# MOLECULAR TAXONOMIC STUDIES OF SELECTED MEMBERS OF THE XYLARIACEAE (FUNGI)

Nuttika Suwannasai

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# การศึกษาอนุกรมวิชานเชิงโมเลกุลของเชื้อรากลุ่ม XYLARIACEAE

นางสาวณัฏฐิกา สุวรรณาศรัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต สาขาวิชาจุลชีววิทยา มหาวิทยาลัยเทคโนโลยีสุรนารี ปีการศึกษา 2548 ISBN 974-533442-1

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การศึกษาเพื่อการระบุและจัดจำแนกชนิดของเชื้อราในกลุ่ม Xylariaceae โดยใช้ข้อมูลทาง ชีววิทยาโมเลกุลนี้เพื่อช่วยแก้ปัญหาที่ไม่สามารถระบุและจัดจำแนกชนิดของเชื้อราที่มีลักษณะทาง สัณฐานที่ใกล้เคียงกันมากและชนิคที่ไม่สามารถเพาะเลี้ยงได้ โดยได้ศึกษาตัวอย่างเชื้อราจากทั้งแหล่ง อ้างอิงจำนวน 31 ตัวอย่าง และแหล่งธรรมชาติจำนวน 338 ตัวอย่าง จาก 14 พื้นที่ใน 11 จังหวัดของ ประเทศไทย ซึ่งจากการศึกษาลักษณะทางสัณฐานและทางเคมีเพื่อระบุชนิดของเชื้อราจากแหล่ง ธรรมชาติพบว่ามีความผันแปรสูงของลักษณะทางสัณฐานและมีข้อจำกัดในการศึกษาแผนภูมิของสาร ทุติยภูมิ ทำให้ไม่สามารถระบุและจัดจำแนกชนิดของตัวอย่างประมาณร้อยละ 30 (จาก 338 ตัวอย่าง) จากนั้นได้สึกษาโดยใช้เทคนิคทางชีววิทยาโมเลกุลโดยหาลำดับนิวคลีโอไทด์ของ 18S rDNA ซึ่ง พบว่ามีขนาดประมาณ 2,000 ถึง 2,200 คู่เบส และ/หรือส่วน Internal transcribed spacer (ITS) 1 และ 2 รวมทั้ง 5.8S rDNA (ITS1-5.8S-ITS2) ซึ่งพบว่ามีขนาดประมาณ 500 ถึง 900 คู่เบส เมื่อ เปรียบเทียบลำดับนิวคลีโอไทค์ภายในกลุ่มของเชื้อราที่ศึกษาและจากฐานข้อมูล GenBank พบว่า ้ลำดับนิวคลีโอไทค์ของเชื้อราที่ศึกษาแต่ละชนิคมีความแตกต่างกันและสามารถระบุชนิดของ ้ตัวอย่างเชื้อราที่มีปัญหาได้อย่างชัดเจน และเมื่อนำลำดับนิวกลีโอไทด์ที่ได้มาจัดแนวความสัมพันธ์ที่ เหมาะสม พบความผันแปรสูงที่สุดในส่วน ITS1 ซึ่งเป็นประโยชน์ในการออกแบบ primers และ probes ที่จำเพาะต่อเชื้อ จากการศึกษาความสัมพันธ์ทางพันธุกรรมในรูปของ Phylogenetic tree ของเชื้อกลุ่มที่ศึกษา พบว่าสามารถอธิบายความสัมพันธ์ของเชื้อราแต่ละชนิดได้และสามารถยืนยัน ้ผลของการพบเชื้อราชนิดใหม่ได้อย่างชัดเจน ทั้งนี้พบว่าเชื้อราที่ได้จากแหล่งธรรมชาติทั้งสิ้นมี 9 สกุล (Astrocystis, Biscogniauxia, Camillea, Daldinia, Hypoxylon, Kretzschmaria, Nemania, Rosellinia และ Xylaria) 59 ชนิด ซึ่งรวมชนิคใหม่ 9 ชนิค คือ Biscogniauxia 1 ชนิด Hypoxylon 5 ชนิด และ Xylaria 3 ชนิด ลำคับนิวคลีโอไทด์ที่ได้ยังเป็นข้อมูลสำคัญในการ สร้างฐานข้อมูลของลำคับนิวกลีโอไทค์ของเชื้อรากลุ่ม Xylariaceae ในประเทศไทย

