2.5.1 Palms and collecting sites

Two species of palms; *C. kerrianus* (rattan) and *W. caryotoides* (taorang) were examined from Huay Kog Maa and Medicinal Plant Garden of Doi Suthep-Pui National Park, Chiang Mai. Both palms are widely distributed in the two collection sites.

Site 1, Huay Kog Ma is about 1000 m above sea level. It is a evergreen hill forest located on the Doi Suthep to Doi Pui Road along a trail from main road to the middle of the forest. The canopy is dense and the palms grow within other plants species.

Site 2, Medicinal Plant Garden is an evergreen forest with a stream passing through it, dividing the forest into two parts. It is about 800 m above sea level, located on the Doi Suthep-Pui Natioal Park (Gardner *et al.*, 2000).

2.5.2 Sampling procedure

Petioles and leaves of the two species of palm were sampled from healthy looking plants for three seasons, starting in the rainy season (July - October, 1999), then cold season (November 1999 - February 2000) and at the end of the hot season (March - May 2000). Petioles were cut and kept in separately plastic bag. Leaflets were cut from rachides and also kept in separate plastic bag. Ten plants from each species of palms were sampled at each site; including five petioles and five leaves. Twenty plants of *C. kerrianus* and 20 plants of *W. caryotoides* were sampled in each season. The samples were returned to the laboratory and immediately washed in running water. Petioles from one plant were treated as one sample, and were cut into 10 mm long pieces. Leaflets of *C. kerrianus* and *W. caryotoides* from one plant were cut into 5 mm diameter leaf discs including the leaf discs of intervein and vein for each sample. The fragments of petiole, intervein and vein were triple sterilized at their surface.

2.5.3 Surface sterilization of petioles and leaves

Surface sterilization techniques were followed Fröhlich (1997). This method was shown to be useful for palms by Rodrigues and Samuels (1990) in a study on the fungal endophyte of *Licuala ramsayi.* In this study, the concentration of sodiumhypocholrite (NaOCl) and time were adjusted following pilot experiment.

Each leaf disc was surface sterilized by dipping in 95% ethanol for 60 seconds, then in a solution of 3.0% NaOCl for 8 minutes and finally in different bath of 95% ethanol for 30 seconds.

In the case of petioles, each of them was dipped in 95% ethanol for 60 seconds, then in a solution of 3.0% NaOCl for 15 minutes and follow by dipping in 95% of ethanol for 30 minutes.

Stemliked petioles and leaf tissues dried on a sterilized tissue paper and the tissue fragments were place on Petri dishes (9 cm of diameter) of half strength of MEA containing 0.003% of rose bengal and 0.003% streptomycin sulphate. The Rose Bengal was used to decrease the growth of faster developing fungi to prevent their growth over other slower growing fungi (Fröhlich, 1997).

2.5.4 Culturing and subculturing

Petri dishes containing leaf discs and petioles fragments were incubated at 30°C. A few day after incubation, when the fungal hyphae grew out from plant tissues, the growing edges of the hyphae were subcultured and put on Petri dishes with MEA and incubated under nUV light to promote development of growth and sporulation.

Fungi which grew under nUV light were subcultured to MEA microtubes as stock cultures and incubated at room temperature.

2.5.5 Identification

The fungi which were cultured in the Petri dishes under nUV light were identified as they sporulated. If after 4 weeks, the culture did not sporulate they were considered to be mycelia sterilia.

2.5.6 Statistical analysis

The colonization and isolation rate were calculated by using these formula (Taylor, 1999)

$$\text{Colonization rate} = \frac{\text{total number of samples yielding} \geq 1 \text{ isolate}}{\text{total number of samples in that trial}} \times 100$$

$$\text{Isolation rate} = \frac{\text{total number of isolates yielding in a given trial}}{\text{total number of samples in that trial}}$$

Isolate prevalence were expressed as percentage (Petrini *et al.*, 1982) and commonly used in the literature. But isolation rates cannot be expressed as percentages, they were used to demonstrate the degree of multiple colonization from the samples in different trials. A chi-square goodness-of-fit test was performed to test whether the colonization rate of twelve trials and isolation rate of thirty-six trials were statistically different. Non parametric statistical tests were used because the data in most cases did not fit the assumptions for parametric statistics. A Kruskal-Wallis procedure was used for multisample analysis, such as the investigation of the number of isolates recovered from petiole, vein and intervein tissues for all plant samples at each site, and for the number of isolates recovered from different tissue types at each site in rainy, cold and hot season (Petrini *et al.*, 1982). In all analyses, P values are described as $P = 0.05$

2.6 Results and discussion

2.6.1 Overall colonization and age effect

A total of 1800 samples from two species of palm, *C. kerrianus* and *W. caryotoides* from Huay Kog Ma and Medicinal Plant Garden, Doi Suthep Pui National Park were collected and processed in rainy, cold and hot season. There were 2619 isolates recovered. The overall colonization rate (%) were not significant difference in each trial (Table 2). These results can be compared with other studied of palm endophytes, which the colonization rate had been reported. The colonization rate of *Licuala ramsayi* and *Licuala* sp. from Australia and Brunei were high as 80.8%-89.2% (Fröhlich, 1997), whereas those reported by Rodrigues (1994) from *Euterpe oleracea* were much lower (21%-30%) and as little as 12.5% in another study (Rodrigues and Samuels, 1990). The colonization rate of *Trachycarpus fortunei* reported by Taylor (1999) were 23.4%-57.3%. In general, the colonization rates were varied between 20%-94% in rainy season, 10%-98% in cold season and 0%-52% in hot season.

Colonization rates in rainy and cold season of *C. kerrianus* and *W. caryotoides* were comparatively high in both sites compare to the colonization rate of *Amomum siamense* which isolated in wet (August 1999) and dry (February 2000) season from the same two sites of Doi Suthep-Pui National Park (Bussaban, 2001). The climate conditions, including humidity and rain fall were high in this study. These condition may influence to colonization rate of fungal endophytes.Taylor *et al.* (1999) investigated that the colonization rates of endophytic fungi in *T. fortunei* declined with decreasing relative humidity and rainfall. Carroll and Carroll (1978) demonstrated that Douglas fir was more

Table 2. The colonization rate (%) of endophytes from 50 samples of petiole segments vein and intervein of *C. kerrianus* and *W. caryotoides*

	Colonization rate		
	Rainy season	Cold season	Hot season
Calamus kerrianus			
Huay Kog Ma Site			
Petioles	82	84	44
Vein	54	82	24
Intervein	66	84	14
Medicinal Plant Garden Site			
Petioles	74	10	34
Vein	94	72	0
Intervein	58	70	4
Wallichia caryotoides			
Huay Kog Ma Site			
Petioles	76	80	52
Vein	88	94	22
Intervein	76	88	4
Medicinal Plant Garden Site			
Petioles	64	92	48
Vein	64	98	42
Intervein	20	80	18

heavily infected in the moist sites than in dry sites and suggested that difference in elevation , humidity, density of canopy cover and innate host susceptibility were likely to cause the observed differences in endophyte infection between sites.

In the hot season the colonization rate of *C. kerrianus* and *W. caryotoides* were low. This result agrees with the study of endophytic fungi in *T. fortunei* by Taylor (1999) and Carroll and Carroll (1978) who study endophytic fungi in Douglas fir. In this season

most of palm leaves dried and were infected by pathogen but the newly expanded leaves were clean and looked healthy and most of samples which collected in hot season were young petioles and leaves. The colonization rate in this season were varied between 0%-52% that lower than the colonization rate in rainy and cold season which most samples were mature. These may be due to the difference of climatic conditions among seasons and leave age of palms. But the result agrees with Rodrigues (1994) who studied endophytic fungi of *E. olerceae* leaves in three age classes (unopened, newly expanded and mature) and found significant variation between all three classes in mature tree, but more variable results in samplings. In a study of *L. ramsayi* (Rodrigues and Samuels, 1990), it was suggested that the few endophytes taxa present in the tightly rolled spear leaf are systemic and transmitted through the seed. The majority found in the expanded frond, incontrast, probably originate frond air-borne propagules. Fröhlich (1997) suggested that most endophytes are believed to enter a plant when a spore lands on a leaf surface and grows into the plant through the stoma or penetrates the host directly. The increase incidence endophyte taxa on the older leaves would have had more time to accumulate endophytes from environment. Older leaves would also have more time to accumulate vertically transmitted colonizers that enter from the petiole.

2.6.2 Effect of tissue type

The result of the Kruskal-Wallis test indicate that there were no significant difference between the number of isolates recovered from petiole, intervein and vein of both two species of palms, *C. kerrianus* and *W. caryotoides* collected from the two sites of Doi Suthep Pui Nional Park (Table 3). However, previous studies had indicated that

endophyte may exhibit tissue specificity (Luginbuhl and Müller 1980, Rodrigues and Samuels 1990, Clay 1992, Rodrigues 1994, Fröhlich *et. al.* 2000). The factors that may contribute to change in the endophytic community with leaf age, include weathering of the leaf cuticle, the presence of wounds, increased exposure to propagules with time, and changes in leaf physiology and chemistry (Petrini and Carroll, 1981; Stone, 1987; Espinosa-García and Longenheim, 1990). For this study, petiole was a tissue that had high frequency of many fungi, but the frequency of some fungi in vein or intervein were higher than in petiole. The frequency of fungi isolated from petiole tissues of *C. kerrianus* ; Mycelia sterilia, *Phomopsis* sp., *Xylaria* sp. 2, *Xylaria* sp. 3, *Xylaria* sp. 4 and *Xylaria* sp. 9 were higher than those isolated from vein and intervein tissues. But the frequency of *Collectotrichum gloeosporioides* in tissue of intervein was higher than in tissues of petiole and vein. In *W. caryotoides*, fungi in petiole tissues; *Colletotrichum gloeosporiordes*, *Phomopsis* sp. and *Xylaria* sp. 3 had higher frequency than in vein and intervein tissues. Mycelia sterilia, *Xylaria* sp. 2, *Xylaria* sp. 4 and *Xylaria* sp. 9 in tissues of vein had higher frequency than tissues of petiole and intervein (Table 4). Some fungi were found exclusively in petiole or vein or intervein, this agree with the study of Photita (2001) who found that some endophytic fungi taxa have an affinity for different tissue types. Previous studies have indicate that endophytes may exhibit tissue specificity (Luginbuhl & Müller, 1980; Rodrigues Samuel, 1990; Clay, 1992; Rodrigues 1994, Frö hlich *et al.*, 2000) Some of endophytic fungi which infected these two palms species may be transmitted through the seed or may be systemic fungi, especially those fungi which

found in every tissue type with high frequency. Rodrigues and Samuel (1990) suggested that endophytic fungi could be a systemic fungi and transmitted through the seed.

Table 3. The relative frequency (%) of some fungal endophytes from different tissue of *C. kerrianus* and *W. caryotoides*

Taxa	*C. kerrianus*			*W. caryotoides*		
	Petiole	Vein	Intervein	Petiole	Vein	Intervein
Colletotrichum gloeosporioides	0.44	1.27	1.56	1.17	1.07	0.49
Mycelia sterilia	3.85	3.61	2.54	1.85	4.00	3.02
Phomopsis sp.	1.80	0.98	0.15	6.54	1.37	0.68
Xylaria sp. 2	6.68	5.12	3.17	4.24	7.12	4.59
Xylaria sp. 3	4.49	2.93	1.61	3.32	3.12	2.24
Xylaria sp. 4	2.05	0.89	0.78	1.56	2.05	0.88
Xylaria sp. 9	2.05	0.30	0.78	0.49	2.83	1.27

2.6.3 Site effect

Huay Kog Ma and Medicinal Plant Garden have similar of tree species but they are difference in canopy cover and elevation that have mentioned in materials and methods. There were twenty taxa of endophytic fungi recovered from Huay Kog Ma including Xylariaceous taxa (18 morphospecies), mycelia sterilial taxa, five unidentified Hyphomycetes and other twelve fungal endophytes taxa; *Cladosporium* sp., *Colletotrichum gloeosporiodes*, *Corynespora*-like sp., *Fusarium* sp., *Guignardia cocoicola*, *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phomopsis* sp., *Phyllosticta* sp. and *Sarcopodium* sp. (Table 4). At Medicinal Plant Garden, fourteen taxa of endophytic fungi recovered including Xylariaceous taxa (17 morphospecies), mycelia sterilial taxa, three unidentified Hyphomycetes, *Colletotrichum gloeosporioides,*

Corynespora-like sp., *Fusarium* sp., *Guignardia cocoicola*, *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phomopsis* sp., *Phyllosticta* sp. and *Sarcopodium* sp. (Table 5).

Table 4. Relative frequency (%) of endophytic fungi in petiole (P), vein (V) and intervein (IV) of *C. kerrianus* and *W. caryotoides* at Huay Kog Ma.

Taxa	*Calamus kerrianus*									*Wallichia caryotoides*								
	Rain			Cold			Hot			Rain			Cold			Hot		
	P	V	IV	P	V	IV	P	V	IV	P	V	IV	P	V	IV	P	V	IV
1. *Cladosporium* sp.	0.1	0.1																
2. *Colletotrichum gloeosporioides*	0.1	0.58	0.68	0.3	0.68	0.9				0.9	0.7	0.1		0.4	0.4	0.2		
3. *Corynespora*-like sp.	0.3	0.1	0.4					0.4										
4. *Fusarium* sp.	0.4			0.1			0.1			0.2			0.2	0.1				
5. *Guignardia cocoicola*	0.5	0.5	0.6		0.2	0.3				0.2								
6. Mycelia sterilia	0.11	0.04								0.1				0.04		0.04		
7. *Paecilomyces* sp.																		
8. *Pestalotiopsis* sp.	0.4				0.1	0.2						0.1			0.1			
9. *Phaialophora* sp.	0.1																	
10. *Phoma* sp.	0.2								0.2	0.6						0.1		
11. *Phoma*-like sp.	0.2															0.1		
12. *Phomopsis* sp.	0.9	0.3		0.4	0.2	0.05	0.4	0.3		2.15			4.09	0.2		0.3		
13. *Phyllosticta* sp.								0.4	0.3									
14. *Sarcopodium* sp.										0.2								
15. Unidentified hyphomycetets	0.05	0.05	0.05							0.05	0.05							
16. *Xylaria* sp. 1			0.3								0.2	0.1	0.4					
17. *Xylaria* sp. 2	0.8	0.5	0.24	0.68	1.7	0.58	0.44			0.8	0.34		0.3		0.44	0.34		
18. *Xylaria* sp. 3	0.1	0.3	0.2	1.4	0.8					0.3			0.3	1.5	01	0.1		
19. *Xylaria sp.* 4	1.2	0.3	0.4	0.2	0.2		0.2			0.4	0.1	0.1	0.1	0.1				
20. *Xylaria sp.* 5	0.3		0.1															
21. *Xylaria sp.* 6		0.1	0.1				0.1			0.1								
22. *Xylaria sp.* 7										0.1								
23. *Xylaria sp.* 8										0.1			0.4	0.2				
24. *Xylaria* sp. 9	1.0	0.2	0.6			0.1	0.3				0.3	0.58	0.1					
25. *Xylaria* sp. 10			0.1		0.3						0.2	0.1	0.1		0.1			
26. *Xylaria* sp. 11			0.2		1.2	0.58							0.1					
27. *Xylaria* sp. 12																		
28. *Xylaria* sp. 13			0.1	0.6	0.2	0.5	0.1			0.2	0.2	0.1						
29. *Xylaria* sp. 14			0.1															
30. *Xylaria* sp. 15										0.21						0.1		
31. *Xylaria* sp. 16			0.1			0.1												
32. *Xylaria* sp. 17		0.1	0.1												0.2			
33. *Xylaria* sp. 18											0.2	0.1						
34. *Xylaria* sp. 19																		
35. *Xylaria* sp. 20											0.1	0.1						

Taxa occuring < 0.4% in each season :

Calamus kerrianus

Rainy season: *Cladosporium* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phyllosticta* sp., *Sarcopodium* sp., Unidentified hyphomycetes, *Xylaria* sp. 1, *Xylaria* sp.6, *Xylaria* sp. 7, *Xylaria* sp. 8, *Xylaria* sp. 10, *Xylaria* sp. 11,

Xylaria sp. 12, *Xylaria* sp. 13, *Xylaria* sp. 14, *Xylaria* sp. 15, *Xylaria* sp. 16, *Xylaria* sp. 17, *Xylaria* sp. 18, *Xylaria* sp. 19 and *Xylaria* sp. 20.

Cold season : *Cladosporium spp.*, *Corynespora*-like sp., *Fusarium* sp., *Guignardia cocoicola* , *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma* like sp., *Phyllosticta* sp., *Sarcopodium* sp., Unidentified Hyphomycetes, *Xylaria* sp. 5, *Xylaria* sp. 6, *Xylaria* sp. 7, *Xylaria* sp. 8, *Xylaria* sp. 10, *Xylaria* sp. 12, *Xylaria* sp. 14, *Xylaria* sp. 15, *Xylaria* sp. 16, *Xylaria* sp. 17, *Xylaria* sp. 18, *Xylaria* sp. 19 *and Xylaria* sp. 20.

Hot season : *Cladosporium* sp., *Colletotrichum gloeosporiodes*., *Fusarium* sp., *Guignardia cocoicola*., *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phyllosticta* sp., *Sarcopodium* sp., Unidentified Hyphomycetes, *Xylaria* sp. 1, *Xylaria* sp. 3, *Xylaria* sp. 4, *Xylaria* sp. 5, *Xylaria* sp. 6, *Xylaria* sp. 7, *Xylaria* sp. 8, *Xylaria* sp. 10, *Xylaria* sp. 12, *Xylaria* sp. 14, *Xylaria* sp. 15, *Xylaria* sp. 16, *Xylaria* sp. 17, *Xylaria* sp. 18, *Xylaria* sp. 19 and *Xylaria* sp. 20.

Wallichia caryotoides

Rainy season: *Cladosporium* sp., *Corynespora*-like sp., *Fusarium* sp., *Guignardia cocoicola, Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phyllosticta* sp., *Sarcopodium* sp., Unidentified Hyphomycetes, *Xylaria* sp. 1, *Xylaria* sp. 3, *Xylaria* sp. 5, *Xylaria* sp. 6, *Xylaria* sp. 7, *Xylaria* sp. 8, *Xylaria* sp. 10, *Xylaria* sp. 11, *Xylaria* sp. 12, *Xylaria* sp. 14, *Xylaria* 15, *Xylaria* 16, *Xylaria* 17, *Xylaria* 18, *Xylaria* 19 and *Xylaria* 20.

Cold season : *Cladosporium* sp., *Corynespora*-like sp., *Fusarium* sp., *Guignardia cocoicola*., *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phyllosticta* sp., *Sarcopodium* sp., Unidentified Hyphomycetes, *Xylaria* sp. 4, *Xylaria* sp. 5, *Xylaria* sp. 6, *Xylaria* sp. 7, *Xylaria* sp. 8, *Xylaria* sp. 9, *Xylaria* sp. 10, *Xylaria* sp. 11, *Xylaria* sp. 12, *Xylaria* sp. 13, *Xylaria* sp. 14, *Xylaria* sp. 15, *Xylaria* sp. 16, *Xylaria* sp. 17, *Xylaria* sp. 18, *Xylaria* sp. 19 and *Xylaria* sp. 20.

Hot season : *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp., *Fusarium* sp., *Guignardia cocoicola*, Mycelia sterilia, *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phyllosticta* sp., *Sarcopodium* sp., Unidentified Hyphomycetes, *Xylaria* sp. 1, *Xylaria* sp. 2, *Xylaria* sp. 3, *Xylaria* sp. 4, *Xylaria* sp. 5, *Xylaria* sp. 6, *Xylaria* sp. 7, *Xylaria* sp. 8, *Xylaria* sp. 9, *Xylaria* sp. 10, *Xylaria* sp. 11, *Xylaria* sp. 12, *Xylaria* sp. 13, *Xylaria* sp. 14, *Xylaria* sp. 15, *Xylaria* sp. 16, *Xylaria* sp. 17, *Xylaria* sp. 18, *Xylaria* sp. 19 and *Xylaria* sp. 20.

Table 5. Relative frequency (%) of endophytic fungi in petiole (P), vein (V) and intervein (IV) of *C. kerrianus* and *W. caryotoides* at Medicinal Plant Garden.

Taxa	*Calamus kerrianus*									*Wallichia caryotoides*								
	Rain			Cold			Hot			Rain			Cold			Hot		
	P	V	IV	P	V	IV	P	V	IV	P	V	IV	P	V	IV	P	V	IV
1. *Cladosporium* sp.																		
2. *Colletotrichum gloeosporioides*							0.05									0.1		
3. *Corynespora*-like sp.						0.2	0.15			0.5								
4. *Fusarium* sp.							0.05										0.1	
5. *Guignardia cocoicola*					0.6	0.2												
6. Mycelia sterilia				0.04														
7. *Paecilomyces* sp.																		
8. *Pestalotiopsis* sp.																		
9. *Phialophora* sp.																		
10. *Phoma* sp.																	0.1	
11. Phoma-like sp.																		
12. *Phomopsis* sp.					0.1	0.1	0.15							0.9	0.1		0.3	0.6
13. *Phyllosticta* sp.																	0.6	
14. *Sarcopodium* sp.																		
15. Unidentified hyphomycetes								0.05			0.15						0.15	
16. *Xylaria* sp. 1	10.5	0.3	0.3							0.1	0.2		0.9	0.1	0.15			
17. *Xylaria* sp. 2	1.46	2.0	1.56	2.5	1.0	0.8	0.9			2.0	4.8	2.7		1.9	1.5	0.9		
18. *Xylaria* sp. 3	1.46	1.56	1.0	1.4	0.3	0.4	0.2	0.05		2.0	1.3	1.4	0.68	0.4	0.8			
19. *Xylaria* sp. 4	0.5	0.1	0.3	0.2	0.3	0.1				0.6	1.2	0.4		0.68	0.4	0.5		
20. *Xylaria* sp. 5																		
21. *Xylaria* sp. 6		0.2	0.1	0.2			0.24						0.2		0.2			
22. *Xylaria* sp. 7																		
23. *Xylaria* sp. 8					0.1							0.2						
24. *Xylaria* sp. 9	0.78		0.1		0.1					0.2	2.5	0.7	0.2					
25. *Xylaria* sp. 10					0.1									0.1				
26. *Xylaria* sp. 11		0.1								0.1	0.1	0.1	0.05					
27. *Xylaria* sp. 12																		
28. *Xylaria* sp. 13			0.1		0.2		0.05					0.1	0.05	0.3	0.4			
29. *Xylaria* sp. 14							0.2											
30. *Xylaria* sp. 15							0.2											
31. *Xylaria* sp. 16												0.1						
32. *Xylaria* sp. 17						0.2				0.3	0.3	0.2						
33. *Xylaria* sp. 18															1.2			
34. *Xylaria* sp. 19											0.1							
35. *Xylaria* sp. 20	0.5	0.2	0.1								0.4				0.2			

Taxa occuring < 0.4% in each season :

***Calamus kerrianus*:**

Rainy season: *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp., Fusarium sp., *Guignardia cocoicola*, Pestalotiopsis sp., Phialophora sp., Phoma sp., Phoma like sp., Phomopsis sp., Phyllosticta sp., Sarcopodium sp., Unidentified Hyphomycetes, *Xylaria* sp. 5, *Xylaria* sp. 6, *Xylaria* sp. 7, *Xylaria* sp. 8, *Xylaria* sp. 10, *Xylaria* sp. 11, *Xylaria* sp. 12, *Xylaria* sp. 13, *Xylaria* sp. 14, *Xylaria* sp. 15, *Xylaria* sp. 16, *Xylaria* sp. 17, *Xylaria* sp. 18, *Xylaria* sp. 19 and *Xylaria* sp. 20.

Cold season : *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp., *Fusarium* sp., *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phyllosticta* sp., *Sarcopodium* sp., Unidentified Hyphomycetes, *Xylaria* sp. 1, *Xylaria* sp. 5, *Xylaria* sp. 6, *Xylaria* sp. 7, *Xylaria* sp. 8, *Xylaria* sp. 10, *Xylaria* sp. 11, *Xylaria* sp. 12, *Xylaria* sp. 13, *Xylaria* sp. 14, *Xylaria* sp. 15, *Xylaria* sp. 16, *Xylaria* sp. 17, *Xylaria* sp. 18, *Xylaria* sp. 19 and *Xylaria* sp. 20.

Hot season : *Cladosporium* spp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp, *Fusarium* sp., *Guignardia cocoicola*, Mycelia sterilia, *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phyllosticta* sp., *Sarcopodium* sp., Unidentified Hyphomycetes, *Xylaria* sp. 1, *Xylaria* 3, *Xylaria* 4, *Xylaria* 5, *Xylaria* 6, *Xylaria* 7, *Xylaria* 8, *Xylaria* 10, *Xylaria* 11, *Xylaria* 12, *Xylaria* 13, *Xylaria* 14, *Xylaria* 15, *Xylaria* 16, *Xylaria* 17, *Xylaria* 18, *Xylaria* 19 and *Xylaria* 20.

***Wallichia caryotoides*:**

Rainy season : *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Fusarium* sp., *Guignardia cocoicola*, Mycelia sterilia, *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Phyllosticta* sp., *Sarcopodium* sp., Unidentified hyphomycete, *Xylaria* sp. 1, *Xylaria* sp. 5, *Xylaria* sp. 6, *Xylaria* sp. 7, *Xylaria* sp. 8, *Xylaria* sp. 10, *Xylaria* sp. 11, *Xylaria* sp. 12, *Xylaria* sp. 13, *Xylaria* sp. 14, *Xylaria* sp. 15, *Xylaria* sp. 16, *Xylaria* sp. 18, *Xylaria* sp. 19 and *Xylaria* sp.20.

Cold season : *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp., *Fusarium* sp., *Guignardia cocoicola.*, *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp, *Phomopsis* sp., *Phyllosticta* sp., *Sarcopodium* spp., Unidentified hyphomycete, *Xylaria* sp. 5, *Xylaria* sp. 7, *Xylaria* sp. 8, *Xylaria* sp. 9, *Xylaria* sp. 10, *Xylaria* sp. 11, *Xylaria* sp. 12, *Xylaria* sp. 14, *Xylaria* sp. 15, *Xylaria* sp. 16, *Xylaria* sp. 17, *Xylaria* sp. 18, *Xylaria* sp. 19 and *Xylaria* sp. 20.

Hot season : *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora*-like sp., *Fusarium* sp., *Guignardia cocoicola*, Mycelia sterilia, *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma*-like sp., *Sarcopodium* sp., Unidentified hyphomycete, *Xylaria* sp.1, *Xylaria* sp. 3, *Xylaria* sp. 5, *Xylaria* sp. 6, *Xylaria* sp. 7, *Xylaria* sp. 8, *Xylaria* sp. 9, *Xylaria* sp. 10, *Xylaria* sp. 11, *Xylaria* sp. 12, *Xylaria* sp. 13, *Xylaria* sp. 14, *Xylaria* sp. 15, *Xylaria* sp. 16, *Xylaria* sp. 17, *Xylaria* sp. 18, *Xylaria* sp. 19 and *Xylaria* sp. 20.

The difference of fungal frequency between two sites study was showed in Table 6. There were no significance difference of colonization and isolation rate when the Kruskal-Wallis were tested. The frequency of *Colletotrichum gloeosporioides* in both *C. kerrianus* and *W. caryotoides* were high in Huay Kog Ma, but there were a few number in Medicinal Plant Garden. While the frequency of *Xylaria* sp. 2 and *Xylaria* sp. 3 at Medicinal Plant Garden were higher than at Huay Kog Ma. This is in agreement with the study of Photita (2001) who reported that the common fungal endophytes isolated from banana sample which collected from five difference sites in Chiang Mai had varied from each site.

Table 6. The relative frequency (%) of some fungal endophytes in *C. kerrianus* and *W. caryotoides* from two different sites.

Taxa	*C. kerrianus*		*W. caryotoides*	
	Huay Kog Ma	Medicinal Plant Garden	Huay Kog Ma	Medicinal Plant Garden
Colletotrichum gloeosporioides	3.22	0.05	2.63	0.02
Mycelia sterilia	4.98	5.02	7.40	1.46
Phomopsis sp.	2.49	0.34	6.73	1.85
Xylaria sp. 2	4.88	10.15	2.20	13.76
Xylaria sp. 3	2.73	6.30	2.24	6.44
Xylaria sp. 4	2.44	1.46	0.78	3.71
Xylaria sp. 9	2.15	0.98	0.98	3.61

2.6.4 Seasonal effect

In *C. kerrianus*, the isolation rates of petioles were higher than those for vein and intervein in both two sites and in all season. Except in the rainy season, vein of *C.*

kerrianus from Medicinal Plant Garden had higher isolation rate than petiole and intervein. *W. caryotoides*, which collected from Huay Kog Ma in cold and hot season, isolation rate of fungal endophytes from petioles were higher than vein and intervein. The isolation rate of *W. caryotoides* which collected from Medicinal Plant Garden in rainy and cold season, vein and intervein had higher isolation rate than petiole. But in the hot season isolation rate of petiole had higher than vein and intervein (Table 7).

Season are effect to the colonization rate and isolation rate of fungal endophyte in both C. kerrianus and W. karyotoides. The number of isolate in each season are significant difference at $P< 0.05$ when the Kruskal Wallis Test were used. It agree with the study of palms by Fröhlich (1997) reported that the isolation rate for the petiole and vein of *L. ramsayi* and *Licuala* sp. were higher than those for vein in all four collecting sites; 1 site in Australia and 3 sites in Brunei. Colonization and isolation rate of endophytic fungi in rainy and cold season were higher than hot season (Table 2, 7). However, the study of endophytic fungi in *A. siamense* from Huay Kog Ma and Medicinal Plant Garden of Doi Suthep Pui National Park (Bussaban, 2001), colonization rate in both wet and dry season were not difference. Because the studied sites had high humidity, temperature and rainfall.

Table 7. Number of isolates of fungal enddophytes from the two species of palms, isolate from different tissue type in three season.

	Petiole	Vein	Intervein	Mean
Calamus kerrianus				
Rainy	7.8	6.2	7.4	7.1[a]
Cold	9.2	7.7	7.7	8.2[a]
Hot	3.9	0.9	1.2	2.0[b]
Wallichia caryotoides				
Rainy	5.8	4.8	7.6	6.1[b]
Cold	8.6	8.4	9.6	8.7[a]
Hot	5.0	1.1	2.1	2.7[c]

In each season, climatic conditions; temperature, moisture content at the study sites are vary. Colonization and isolation rate were not much difference in rainy and cold season because at the study sites, the moisture content in cold season are high but not as high as in rainy season. In hot season, the moisture in the air are very low, this effect to the colonization and number of isolation rate in this season, there were less colonization and isolation rate compared with those rate in other two season. There are some fungi which had high fequency of isolation (Table 8). In *C. kerrianus* and *W. caryotoides*; *Colletotrichum gloeosporioides*, *Xylaria* sp. 2, *Xylaria* sp. 3, *Xylaria* sp. 4 and *Xylaria* sp. 9 had distinct frequency in rainy season but *Phomopsis* sp. had high frequency in cold season.

Table 8. The relative frequency (%) of some fungal endophytes recovered from *C. kerrianus* and *W. caryotoides* in three seasons.

Taxa	*C. kerrianus*			*W. caryotoides*		
	Rain	Cold	Hot	Rain	Cold	Hot
Colletotrichum gloeosporioides	1.37	1.85	0.05	1.66	0.78	0.29
Mycelia sterilia	8.00	1.56	0.44	6.92	1.56	0.39
Phomopsis sp.	1.17	0.83	0.83	2.15	5.26	1.17
Xylaria sp. 2	6.54	7.17	1.32	10.63	4.10	1.22
Xylaria sp. 3	4.56	4.20	0.24	4.88	3.71	0.10
Xylaria sp. 4	2.73	0.98	0.20	2.73	1.27	0.4
Xylaria sp. 9	2.63	0.20	0.29	4.29	0.39	0

2.7 Conclusions

Mycelia sterilia with 11 species, Xylaria with 1655 isolates (20 morphospecies), eight unidentified Hyphomycetes, twelve fungal taxa; *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Corynespora* like, *Fusarium* sp., *Guignardia cocoicola*, *Pestalotiopsis* sp., *Phialophora* sp., *Phoma* sp., *Phoma* like, *Phomopsis* sp., *Phyllosticta* sp., and *Sarcopodium* sp. were recovered from Huay Kog Ma and Medicinal Plant Garden. Fungi in Xylariaceous group had highest frequency. Season are effect to the colonization rate and isolation rate of fungal endophytes. But site study and tissue of host were not effect to the colonization and isolation rate in this study. This study had carried out in one year to study three factors; tissue, site study and season that may effect to endophytic fungi in two species of palms. A period of one year is too short to determine that the factors were not effect to endophytic fungi. There were 2050 isolates of fungi recovered and 11

isolates were classified as mycelia sterilia after incubation only 4 weeks. They need longer time to produce their spores. The induction of sporulation should be investigate.

The step of surface sterilization; concentration of NaOCl may be too high (3.0% for both petioles and leaves) and the period of time when the tissue fragments were dip into the solution was too long (15 minutes for petioles and 8 minutes for leaves). This step may kill susceptible fungi, left just the resistance isolates and those killed fungi might be valuable. However, hopefully this study will be advantage to those who interested in endophytic fungi from palms.

Chapter 3

Saprobes on palms

3.1 What are saprobes

Fungal saprobes are a decomposers of organic matter. They can degrade complex structural materials such as wood and insect cuticles by producing several enzymes. The species of saprobic fungi may be difference. They are members of class Ascomycetes, Basidiomycetes, Hyphomycetes or are often lower fungi (Deacon, 1997). In this study saprophyte as a higher fungi were investigate.

K. D. Hyde is one of the leading mycologists who has been studying the fungi associated with palms. Many papers are about saprobic fungi on tropical palms. He suggested in 'The Biodiversity of Tropical Microfungi', Hyde *et al.* (1997) that the number of saprobic palm fungi are large and plams tissue are substrates that support growth of a diverse group of fungi. The larger palms produce large quantities of woody material and a larger diversity of fungi can identified from such palms. The petioles and rachides are a better resource of fungi diversity than trunks. Leaf tissues also support a great fungal diversity, but mostly are a different group of fungi, including pathogens. Mycologists who have been studying fungi in the tropics considered that many fungi in tropical forests are not yet discovered (Hyde *et al.*, 1997; Rodrigues, 1996; Rossman, 1997). Approximately 30% of ascomycete species described from palms in temperate regions (Hyde *et al.*,1997), while most of palms are located in tropics (Jones, 1994).

Fungi associated with palms have been studied by a small number of mycologists. Hyde *et al.*,(1997) considered that the ratio of fungi to palm is higher than the host species to fungus ratio of 1:6 for other plants that has generally been advocated by Hawksworth (1991). This has shown the great diversity of fungi in tropical forests. In this study, fungi will be collected and isolated from decaying *Calamus kerrianus* and *Wallichia caryotoides* petioles.

3.2 Objectives of study

To study the biodiversity of saprobic fungi on two species of palms at Huay Kog Ma and Medicinal Plant Garden in rainy season (July - October 2000).

3.3 Materials and methods

3.3.1 Palms, collecting sites and collection dates

Dead petioles of *Calamus kerrianus* and *Wallichia caryotoides* were sampled from Huay Kog Ma and Medicinal Plant Garden during the rainy season of July - October of year 2000. Ten dead petioles of each palm were collected from each site. Samples were kept separately in plastic bags and returned to laboratory.