สาขาวิชาจุลชีววิทยา ปีการศึกษา 2548 ลายมือชื่อนักศึกษา <u>ณิรูรู้ ก</u> *พอรร กาอโร* ลายมือชื่ออาจารย์ที่ปรึกษา <u>borner</u> ลายมือชื่ออาจารย์ที่ปรึกษาร่วม **AVFW cler** ลายมือชื่ออาจารย์ที่ปรึกษาร่วม <u>AVFW cler</u>

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### XYLARIACEAE/NUCLEOTIDE SEQUENCE/PHYLOGENY/INTERNAL TRANSCRIBED SPACER REGIONS

Species identification and classification of the fungi in the family Xylariaceae based on their molecular data were studied for resolving undescribed species, which were closely related in their morphological characteristics, and some were uncultured specimens. In this study, thirty one specimens from reference sources and three hundred and thirty eight specimens from natural habitats of 14 localities in different 11 provinces of Thailand were examined. Morphological and chemical characterisation results showed high morphological variations and limitations in their secondary metabolite profiles. Approximately 30 % of all collected specimens could not be identified. The molecular technique was then performed. Nucleotide sequences of 18S rDNA having approximately 2,000 to 2,200 bp, and/or the internal transcribed spacer (ITS) 1 and 2 regions including 5.8S rDNA (ITS1-5.8S-ITS2) having approximately 500 to 900 bp, were achieved. The comparison of these nucleotide sequences within specimens examined and sequences from GenBank database exhibited clearly separations among xylariaceous species and these sequences can be used to identify the problem fungi. When the whole ITS sequences were aligned, they revealed the greatest variation in ITS1 region, which was suitable to design specific primers and probes for these particular strains. The phylogenetic trees showed clear relationships within xylariaceous species and also could be used to confirm results of the finding of new species. From this study, the xylariaceous fungi were identified as belonging to nine genera; *Astrocystis, Biscogniauxia, Camillea, Daldinia, Hypoxylon, Kretzschmaria, Nemania, Rosellinia* and *Xylaria*, and were represented by fifty nine species, including nine new species, which one, five, and three species belonged to *Biscogniauxia, Hypoxylon,* and *Xylaria* respectively. In addition, these molecular data are valuable for the creation of the DNA sequence database of the xylariaceous fungi found in Thailand.

School of Microbiology

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### LIST OF ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool	
bp	Base pair	
°C	Degree Celsius	
dATP	Deoxyadenosine triphosphate	
dCTP	Deoxycytidine triphosphate	
dGTP	Deoxyguanosine triphosphate	
dNTPs	Deoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP)	
dTTP	Deoxythymidine triphosphate	
DNA	Deoxyribonucleic acid	
et al.	et alia (and others)	
(m, µ) g	(milli, micro) Gram	
h	Hour	
(m, µ) L	(milli, micro) Litre	
(m, µ) M	(milli, micro) Molar	
(c, m) m	(centri, milli) Metre	
min	Minute	
(m, µ) mol	(milli, micro) Mole	
%	Percent	
PAUP	Phylogenetic analysis using parsimony	
PCR	Polymerase chain reaction	

## LIST OF ABBREVIATIONS (Continued)

PDA	Potato dextrose agar
rDNA	Ribosomal deoxyribonucleic acid
rpm	Round per minute
sp.	Species
TLC	Thin layer chromatography
v/v	Volume by volume
UV	Ultraviolet

#### **CHAPTER I**

#### **INTRODUCTION**

#### **1.1** Significance of the study

The Xylariaceae is a large and relatively well-known fungal family which is represented in most countries of the world especially in the tropics and subtropics. The fungi in this group play an important role in the natural functions of forest ecosystems. They are wood-decay fungi that are able to break down the major components of wood, and play a role in nutrient cycling in the forest. In addition, the Xylariaceae is known to contain phytopathogens and also endophytes. It has been well investigated for secondary metabolite production (Whalley and Edwards, 1995; Andersen *et al.*, 2001; Stadler *et al.*, 2001; Mühlbauer *et al.*, 2002; Quang *et al.*, 2002; Stadler *et al.*, 2004).