In the laboratory, the dead palms petioles were cut into 10 cm lengths, and placed in moist plastic boxes and incubated at 20° C for a week. The materials were examined and study under stereo and compound microscopes. Spores were measured, photographed, drawn and permanent slides were prepared. The material was dried and placed in the envelop paper for herbarium.

3.3.2 Isolation

In this study most saprobic fungi were isolated by using a sterile blade cut to open the ascomata (the majority of saprobes were ascomycetes) and sterile fine forceps were used to transfer ascospores from inside the ascomata, then put onto water agar (WA) and incubated at room temperature (20-23° C). When the fungi germinated, they were transferred to MEA Petri dishes and MEA testtubes.

3.3.3 Statistical analysis

$$\text{Percentage of occurrence} = \frac{\text{number of leaves or petiole samples from which the fungus was detected}}{\text{total number of leaves or petiole sample examined in each site}} \times 100$$

Similarity index = 2C/ A+B

A: The number of species in habitat A

B: The number of species in habitat B

C: The number of species in common in habitat A and B

3.4 Results

The taxa identified on the petioles of palms; *C. kerrianus* and *W.caryotoides* from each site and their frequency occurrence are given in Table9. The description and pictures of all taxa were performed on page 33-65. Thirty-three fungal taxa were recovered, comprising 28 ascomycetes and 5 mitosporic taxa (4 hyphomycetes and 1 unidentified ceolomycetes).The most common species were *Oxydothis* sp. 1 (Occuring on 25% of sample) and *Oxydothis* sp. 2 (22.5%). There were 21 species recovered from Huay Kog Ma and 19 species recovered from Medicinal Plant garden.

Fungal communities were found on petioles of *C. kerrianus* and *W. caryotoides*, the difference of those fungal species is due to the species of palm. *Astrosphaerella* sp., *Orbilia* sp., *Ornartispora* sp., *Tubuefia* sp. 1, *Tubuefia* sp. 2, were found exclusively from *C. kerrianus* *Astrosphaerella* sp., and *Byssosphaera* sp., *Caralisporium* sp., *Iodosphaeria* sp., *Kostermasinda* sp., *Lophiostoma* sp. 1, *Massarina* sp. 1 and *Massarina* sp. 2 were found only on petioles of *W. caryotoides*.

Percentage similarity of fungi on *C. kerrianus* between Huay Kog Ma and Medicinal Plant Garden is 0.33%; *Lophiostoma* sp. 2, *Nectria* sp., *Oxydothis* sp. 2 and *Morenoina* sp. were found in both sites. Percentage similarity of fungi on *W. caryotoides* between Huay Kog Ma and Medicinal Plant Garden were 0.21%; *Oxydothis* sp. 1, *Oxydothis* sp. 2 and *Stictis* sp., were found in both sites. Taylor *et. al.* (2000) examined fungi on *Anchontophonix alexandre* in Hong Kong, north Queensland and Malaysia and found very few overlaping fungi.

For the isolation and culture of fungal saprobe, six fungi were isolated including *Astrosphearella* sp., *Byssosphaeria* sp., *Diaporthe* sp., *Kostermasinda* sp., *Stictis* sp.and unidentified ascomycetes.

Table 9. Percentage occurrence of fungal taxa on *C. kerrianus* and *W. caryotoides*

Fungal taxa	*Calamus kerrianus*		*Wallichia caryotoides*		Overall percentage occurence
	HKM	MPG	HKM	MPG	
Anthostomella ludoviciana		10		30	10.0
Astrosphaerella fissurisstoma		30			7.5
Bionectria sp.	10		10		5.0
Byssosphaera schneidermayriana				10	2.5
Capsulospora sp.	40	10	10		15.0
Canalisporium sp.				10	2.5
Chaetosphaeria sp.		40		10	12.5
Diaporthe sp.			10		2.5
Dictyosporium sp.				10	2.5
Didymella sp.	20				5.0
Iodosphaeria hongkongensis				10	2.5
Kostermansinda minima				20	5.0
Lepteutypa sp.			10	10	5.0
Lophiostoma sp. 1				30	7.5
Lophiostoma sp. 2	30	30			15.0
Massarina sp. 1				10	2.5
Massarina sp. 2				10	2.5
Massarina sp. 3	20		10		7.5
Morenoina sp.	20	40			15.0
Nectria sp.	20	10	10		10.0
Nectriopsis sp.	10		20		7.5
Ophioceras sp. 1	20		10		7.5
Ophioceras sp. 2			30		7.5
Orbilia sp.	10	10			2.5
Ornatispora sp.	10	10			2.5

Table 9. (Continue)

Fungal taxa	*Calamus kerrianus*		*Wallichia caryotoides*		Overall percentage occurence
	HKM	MPG	HKM	MPG	
Oxydothis sp. 1		10	50	40	25.0
Oxydothis sp. 2	30		30	10	22.5
Stachylidium bicolor			10		2.5
Stictis sp.			10	20	7.5
Stilbohypoxylon rehmii	50			10	15.0
Tubeufia sp.	10	10			2.5
Unidentified coelomycetes			10		2.5
Total (33 taxa)	**15**	**9**	**14**	**15**	

A Descriptions of Fungal Saprobes from *C. kerrianus* and *W. caryotoides*

(The descriptions of fungi in this study follow those given by Ellis, 1971 in Dematiaceous Hyphomycetes and those given by Hyde *et al.*, 2000 in Genera of Ascomycetes from Palms).

***Anthostomella ludoviciana* Ellis. (*Xylariaceae*: Xylariales)**

Description: *Ascomata* immersed beneath a clypeus, visible as a conical blakened crust with a minute ostiolar dots, carbonaceous, black and solitary. *Ostiole* center, papillate. *Asci* 8-spored, 60x10 μm, narrowly cylindrical, apical round, pedicellate, unitunicate with J+ apical. *Ascospores* 6.25-8 x 2.5-3 μm. uniserate, 1-celled, brown with a smooth wall.

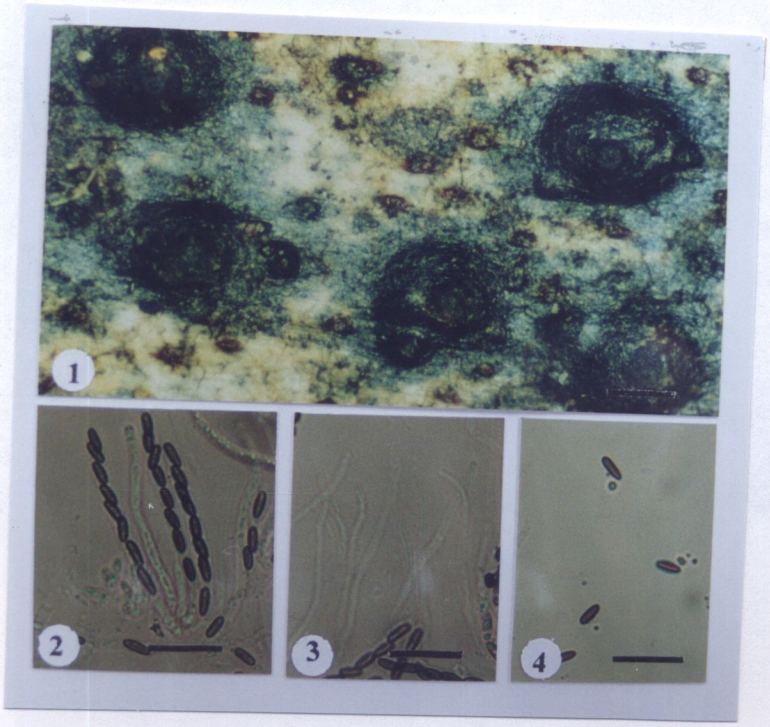
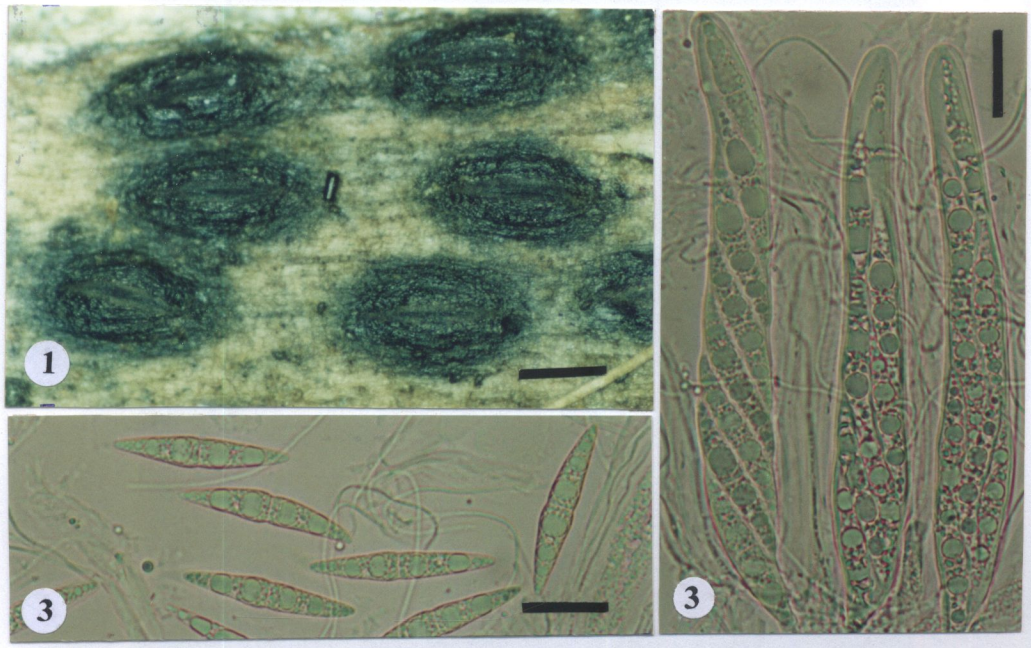


Fig. 4, 1-4. *Anthostomella ludoviciana* **1.** Ascomata on host surface. **2.** Asci **3.** Paraphyses. **4.** Ascospores. Barlines : 1= 200 μm, 2-4 = 20 μm.

***Astrosphaeriella fissuristoma* J. Fröhlich, K. D. Hyde (*Melanommataceae*: DOTHIDEALES)**

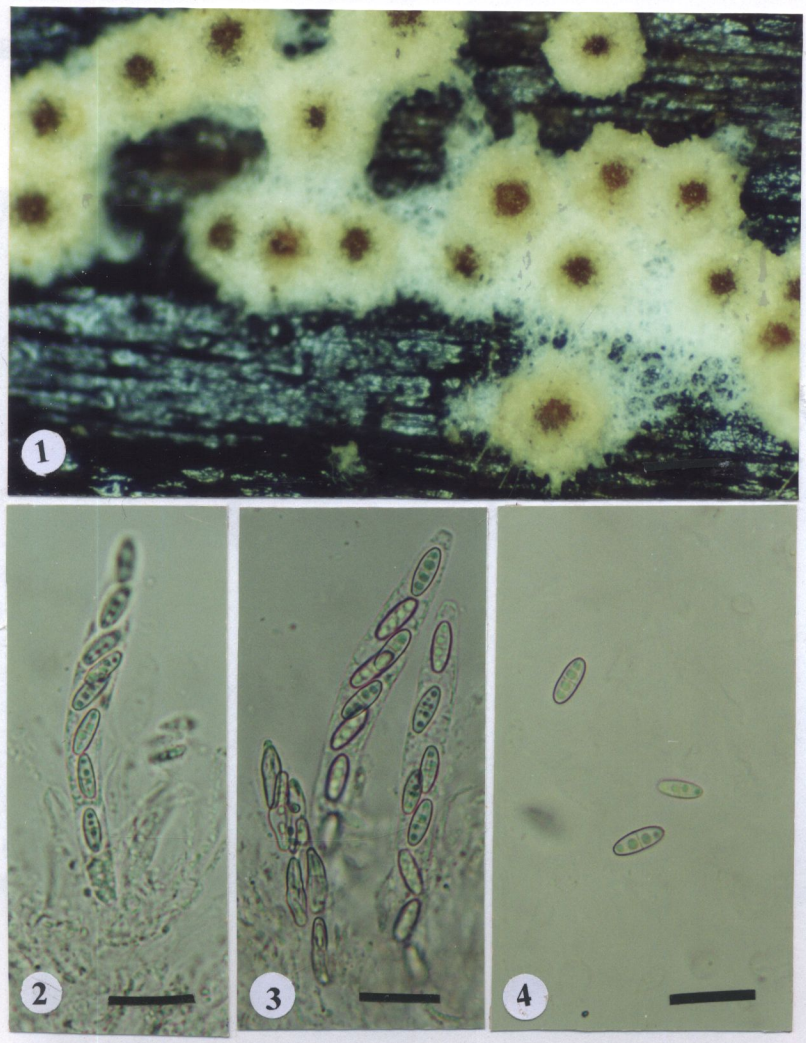
Description: *Ascomata* Developing beneath the host cuticle but appear at the superfecial, hemispherical, base flattened, black, solitary. *Ostiole* central, slit-like. *Asci* 8-spored, 145-160 x 20 μm, narrowly obclavate, pedicellate and bitunicate. *Ascospores* 45-50 x 5-10 μm, 2-3-seriate, fusiform, mostly straight, 2 celled, euseptate, hyaline when young and become reddish-brown when mature, wall smooth with mucilaginous sheaths.



Figs. 5, 1-3. *Astrosphaeriella fissuristoma* **1.** Ascomata on host surface **2.** Asci **3.** Ascospores. Barlines: 1 = 200 μm, 2-3 = 20 μm.

Bionectria (*Bionectriaceae*: HYPOCREALES)

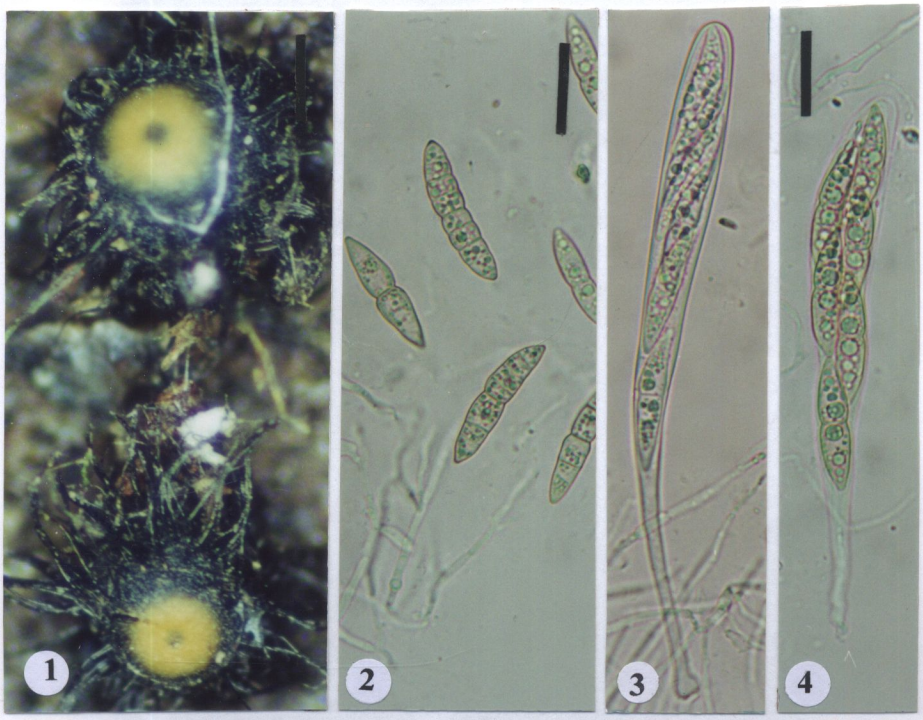
Description: *Ascomata* superficial, tan stroma covered by bright yellowish hair, globose, membraneous and scaly or tuberculate, solitary or gregarious. *Ostiole* central. *Asci* 8-spored, 95-100 x 10 μm, narrowly clavate, unitunicate with short pedicellate. *Ascospores* 11.25-15 x 3.75-5.75 μm, overlaping uniseriate, ellipsoidal, equally 2-celled sepatate, hyaline, wall smooth.



Figs 6, 1-4. *Bionectria* sp. **1.** Ascomata on host surface. **2-3.** Asci. **4.** Ascospores. Barlines: 1 = 200 μm, 2-4 = 20 μm.

***Byssosphaeria schniedermayrina* (*Melanommataceae*: DOTHIDEALES)**

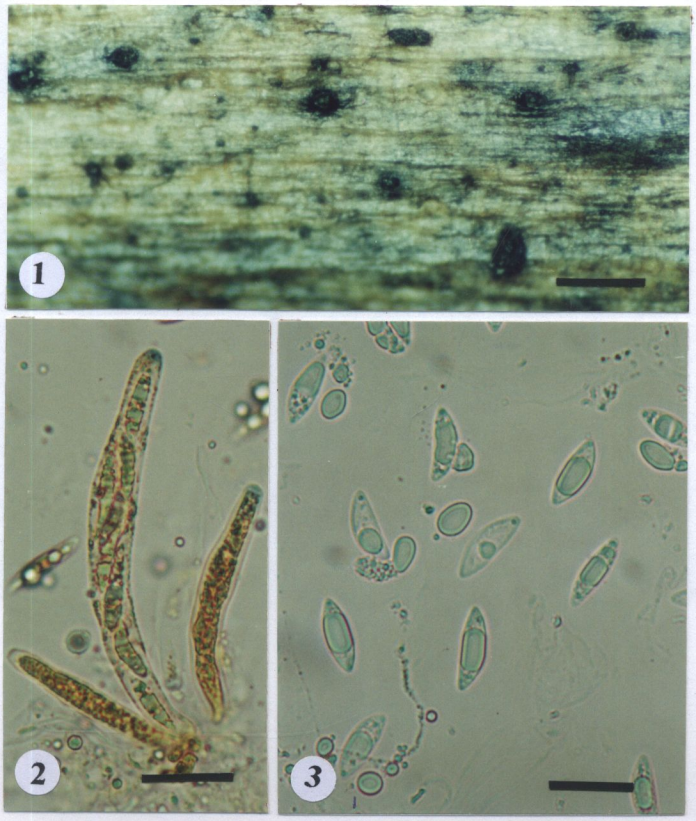
Description: *Ascomata* superficial on a subiculum, subglobose, black, separate or mostly cluster, covered with black hyphal appendages that merge as the subiculum below. *Ostiole* central, with a minute papilla, surrounded by a rim of yellow tissue. *Asci* 8-sporesd, 95-170 x 19 μm, cylindric-clavate, pedicellate, apically rounded. *Ascospores* 2-3-seriate, 15-21.25 x 5-6.25 μm, ellipsoidal, 2 to multi-celled, euseptate, hyaline becoming light brown, smooth walled surrounded by mucilaginous sheath.



Figs 7, 1-4. *Byssosphaeria schneidermayrina* **1.** Ascomata on host surface. **2.** Ascospores. **3.** Young ascus. **4.** Mature ascus. Barlines: 1 ≡ 200 μm, 2-4 ≡ 20 μm.

***Capsulospora* sp. nov. (*Clyeosphaeriaceae*: XYLARIALES)**

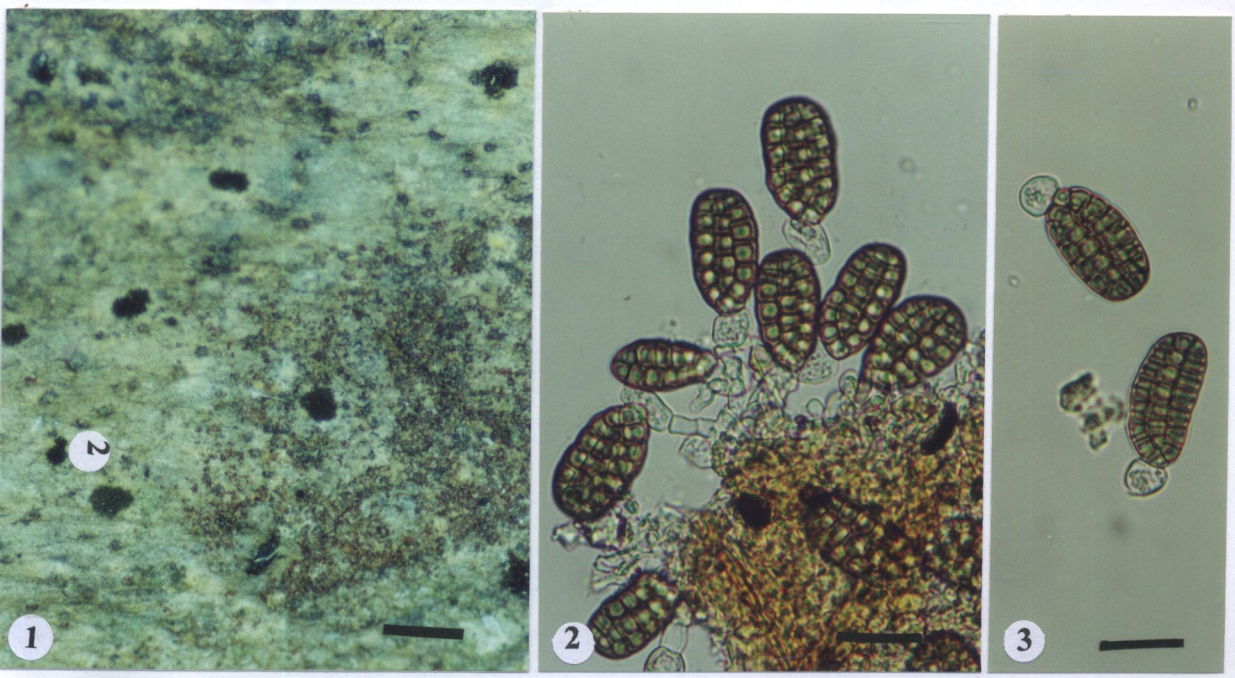
Description: *Ascomata* immersed under clypeus, visible as minute ostiolar dots, coriaceous, solitary. *Ostiole* central, umbilicate, brown and aperiphysate. *Asci* 8-spored, 100 x 10 μm, cylindrical, pedicellate, unitunicate, apically rounded. *Ascospores* 20-22.5 x 5-7.5 μm overlapping uniseriate, ellipsoidal, 1-celled, hyaline, surrounded by a layered mucilaginous sheath.



Figs. 8, 1-3. *Capsulospora* sp. **1.** Ascomata on host surface. **2.** Asci **3.** Ascospores. Barlines: 1= 200 μm, 2-3 = 20 μm.

Canalisporium

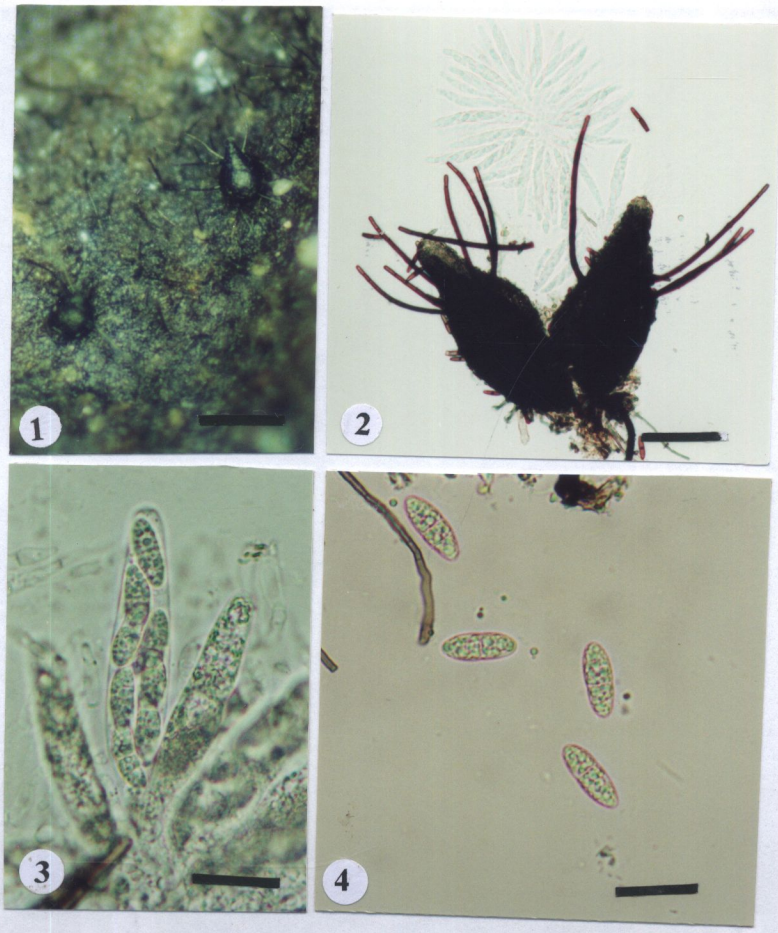
Description: *Conidia* on host surface are cluster, 30-35 x 17.5-20 μm, not flattened, brown, obovoid with multi perferate septum. *Conidiogenous cell* broadly ellipsoid, hyaline.



Figs. 9, 1-3. *Canalisporium* sp. **1.** Colonies on host surface. **2.** Conidia on conidiophore. **3.** Conidia. Barlines: 1 = 200 μm, 2-3 = 20 μm.

Chaetosphaeria (*Chaetosphaeriaceae*: SORDARIALES)

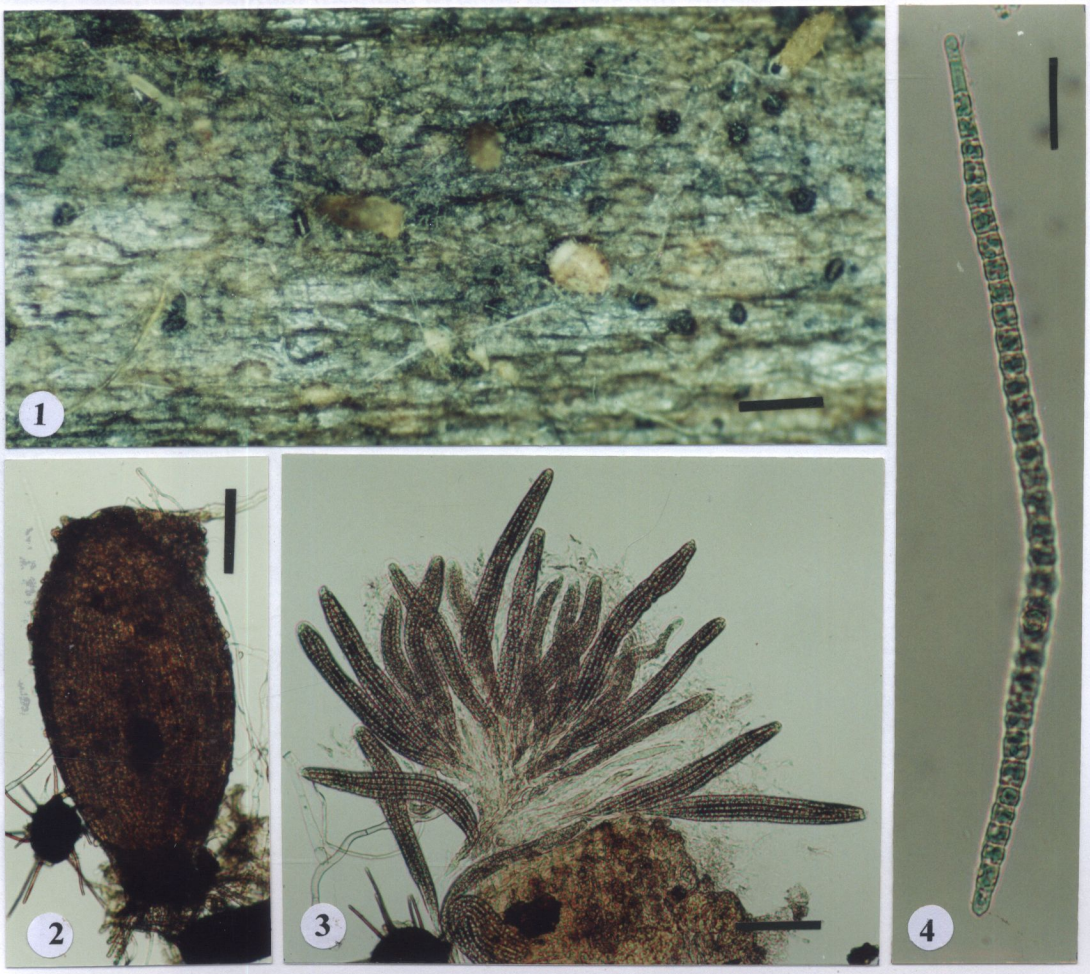
Description: *Ascomata* superficial, pyriform, dark brown to black with setae, solitary. *Papilla* central, short. *Asci* 8-spored, 95 x 12 μm, cylindric-clavate, short pedicellate, unitunicate, apically rounded, discoid. *Ascospores* 20-18.75 x 5-6.25 μm, biseriate, fusiform, 8 celled, euseptate, hyaline and smooth walled.



Figs. 10, 1-4. *Chaetosphaeria* sp. **1.** Ascomata on host surface. **2.** Ascoma and asci. **3.** Asci. **4.** Ascospores. Barlines: 1 = 200 μm, 2 = 50 μm, 3-4 = 20 μm.

***Chaetosphaerulina* sp.**

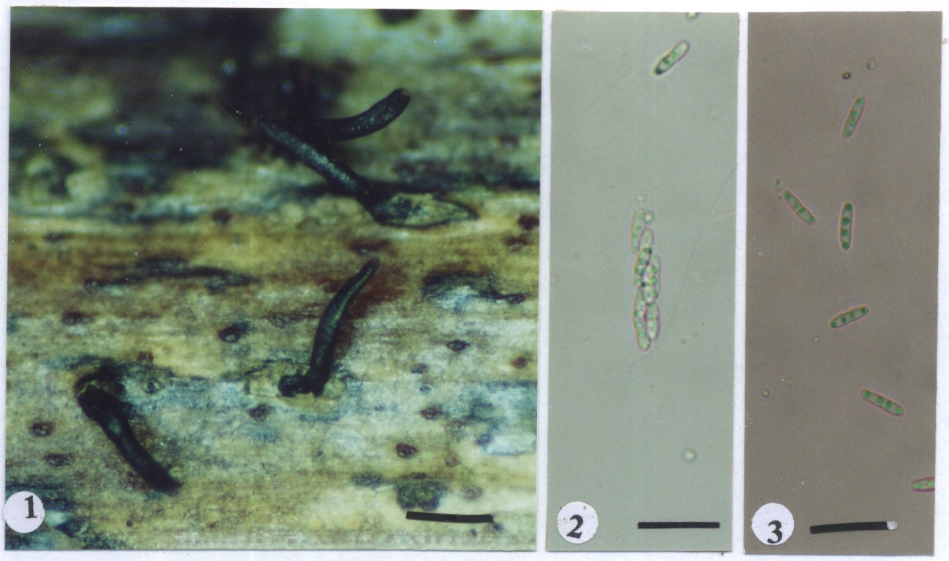
Description: *Ascomata* superficial, ovoid membraneous to coriaceous, pale pigmented, opening by pore, solitary or clustered. *Asci* 8-spored, 250 x 30 μm, broadly cylindric to clavate, apically rounded. *Ascospores* 5-7.5 x 57.5-225 μm elongate fusoid often slightly curved, multi-celled, euseptate, hyaline, light-brown after discharge, guttulate, smooth.



Figs. 11, 1-4. *Chaetoshaerulina* sp. **1.** Ascomata on host surface. **2.** Ascoma. **3.** Asci **4-5.** Ascospores. Barlines: 1 = 200 μm, 2-3 = 200 μm, 4 = 20 μm.

DIAPORTHE (*Valsaceae*: DIAPORTHALES)

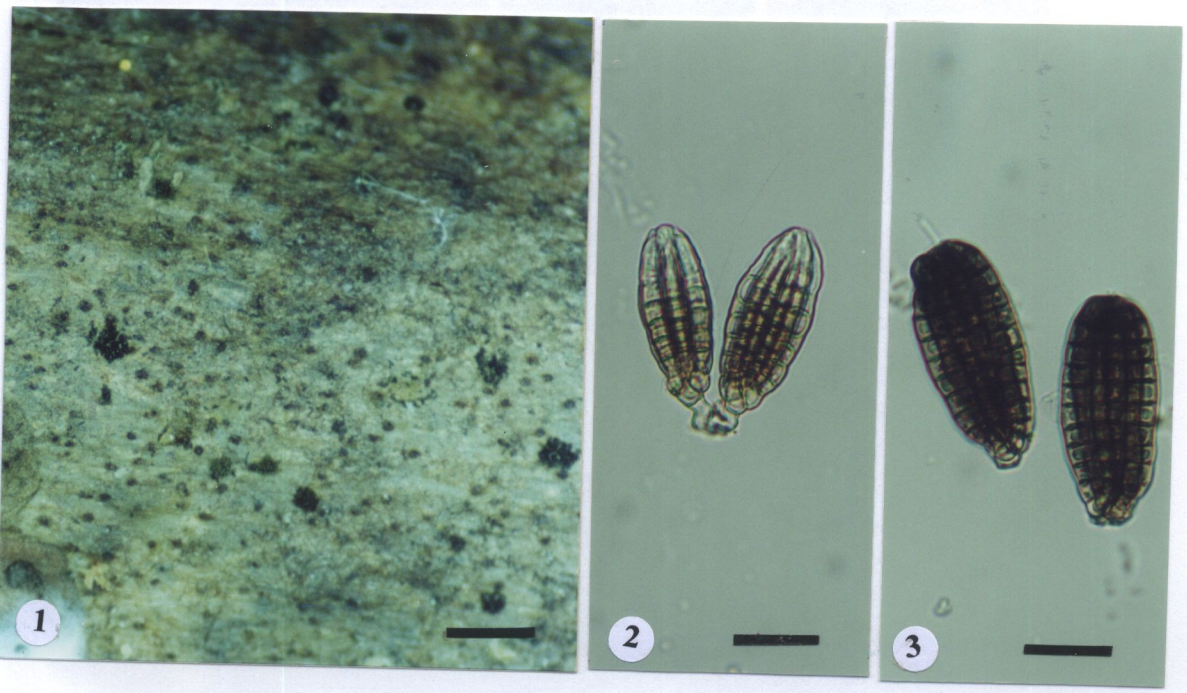
Description: *Ascomata* immersed, becoming erumpent, obpyriform, coriaceous, black, solitary or irregularly clustered. *Ostiole* central and elongate. *Asci* 8-spored, 40 x 8 μm, clavate, apedicellate becoming free within the ascomatal cavity, unitunicate, appically rounded. *Ascospores* 10.0-12.5 x 2.5 x 3.75 μm, biseriate, ellipsoid, straight or curved, 2-celled, euseptate, septum median, hyaline and smooth walled.



Figs 12, 1-3. *Diaporthe* sp. **1.** Ascomata on host surface. **2.** Asci. **3.** Ascospores. Barlines: 1 = 200 μm, 2-3 = 20 μm.

Dictyosporium

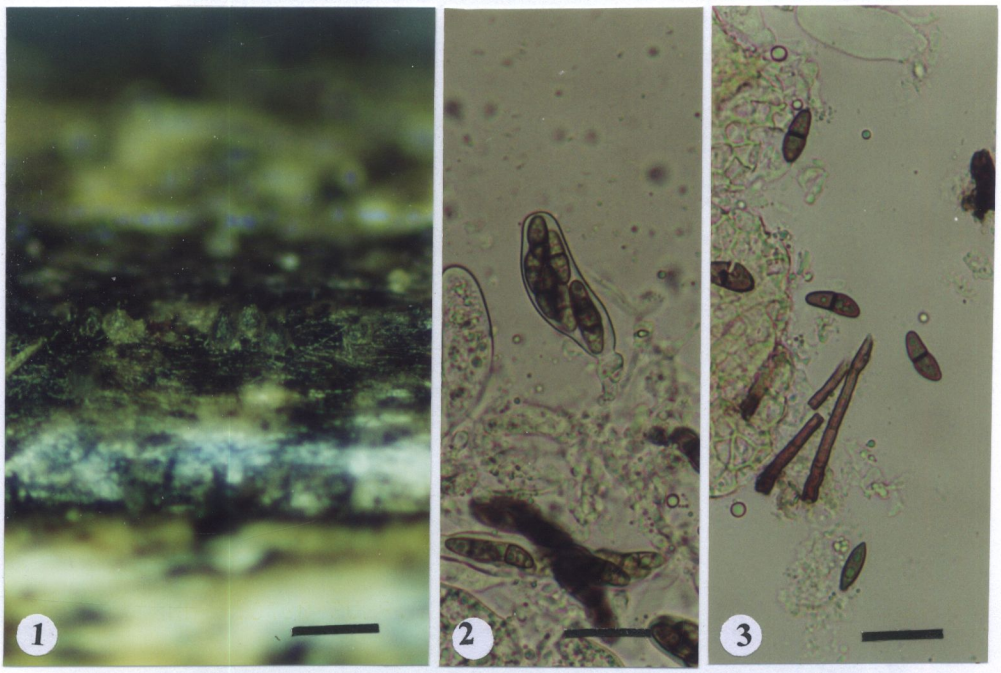
Description: *Colonies* compact, black, often granular. *Conidiophores* micronematous, mononematous, irregulary branched, hyaline to pale brown, short and smooth. *Conidiogenous* cells monoblastic, integrated, terminal and sometimes also intecalary, determinate, cylindrical, doliform. *Conidia* 43.75-60 x 22.5-40 μm fusiform, macrociphalic, brown.



Figs. 13, 1-3. *Dictyosporium* sp. **1.** Colonies on host surface. 2. Young conidia. **3.** Mature conidia. Barlines: 1 = 200 μm, 2-3 = 20 μm.

DIDYMELLA (*DOTHIDEALES*: Incertae Setae)

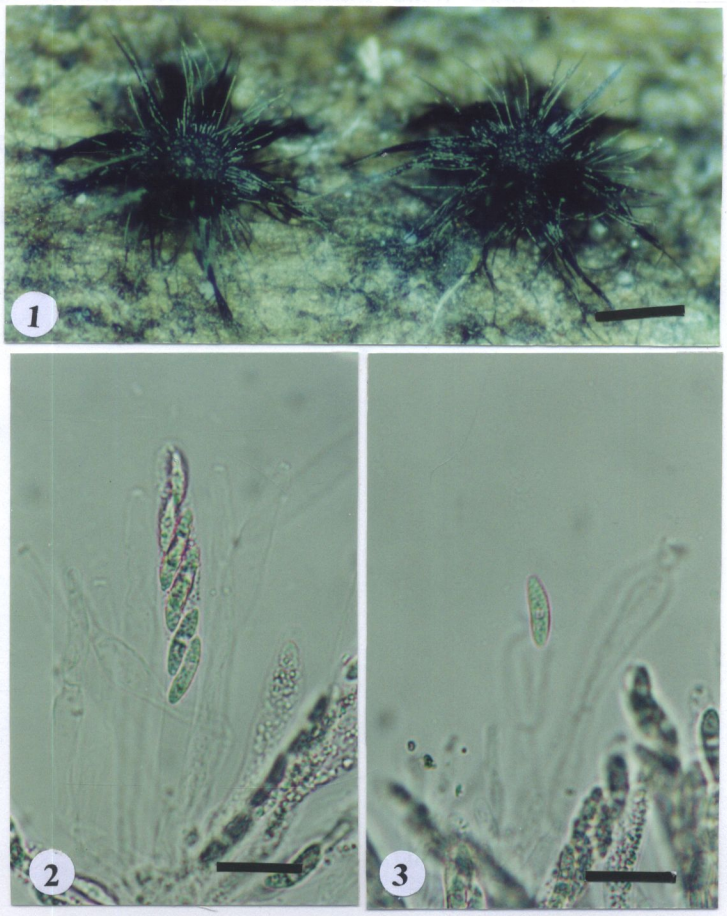
Description: *Ascomata* superficial, pyriform, hyaline to pale brown, solitary. *Ostiole* central, papillate. *Asci* 8-spored, 45 x 15 μm, unitunicate, clavate, short pedicellate, apically rounded. *Ascospores* 12.5-16.25 x 5-6.25 μm, biseriate, fusiform, 2-celled with a septum in the middle of the cell, pale brown, smooth-walled, without a mucilaginous sheath.



Figs. 14, 1-3. *Didymella* sp. **1.** Ascoma on host surface. **2.** Asci. **3.** Ascospores. Barlines: 1 = 200 μm, 2-3 = 20 μm.

***Iodosphaeria hongkongensis* (*Trichosphaeriaceae*: XYLARIALES)**

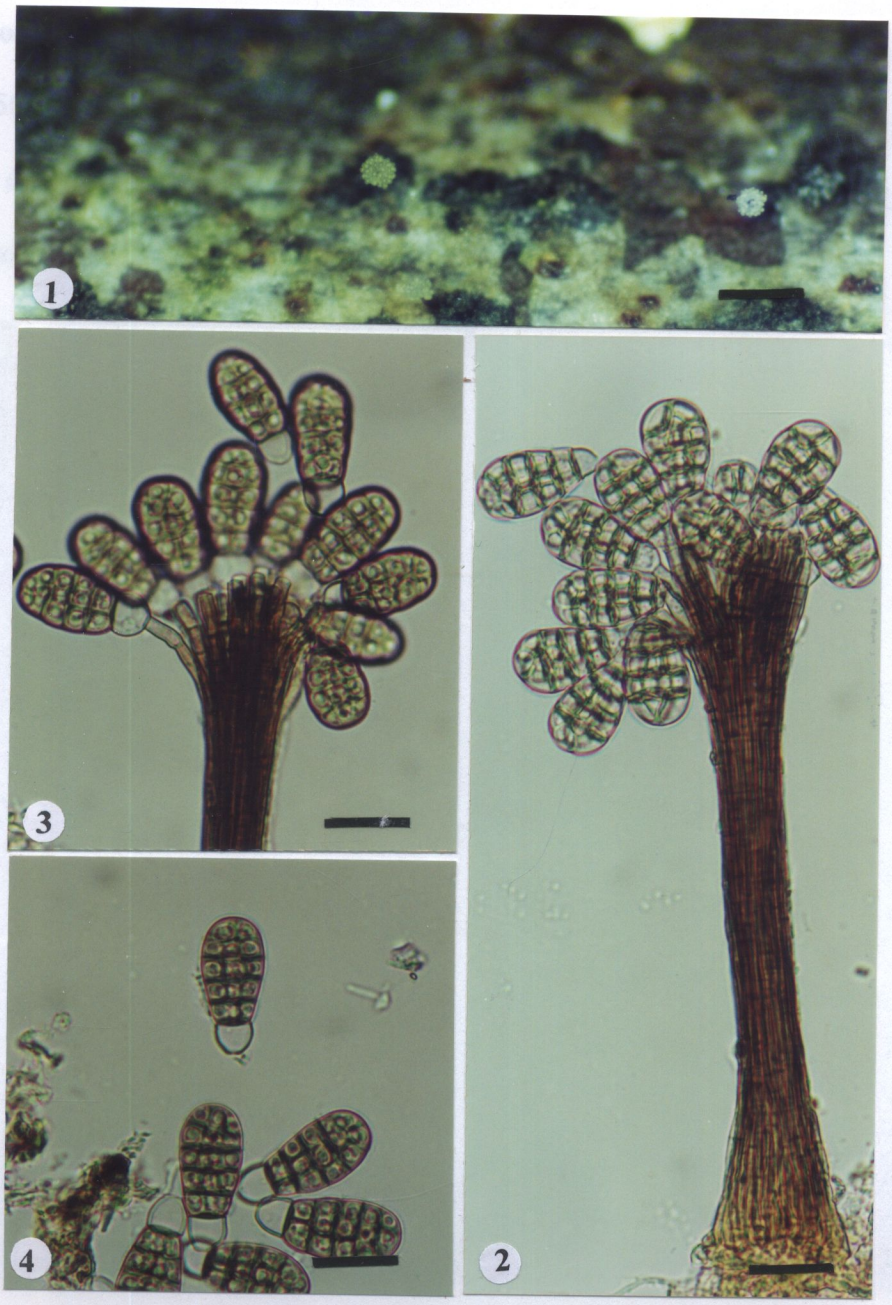
Description: *Ascomata* superficial, solitary, globose, with a flat top, coriaceous, black, with numerous long unbranched hairs. *Ostiole* apapillate. *Asci* 8-spored, 105 x 10 μm, cylindrical to narrowly clavate, short pedicellate, unitunicate, apically rounded. *Ascospores* 15-21 x 5-6.25 μm overlapping uniseriate or biseriate, fusiform, 1-celled, hyaline, wall smooth.



Figs. 15, 1-2. *Iodosphaeria hongkongensis* **1.** Ascomata on host surface. **2.** Asci and ascospores. Barlines: 1 = 200 μm, 2 = 20 μm.

***Kostermansinda minima* Cabello&Aramberri, sp. nov.**

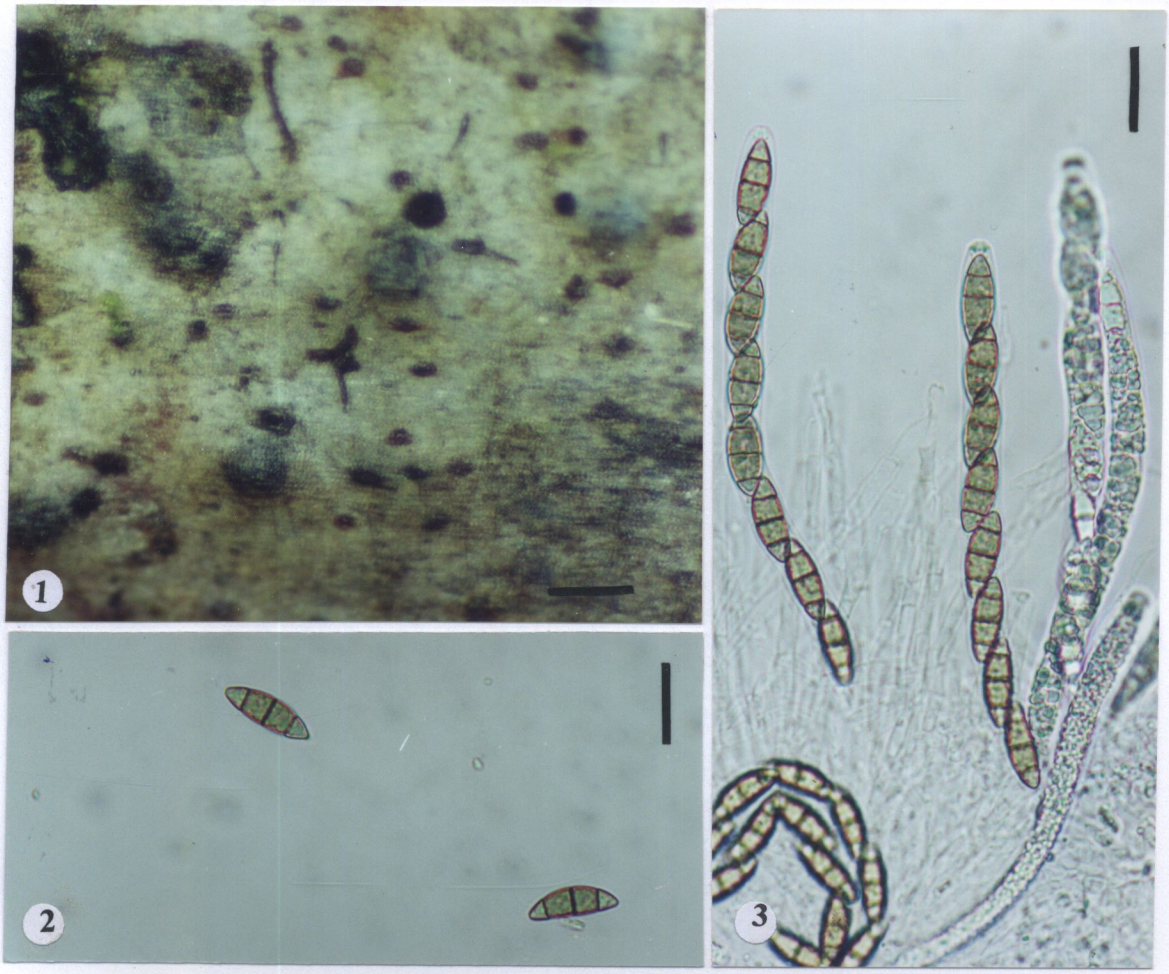
Description: colonies greenish black, with large synnamata clearly visible under a binocular dissecting microscope. *Conidiophores* macronematous, synnematous, erect, dark blackish brown; individual threads unbranched, splaying out to form ahead, straight or flexuous, smooth, brown, paler and swollen at the apex. *Conidiogenous* cells monoblastic, determinate, cylindrical and slightly swallen at the apex. *Conidia* solitary, dry, acrogenous, simple, clavate, smooth, each made up of large, golden brown, muriform terminal part and a smaller, non-septate, pale basal vesicle. *Conidia* are liberated by a break across the wall of the conidiogenous cell just below the vesicle, 15-17.5 x7.5-10 μm.



gs. 16, 1-4. *Kostermansinda minima* **1.** Synnemata on host surface. **2.** Synema. **3.** nidiogenous cell. **4.** Conidia. Barlines: 1 = 200 μm, 2-3 = 20 μm.

LEPTEUTYPA (*Amphisphaeriaceae*: XYLARIALES)

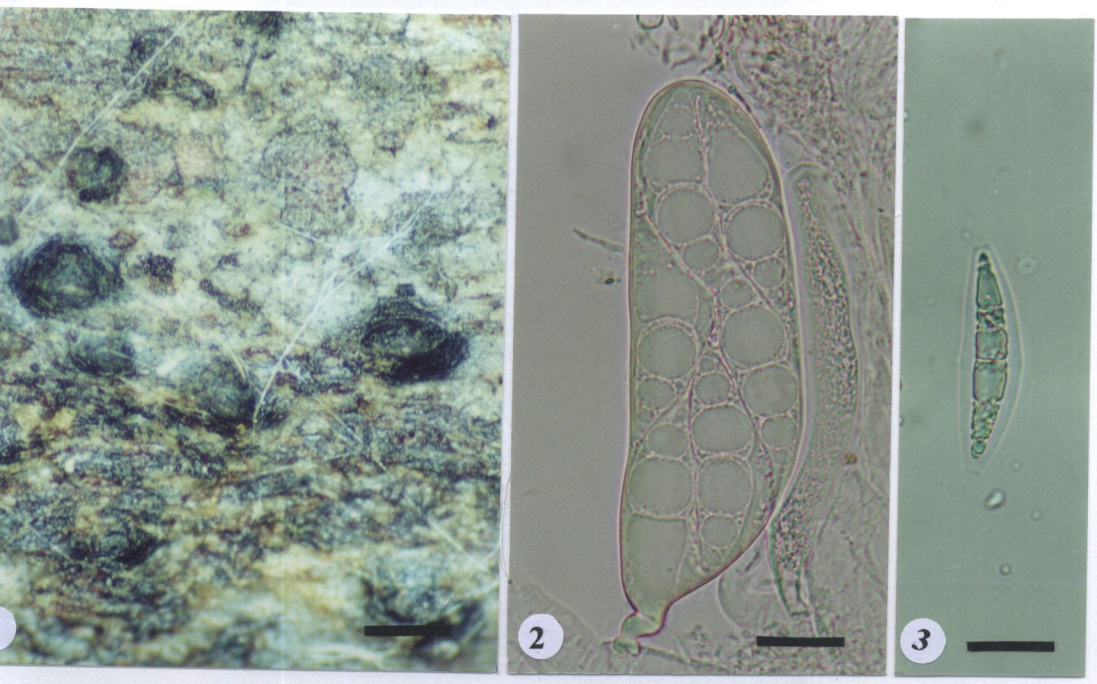
Description: *Ascomata* immersed, clustered, clypeate in some, subglobose. *Asci* 8-spored, 150 x 10 μm, long cylindrical, short pedicellate, unitunicate, apically rounded, with a J+ discoid subapical ring. *Ascospores* 6.25-7.5 x 18.75-23.75 μm uniseriate, 4-celled, euseptate, brown to dark brown, smooth-walled.



Figs 17, 1-3. *Lepteutypa* sp. **1.** Ascomata on host surface. **2.** Ascospores. **3.** Asci. Barlines: 1 = 200 μm. 2-3 = 20 μm.

.ophiostoma sp. 1 (*Lophiostomaceae*: DOTHIDEALES)

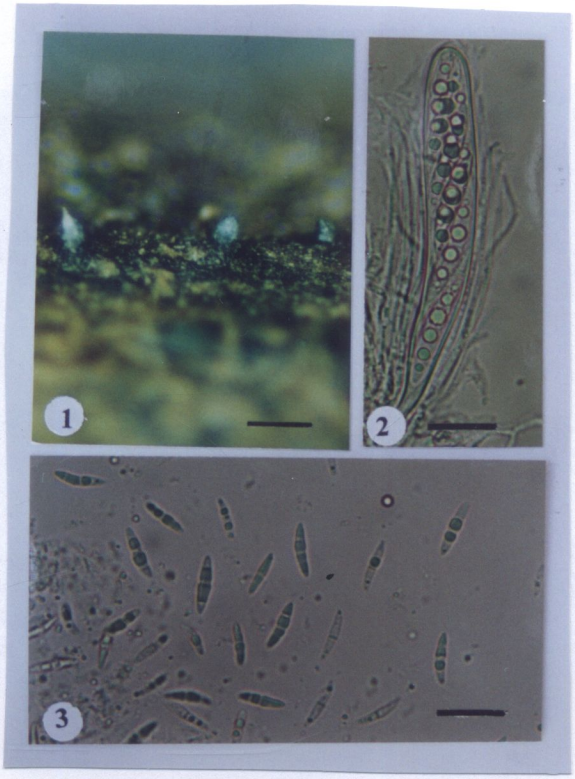
)escription: *Ascomata* immersed to erumpent, becoming superficial by weathering of ost epidermis, conical to hemispherical, carbonceous, black, opening by a slot-like stiole, solitary or clustered. *Asci* 8-spored, 145 x 45 μm, clavate to cylindric-clavate, ort pedicellate, bitunicate. *Ascospores* 45-50 x 5-10 μm, 2-seriate, fusiform, 2 to multi-lled, euseptate, hyaline when young and brown when mature, smooth, verrucose or aite, surrounded by a mucilaginous sheath.



. 18, 1-3. *Lophiostoma* sp. 1, **1.** Ascomata on host surface. **2.** Young asci. **3.** Young spores. Barlines: 1 = 200 μm, 2-3 = 20 μm.

***Lophiostoma* sp. 2 (*Lophiostomaceae*: DOTHIDEALES)**

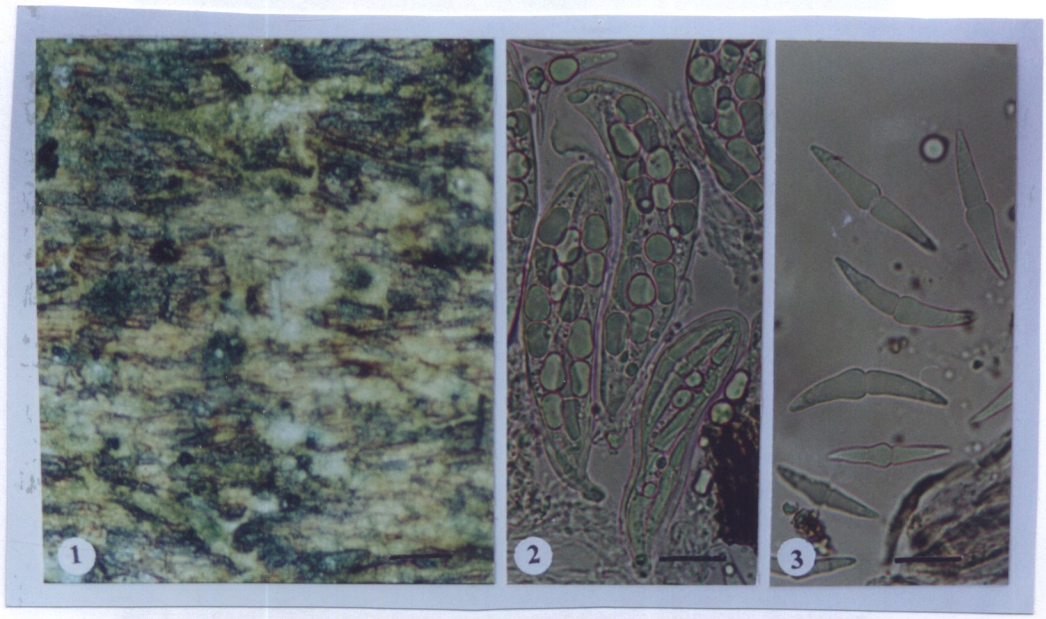
Description: *Ascomata* immersed to erumpent, becoming superficial by weathering of host epidermis, conical to hemispherical, carbonceous, black, opening by a slot-like ostiole, solitary or clustered. *Asci* 8-spored, 115 x 15 μm, cylindric-clavate, short pedicellate, bitunicate. *Ascospores* 41.25-52.5 x 7-7.5 μm, 2-seriate, narrowly fusiform, 2 to multi-celled, euseptate, hyaline and smooth.



Figs. 19, 1-3. *Lophiostoma* sp. 2, **1.** Ascomata on host surface. **2.** Asci. **3.** Ascospores. Barlines: 1 = 200 μm, 2-3 = 20 μm.

***Massarina* sp. 1 (Lophiostomataceae: DOTHIDEALES)**

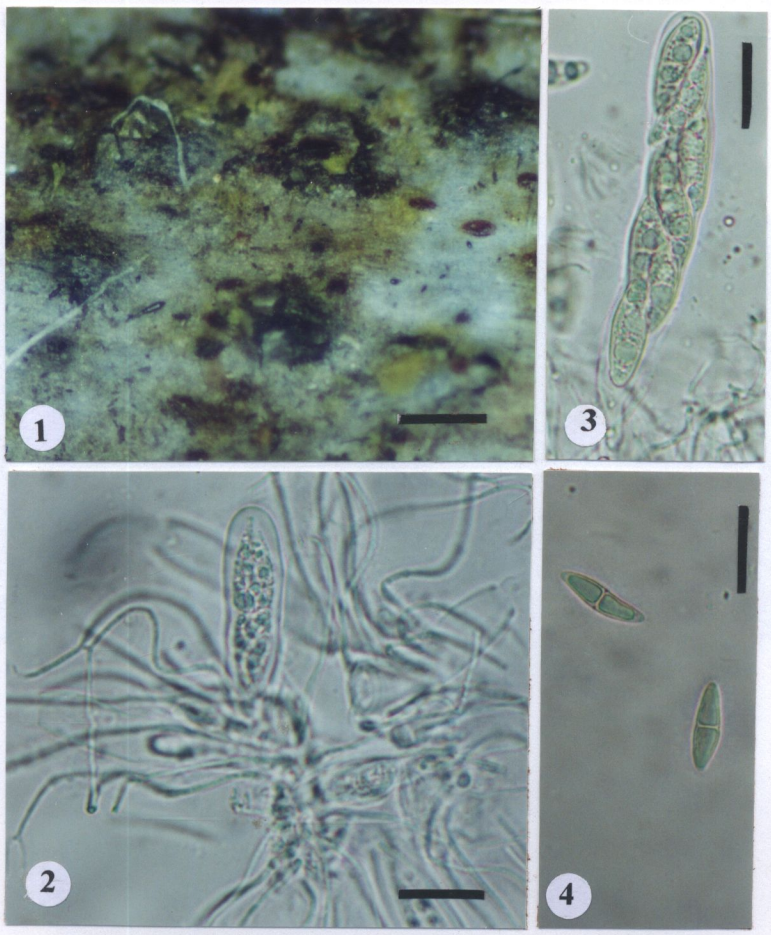
Description: *Ascomata* immersed, occasionally becoming superfecial with only the base remaining immerse at maturity, hemisphaerical or conical, coriaceous, black, solitary or gregacious. *Ostiole* central, papillate, rounded or slot-like. *Asci* 8-spored, 100 x 25 μm, narrowly to broadly clavate, bitunicate. *Ascospores* 37.5-52.5 x 5-10 μm, irregularly multiseriate, fusiform to long ellipsoidal, 2 celled, euseptate, hyaline, becoming pale to dark brown when old, wall smooth.



gs. 20, 1-3. *Massarina* sp. 1, **1.** Ascomata on host surface. **2.** Asci. **3.** Ascospores. rlines: 1 =200 μm, 2-3 = 20 μm.

Massarina sp. 2 (Lophiostomataceae: DOTHIDEALES)

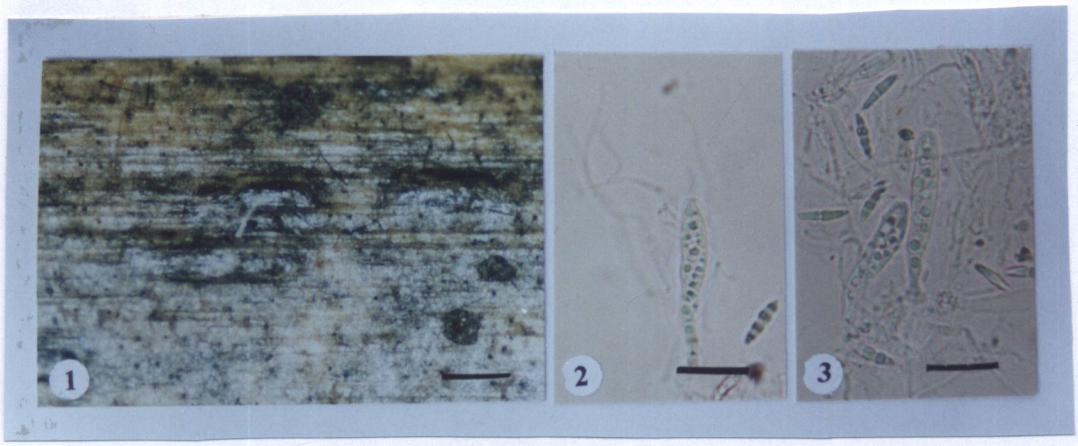
Description: *Ascomata* immersed, occasionally becoming superfecial with only the base remaining immerse at maturity, hemisphaerical or conical, coriaceous, black, solitary or gregacious. *Ostiole* central, papillate, rounded or slot-like. *Asci* 8-spored, 105 x 15 μm, broadly clavate, bitunicate. *Ascospores* 25-28.75 x 7.5-10 μm, irregularly multiseriate, fusiform , 2 celled, euseptate, hyaline, wall smooth.



Figs. 21, 1-4. *Massarina* sp. 2, **1.** Ascomata on host surface. **2.** Mature ascus. **3.** Young asci and paraphyses. **4.** Ascospore. Barlines: 1 = 200 μm, 2-4 = 20 μm.

***Massarina* sp. 3 (Lophiostomataceae: DOTHIDEALES)**

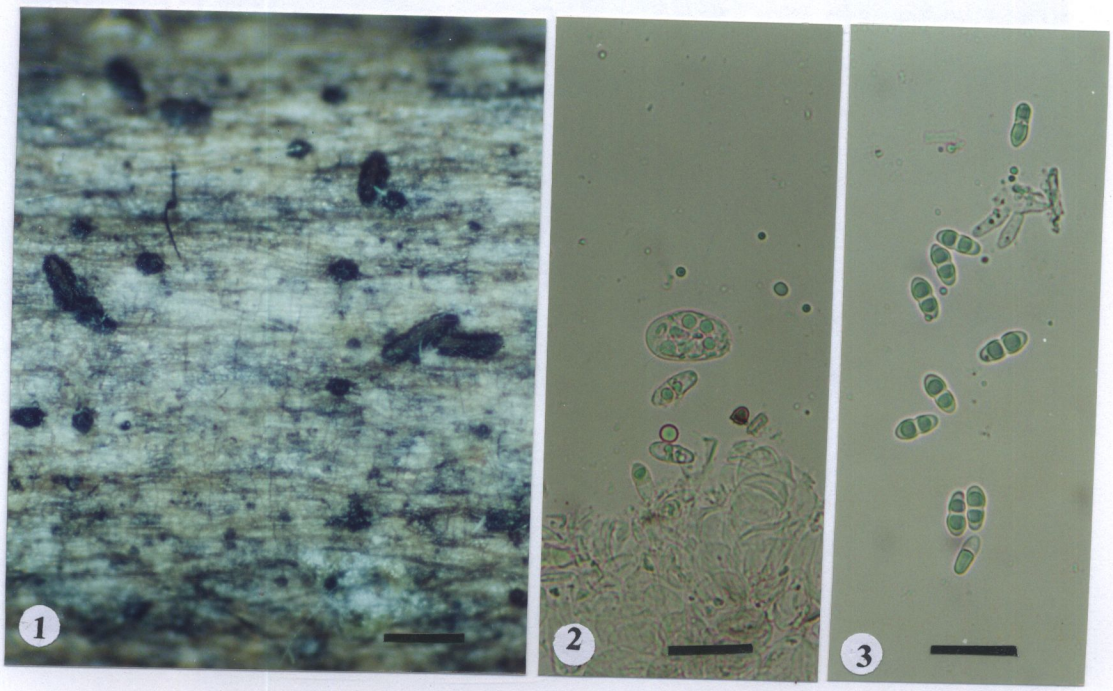
Description: *Ascomata* immersed, occasionally becoming superfecial with only the base remaining immerse at maturity, hemisphaerical or conical, coriaceous, black, solitary or gregacious. *Ostiole* central, papillate, rounded or slot-like. *Asci* 8-spored, 100 x 10 μm, narrowly to broadly clavate, bitunicate. *Ascospore* irregularly multiseriate, fusiform to long ellipsoidal, 2 celled, euseptate, hyaline, becoming pale to dark brown when old, wall smooth.



Figs. 22, 1-3. *Massarina* sp. 3, **1.** Ascomata on host surface. **2.** Ascus and paraphyses. **3.** Asci and ascospore. Barlines: 1 = 200 μm, 2-4 = 20 μm.

MORENOINA (*Asterinaceae*?: DOTHIDALES)

Description: *Ascomata* thyriothecia, black, elongated, opening by an irregular split. *Psuedoparaphyses* comprising amorphous gelatinous material between the asci. *Asci* 8-spored, 15 x 22 μm, ovoid, sessile with a short pedicellate, bitunicate, attached to the thin base. *Ascospores* 12 x 16 μm, overlapping, multi-seriate, 2-celled, upper cell wider than lower cell, euseptate, initially hyaline and guttulate.

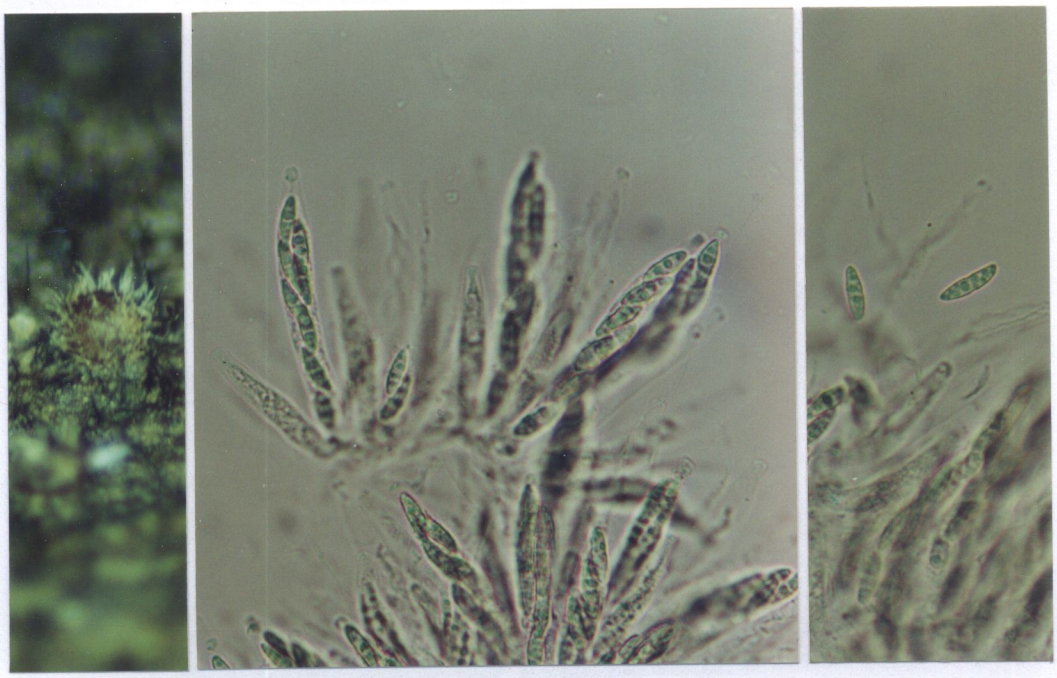


gs. 23, 1-3. *Morenoina* sp. **1.** Ascomata on host surface. **2.** Ascus. **3.** Ascospores.

arlines: 1 = 200 μm, 2-3 = 20 μm.

NECTRIA

Description: *Ascomata* superficial, globose , have a pale brown pigmentation, composed of angular cells, covered with a hyphal appendages. *Asci* 8 spored, 70 x 9 μm, clavate, with and apical ring. *Ascospores* 27.5-32.5 x 3.75 μm, ellipsoid, septate, septa generally transverse, hyaline to pale green and wall smooth.



Figs. 24, 1-3. *Nectria* sp. **1.** Ascomata on host surface. **2.** Asci. **3.** Ascospores. Barlines: 1 = 200 μm, 2-3 = 20 μm.

NECTRIOPSIS (*Bionectriaceae*: HYPOCREALES)

Description: *Ascomata* superficial, not conspicuously stromatic, globose and smooth. *Ostiole* central. *Asci* 8-spored, 13 x 75 μm, clavate, pedicellate, unitunicate. *Ascospores* 30.0-45.0 x 3.75-6.25 μm 1-2-seriate, 2-celled, euseptate, constricted or not constrict at the septum, hyaline, smooth.

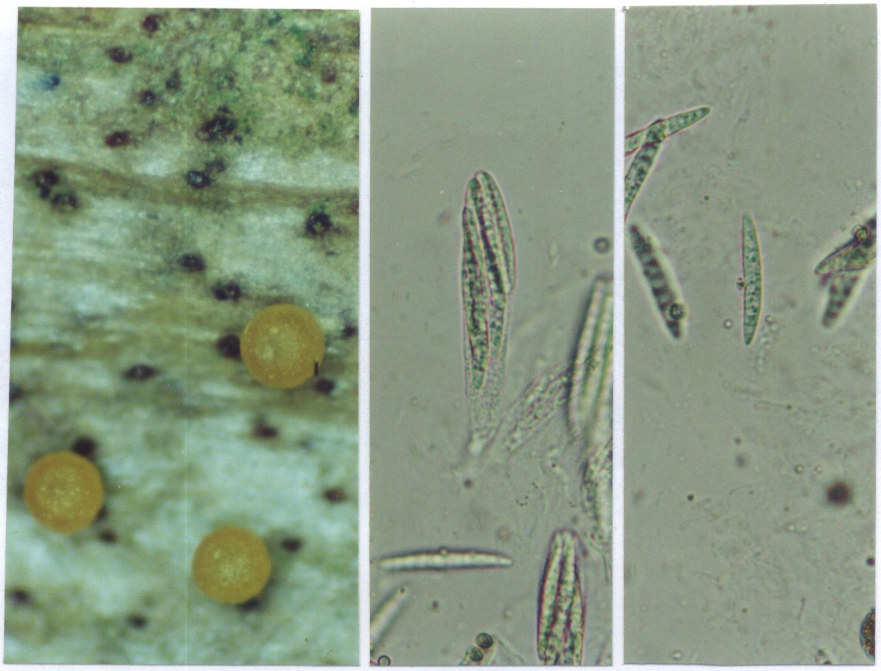
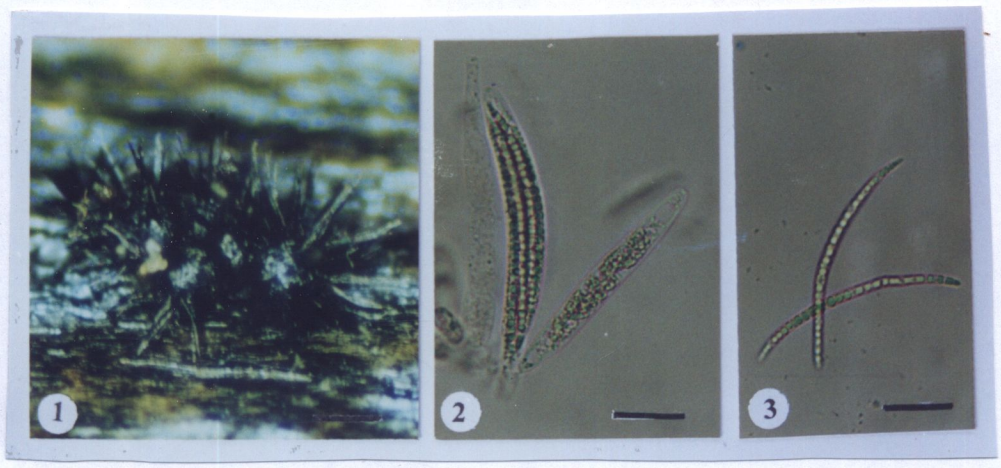


Fig. 25, 1-3. *Nectriopsis* sp. **1.** Ascomata on host surface. 2. Ascus. **3.** Ascospores. Scales: 1 = 200 μm, 2-4 = 20 μm.

***Ophioceras sp.* 1 (*Magnaporthaceae*: Familia Incerate Sedis)**

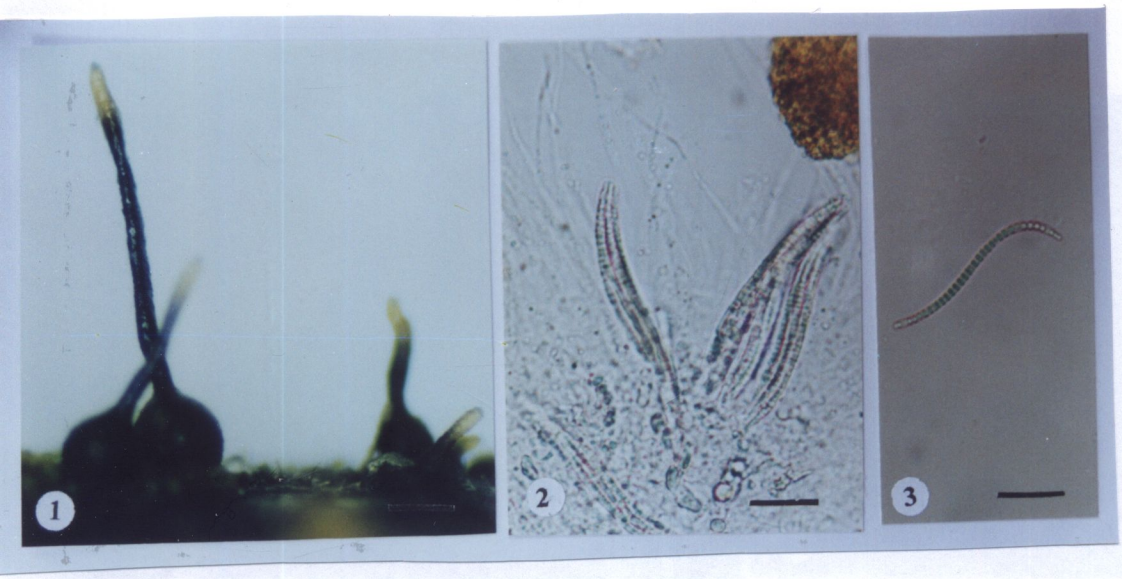
Description: *Ascomata* superficial, globose, carbonaceous, black, solitary or gregarious. *Neck* elongate. *Asci* 8-spored, 160 x 10 μm, cylindrical, apedicellate, unitunicate, apically rounded, with a J-, thimble-shaped. *Ascospores* 62.5-77.5 x 2.5-3.75 μm, filiform, mostly curved, multi-celled, euseptate, hyaline.



Figs. 26, 1-3. *Ophioceras* sp. 1, **1.** Ascomata on host surface. **2.** Asci. **3.** Ascospores. Barlines: 1 = 200 μm, 2-3 = 20 μm.

***Ophioceras sp.* 2 (*Magnaporthaceae*: Familia Incerate Sedis)**

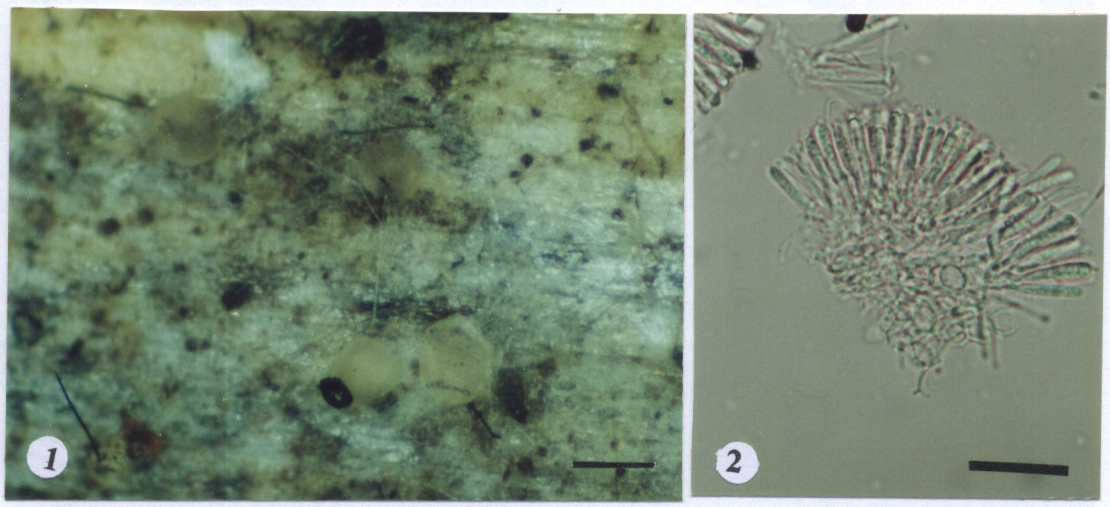
Description: *Ascomata* superficial, globose, carbonaceous, black, solitary or gregarious. *Neck* elongate. *Asci* 8-spored, 90 x 10 μm, cylindrical, apedicellate, unitunicate, apical rounded, with a J-, thimble-shaped. *Ascospores* 60 x 1 μm, filiform, mostly curved, multi-celled, euseptate, hyaline.



Figs. 27, 1-3. *Ophioceras* sp. 2, **1.** Ascomata on host surface. **2.** Asci. **3.** Ascospores. Barlines: 1 = 200 μm, 2-3 = 20 μm.

ORBILIA (*Orbiliaceae*: LEOTIALES)

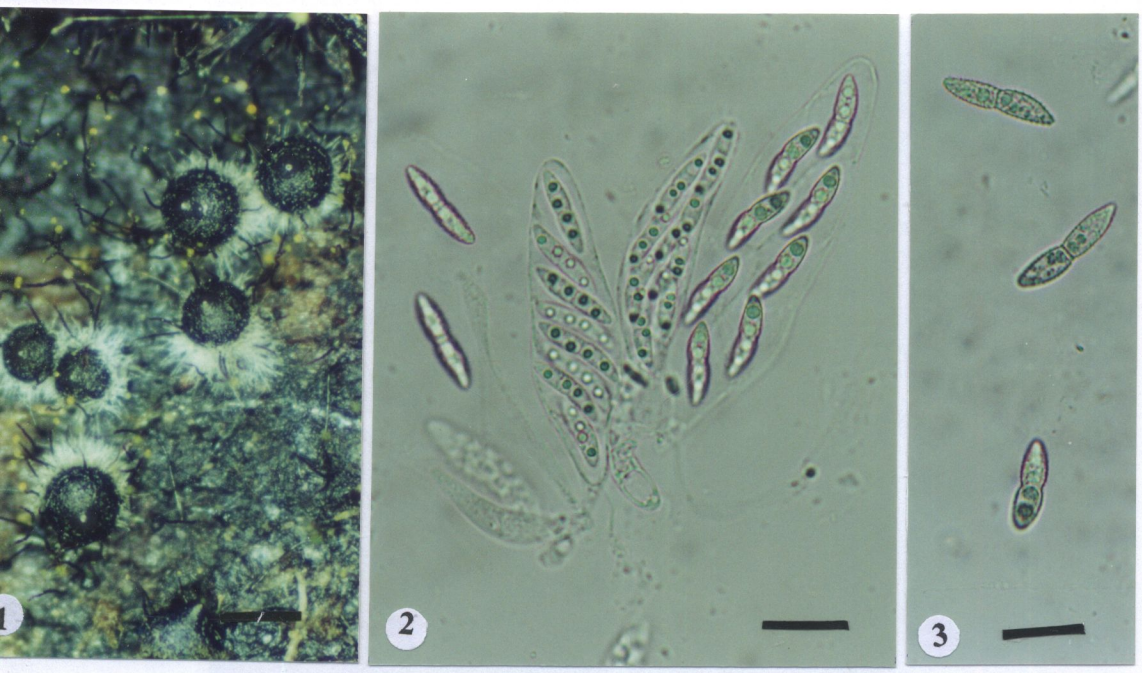
Description: *Apothecia* superficial, subsessile, pale, often translucent when fresh, with a waxy consistency, often gregarious. *Disc* flattened, irregulary convex, smooth, with and even margin. *Asci* 8-spored, 6 μm long, cylindric-clavate, apically truncate, sessile, unitunicate. *Ascospores* smaller than 1 μm, overlaping uniseriate, allantiod, 1-celled, hyaline, wall smooth.



Figs. 28, 1-2. *Orbilia* sp. **1.** Apothecia. **2.** Asci. Barlines: 1 = 200 μm, 2 = 10 μm.

Ornatispora

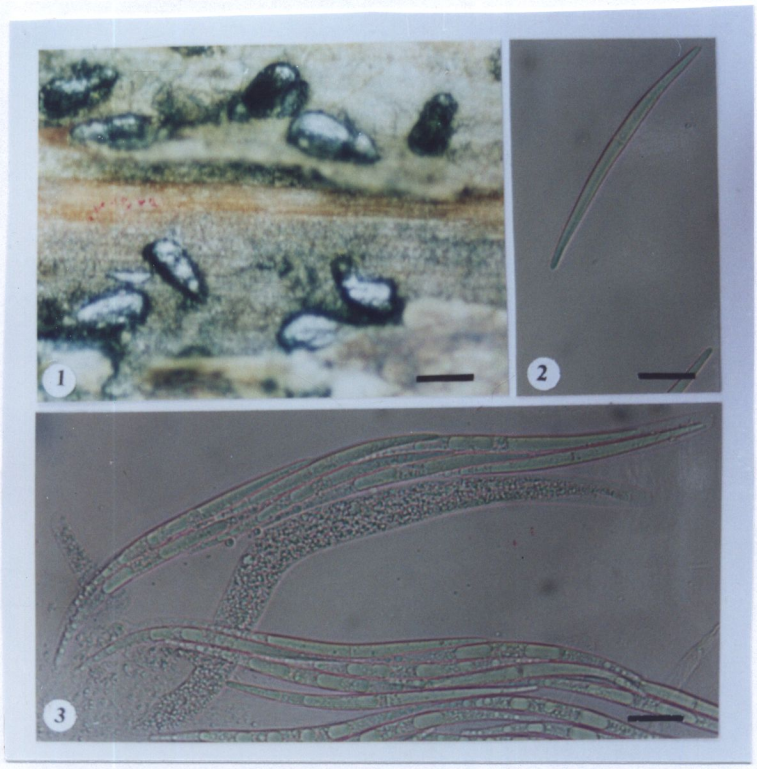
Description: *Ascomata* superficial, globose, coriaceous, black, covered with numerous pale setae. *Papilla* short, black and shiny. *Asci* 8-spored, 110 x 30 μm, clavate, short pedicellate. *Ascospores* 28.25-32.5 x 5-7.5 μm, biseriate, fusiform, 2-celled, euseptate, hyaline, wall verrucous and surrounded by a mucilaginous sheath.



Figs. 29, 1-3. *Ornatispora* sp. **1.** Ascomata on host surface. **2.** Asci. **3.** Ascospore. Barlines: 1 = 200 μm, 2-3 = 20 μm.

***Oxydothis sp.*1 (*Hyponectriaceae*?: Familia Incertae Sedis)**

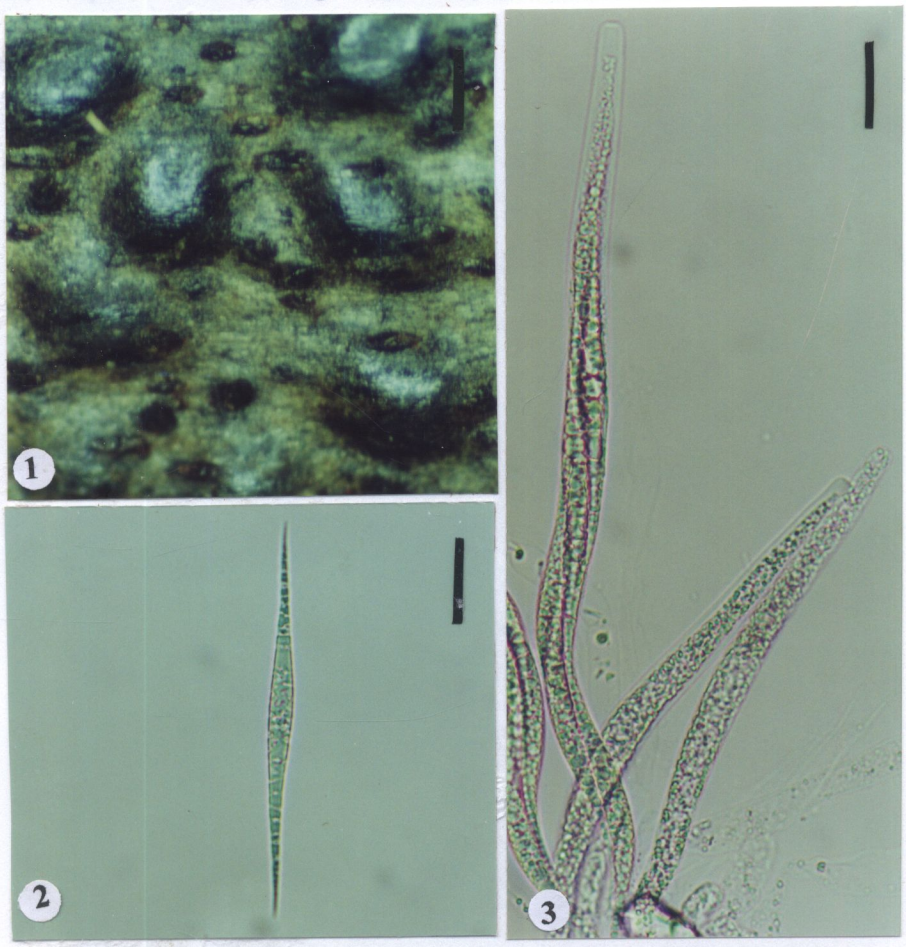
Description: *Ascomata* immersed in psuedostroma, ascomata develop below the raised epidermis, darkened with an eccentric ostioles that protrude through cracks at the edge of the uplifted host epidermis; solitary or clustered. *Asci* 8-spored, 250 x 20 μm, cylindrical, pedicellate, unitunicate, apically or truncate, with a J+, subapical ring, *Ascospore* 6.25-4.5 x 70-105 μm, fusiform and filiform, 2-celled, euseptate with a central, non constricted septum, hyaline.



Figs. 30, 1-3. *Oxydothis* sp 1., **1.** Ascomata on host surface. **2.** Asci. **3.** Ascospores. Barlines: 1 = 200 μm, 2 = 20 μm.

***Oxydothis sp.* 2 (*Hyponectriaceae*?: Familia Incertae Sedis)**

Description: *Ascomata* immersed in psuedostroma, ascomata develop below the raised epidermis, darkened with an eccentric ostioles that protrude through cracks at the edge of the uplifted host epidermis; solitary or clustered. *Asci* 8-spored, 230 x 10 μm, cylindrical, pedicellate, unitunicate, apically or truncate, with a J+, subapical ring, *Ascospore* 70-100 x 5-7.5 μm, fusiform and filiform, 2-celled, euseptate with a central, non constricted septum, hyaline, either gradually tapering from the centre but trapering abruptly near the ends to form long to spine like process.



Figs. 31, 1-3. *Oxydothis* sp 2., **1.** Ascomata on host surface. **2.** Ascospores. **3.** Asci. Barlines: 1 = 200 μm, 2-3 = 20 μm.

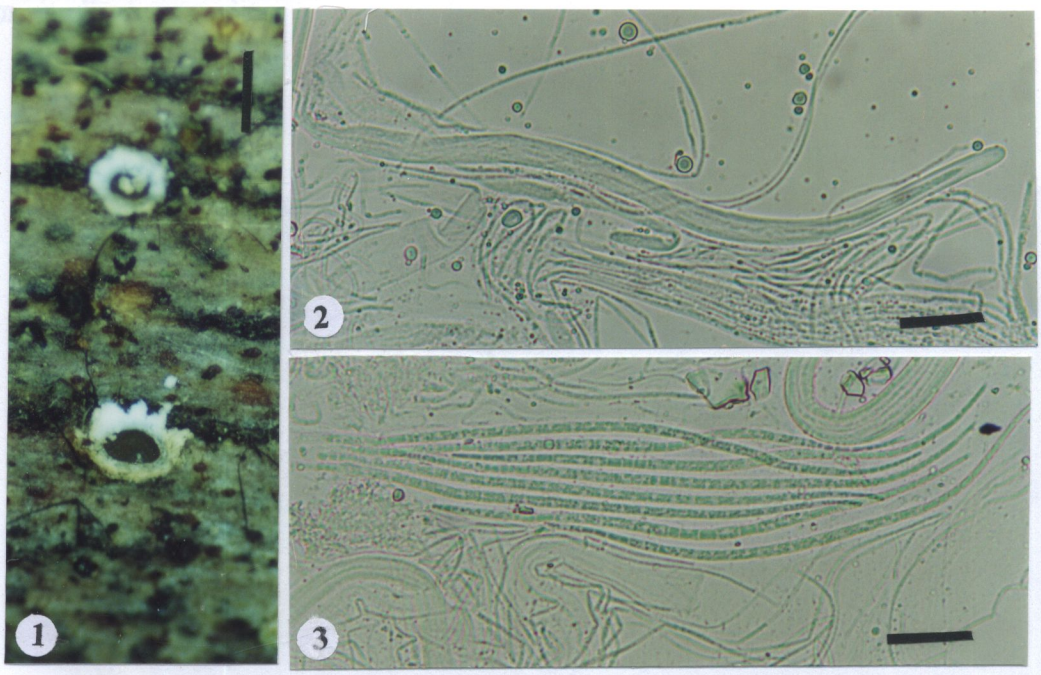
Stachylidium bicolor

Description: *Colonies* grey. *Setae* and hyphopodia absent. *Conidiophores* macronematous, mononematous, unbranched or branched, lower part of stipe sterile, brown or olivaceous brown. Upper part and branched hyaline or olivaceous, with verticallately arranged phialides. *Conidiogenous* cells mono- phialidic, discrete, arranged in verticils. determinate. cylindrical rounded at the apex or narrowly ellipsoidal. with minute opening and no collarette. *Conidia* 3-8 μm aggregated in slimmy heads, acrogenous, simple, narrowly ellipsoidal or cylindrical with round ends, hyaline to pale olive, smooth, 0-septate.

Figs. 32, 1. ***Stachylidium bicolor*** **1.** Conidia on conidiophore. Barline: 1 = 20 μm.

STICTIS (*Stictidaceae*: OSTROPALES)

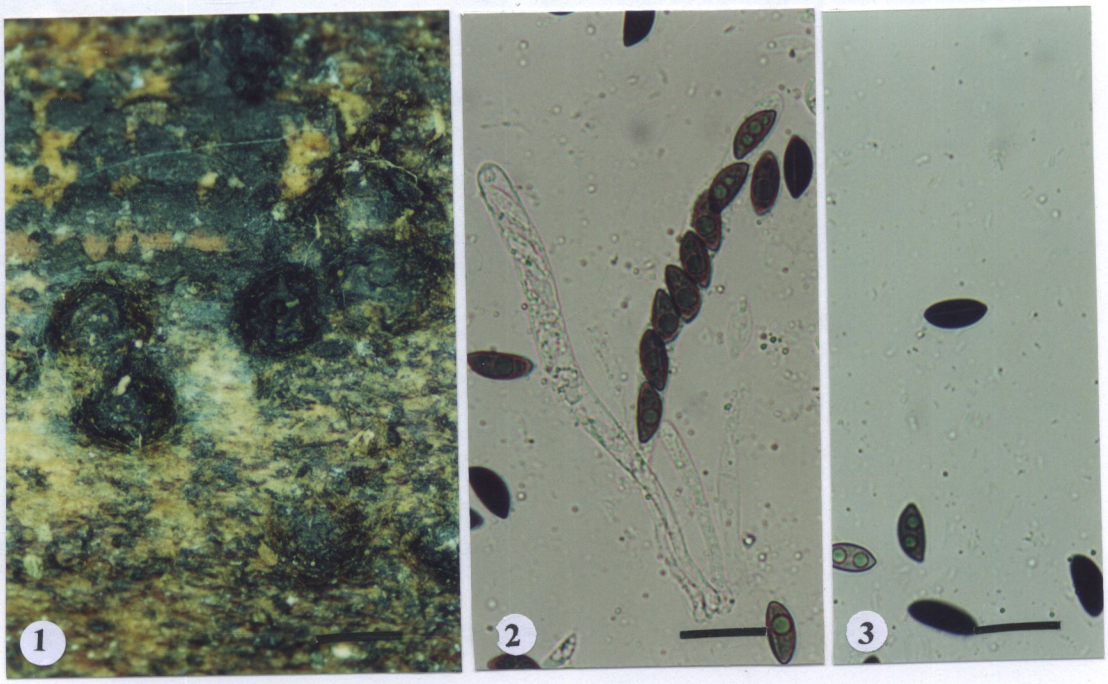
Description: *Apothecia* immersed, becoming splitting the overlying substrate, with an white lacerate margin. *Asci* 8-spores, 750 x 338 μm, cylindrical, pedicellate, unitunicate. *Ascospores* 102.5-187.5 x 2.5-3 μm, filiform, hyaline.



Figs. 33, 1-3. *Stictis* sp. **1.** Ascomata on host surface. **2.** Ascus. **3.** Ascospore. Barlines: 1 = 200 μm, 2-3 = 100 μm.

***Stilbohypoxylon rehmii* (*Xylariaceae*: Xylariales)**

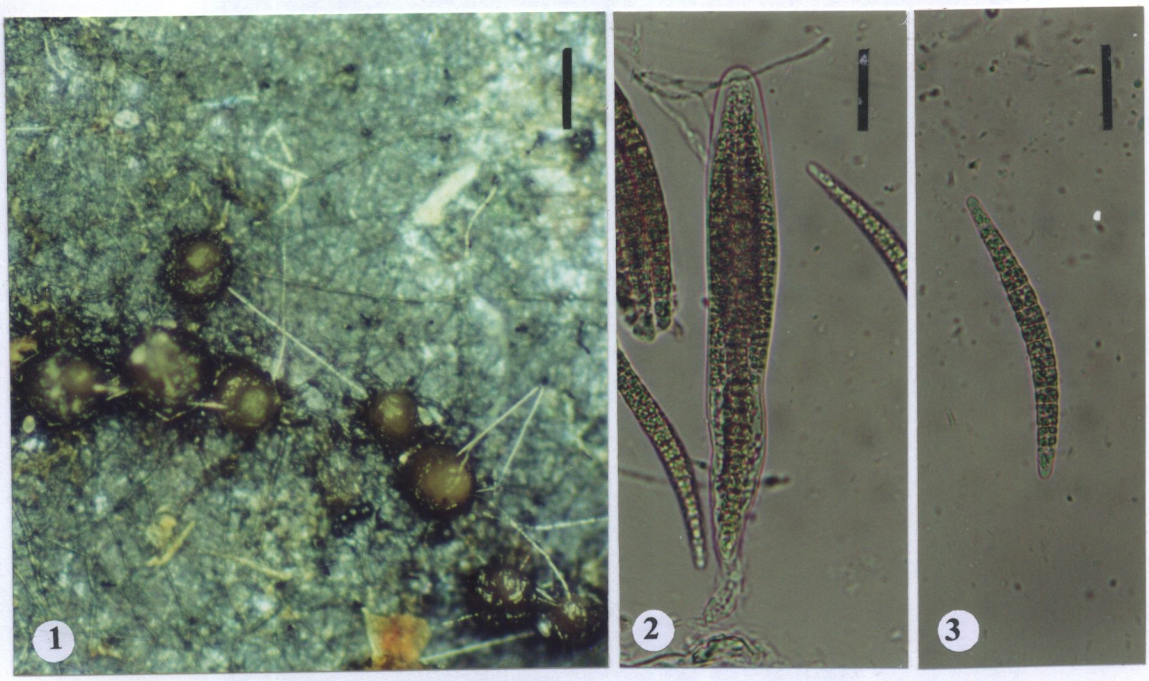
Description: *Stromata* few multi-peritheciate, developing beneath the host cuticle but appearing superficial, hemispherical, mostly solitary, black with a minutely papillate. *Ostiole* central papillate. *Asci* 8-spored, 130-145 x 10 μm, cylindrical, pedicellate, unitunicate, apically rounded with a J+. *Ascospores* 15-17.5 x 6.25-7.5 μm, uniseriate, ellipsoidal with beak at both ends, 1-celled, dark brown, wall smooth with a straight longitudinal germ slit.



Figs. 34, 1-3. *Stilbohypoxylon rehmii* **1.** Ascomata on host surface. **2.** Asci. **3.** Ascospores. Barlines: 1 = 200 μm, 2-3 = 20 μm

TUBEUFIA (*Tubeufiaceae*: DOTHIDEALES)

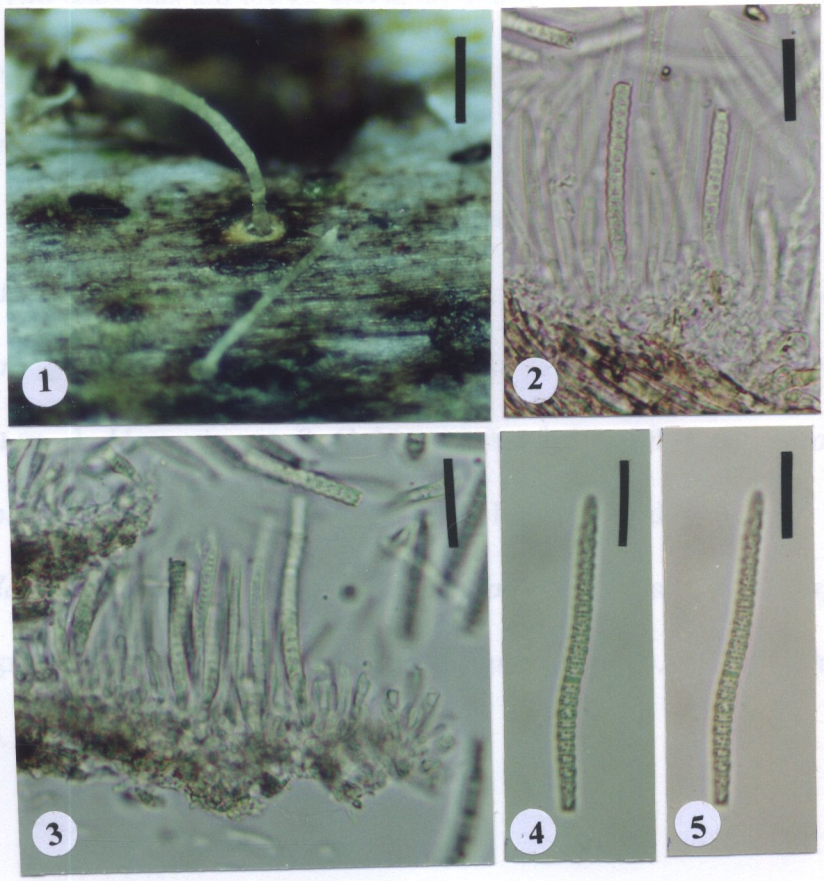
Description: *Ascomata* superficial, ovoid membraneous to coriaceous, pale pigmented, opening by pore, solitary or clustered. *Asci* 8-spores, 140 x 20 μm, clavate, *Ascospores* 150 x 10 μm elongate fusoid often slightly curved, multi-celled, euseptate, hyaline, light-brown after discharge, guttulate, smooth.



Figs. 35, 1-3. *Tubeufia* sp. **1.** Ascomata on host surface. **2.** Ascus **3.** Asci. Barlines: 1 = 200 μm. 2-3 = 20 μm.

Unidentified coelomycetes

Description : *Pycneidia* immerse in host tissue. *Conidia* were release through a wide opened pore as along chain, hyaline, 4.5-6.25 x 70-102.5 μm, filiform straight or irregular, multiseptate and easy to break, rough wall. *Conidiogenous* cell may be enteroblastic.



Figs. 36, 1-5. Unidentified coelomycete. **1.** Pycnedia on host surface. **2-3.** Conidia on Conidiophore. **4-5.** Conidia. Barlines: 1 = 200 μm, 2-5 = 20 μm.

3.5 Conclusions

Thirty-three fungal saprophyte taxa were recovered from dead petioles of *C. kerrianus* and *W. caryotoides* which collected from Huay Kog Ma and Medicinal Plant Garden, Doi Suthep-Pui National Park, during rainy season (July-October 2000). There were 28 Ascomycetes, four Hyphomycetes and one Coelomycete. The similarlity index between the two sites was 0.6 for *C. kerrianus* and 0.28 for *W. caryotoides*. Eight fungi were found exclusively on *C. kerrianus*; *Astrosphaerella fissurisstoma*, *Chaetosphaerulina* sp., *Didymella* sp., *Lophiostoma* sp. 2, *Morenoina* sp., *Orbilia* sp., *Ornatispora* sp., and *Stylbohypoxylon rehmii* and 15 fungi only on *W. caryotoides*; *Byssosphaeria schneidermayiana*, *Canalisporium sp.*, *Diaporthe sp.*, *Dictyosporium sp.*, *Iodosphaeria hongkongensis*, *Kostermansinda minima*, *Lepteutypa* sp., *Lophiostoma* sp. 1, *Massarina* sp., 1, *Massarina* sp. 2, *Ophioceras* sp. 2, *Stachylidium bicolor*, *Stictis* sp., *Tubeufia* sp. and unidentified coelomycete. Fungi found on both palms were *Anthostomella ludoviciana*, *Bionectria* sp., *Capsulospora* sp., *Chaetosphaeria* sp., *Massarina* sp. 3, *Nectria* sp., *Nectriopsis* sp., *Ophioceras* sp. 1, *Oxydothis* sp. 1 and *Oxydothis* sp. 2.

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Appendix

Half strengh malt extract agar

Malt extract	1.0	%
Yeast extract	0.1	%
Agar	1.5	%
Distilled water	100	ml

3.0% Sodium hypocholride solution

10% Sodium hypocholride solution	3	ml
Distilled water	7	ml

Statistical analysis

Kruskal Wallis Test for isolation rate of endophytic fungi

Ranks

Plant	N	Mean Rank
Number Calamus	90	89.76
Wallichia	90	91.24
Total	180	

Test Statistic[a,b]

	Number
Chi-square	0.037
df	1
Asymp. Sig.	0.847

a. Kruskal Wallis Test

b. Grouping Variable: Plant

Ranks

Site	N	Mean Rank
Number Huay kog ma Medicinal Plant Garden	90	91.24
	90	89.76
Total	180	

Test Statistic[a,b]

	Number
Chi-square	0.037
df	1
Asymp. Sig.	0.847

a. Kruskal Wallis Test

b. Grouping Variable: Site

Ranks

Season	N	Mean Rank
Calamus 1.00	30	54.47
2.00	30	62.35
3.00	30	19.68
Total	90	

Test Statistic[a,b]

	Number
Chi-square	46.152
df	2
Asymp. Sig.	0.000

a. Kruskal Wallis Test

b. Grouping Variable: Season

Ranks

Tissue	N	Mean Rank
Calamus 1.00	30	53.48
2.00	30	43.65
3.00	30	39.37
Total	90	

Test Statistic[a,b]

	Number
Chi-square	4.691
df	2
Asymp. Sig.	0.096

a. Kruskal Wallis Test

b. Grouping Variable: Tissue

Ranks

Site	N	Mean Rank
Wallichia 1.00	45	45.8
2.00	45	45.2
Total	90	

Test Statistic[a,b]

	Number
Chi-square	0.12
df	1
Asymp. Sig.	0.913

a. Kruskal Wallis Test.

b. Grouping Variable: Site

Table 10. Endophytic fungi from the experiment which lose and remain

Remaining Taxa	Lost Taxa
Colletotrichum gloeosporioides	*Cladosporium* sp.
Corynespora-like sp.	*Paecilomyces* sp.
Fusarium sp.	*Pestalotiopsis* sp.
Guignardia cocoicola	*Phaialophora* sp.
Mycelia sterilia	Unidentified hyphomycetes
Phoma sp.	*Xylaria* sp.5
Phoma-like sp.	*Xylaria* sp.6
Phomopsis sp.	*Xylaria* sp.7
Phyllosticts sp.	*Xylaria* sp.10
Sarcopodium sp.	*Xylaria* sp.11
Xylaria sp.1	*Xylaria* sp.12
Xylaria sp.2	*Xylaria* sp.13
Xylaria sp.3	*Xylaria* sp.14
Xylaria sp.4	*Xylaria* sp.15
Xylaria sp.8	*Xylaria* sp.16
Xylaria sp.9	*Xylaria* sp.17

Table 10.(Continue)

Remaining Taxa	Lost Taxa
Xylaria sp.20	*Xylaria* sp.18 *Xylaria* sp.19 *Xylaria* sp.20

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