In taxonomic studies of xylariaceous fungi, conventional methods, including morphological, cultural, and chemical features, have been used. They are still frequently used although these methods have limitations regarding very closely related species. Thus, conventional methods are unable to resolve the problem and the confusion in some areas of investigation of the family. Many species are cosmopolitan, and have been frequently reported from different localities and at different stages of development. The xylariaceous fungi also show a great variation in their morphology, and some do not form a teleomorph stage, which causes a difficulty in identification and classification. Therefore, molecular techniques have been chosen to resolve these problems. The nucleic acid sequence data have been successfully applied for the study of evolutionary patterns and phylogeny in fungi. The aim of this study is to apply nucleic acid data based on ribosomal DNA sequences to resolve and clarify the situation regarding selected xylariaceous fungi, where conventional methods have been unsuccessful.

#### **1.2 Research objectives**

This study was undertaken to resolve the selected members of xylariaceous fungi, which are difficult to identify. Therefore, three specific objectives were investigated as follows:

1) to investigate species boundaries in problem species complexes, where traditional taxonomic methodology has failed to resolve the problems,

2) to develop a database for identification of anamorphic isolates of endophytic Xylariaceae, which can not be identified by conventional methods, and

3) to apply molecular techniques to clarify taxonomic relationships in certain genera.

#### **1.3** Scope and limitations of the study

The species complex of selected xylariaceous fungi were studied based on the 18S ribosomal DNA sequence and/or the internal transcribed spacers (ITS) 1 and 2 including 5.8S ribosomal DNA sequence. The nucleotide sequence results were compared to the morphological results. Xylariaceous fungal specimens were collected from forests in Thailand whilst the reference species were obtained from the Royal Forest Department, Thailand, the Liverpool John Moore University, U.K., and the University of Taiwan, Taiwan. The morphological characteristics of the selected xylariaceous fungi were observed. Their chemical characteristics were analyzed by secondary metabolite profiles and compared to the xylariaceous endophytes. Then, this study attempted to resolve and analyze the genetic relationships of selected xylariaceous fungi using different techniques suitable for each genus and species.

#### **1.4 Expected results**

From this study, the nucleotide sequences of the xylariaceous fungi could clearly explain the identification and classification among the problematic genera and/or species. The phylogenetic analysis could help for better understanding of taxonomic and evolutionary relationships among xylariaceous fungi by means of ribosomal DNA sequence analysis. The information of DNA sequences could also be used to design specific primers as well as probes for the detection of specific xylariaceous species in further application. Moreover, the nucleotide sequence database of xylariaceous fungi collected in Thailand would then be developed.

#### **CHAPTER II**

#### LITERATURE REVIEW

#### 2.1 The Xylariaceae

The Xylariaceae is a fungus family belonging to Phylum Ascomycota, and is commonly found throughout the temperate and tropical regions of the world (Ju and Rogers, 1996; Whalley, 1996; Rogers, 2000). The xylariaceous fungus generally has a paraphysate hamathecium and an ascus with the apical apparatus containing 4 to 8 ascospores, one-celled ascospores with a germination slit in each spore, and (Rogers, 1994; Ju and Rogers, 1996; Whalley, 1996). Their habitats are mostly on wood, litter, leaves, seeds, dung, and soil. Some are associated with insect nests. Many species exhibit strong host selectivity and in some cases are host specific (Whalley, 1996).

#### 2.1.1 Ecology and host preference of the xylariaceous fungi

A major role of the Xylariaceae is wood decomposition, and most are reported as white-rot fungi which can produce enzymes to degrade all the major wood components (cellulose and lignin) (Nilsson *et al.*, 1989; Rogers, 2000). The wooddecay fungi in this family are similar to basidiomycete white-rot fungi (Sutherland and Crawford, 1981; Rogers *et al.*, 1997) but they decompose more slowly as found for *Daldinia concentrica* (Bolt.: Fr.) Ces. & De Not. (Merrill *et al.*, 1964; Rogers *et al.*, 1997).

Some xylariaceous species occur on a wide host range such as Hypoxylon rubiginosum Pers.: Fr. and Nemania bipapillata (Berk.) Pouzar, which have been found from several kinds of plants whilst some show a strong host specificity. For example, *Rosellinia buxi* Fabre has only been found on *Buxus sempervirens* L. (Whalley and Hammelev, 1988; Petrini, 1992; Whalley, 1996). *Hypoxylon fraxinophilum* (Bull.: Fr.) Kuntze is always found on *Fraxinus* (Pouzar, 1972) and *Biscogniauxia nummularia* (Bull.: Fr.) Küntze appears restricted to *Fagus* (Whalley and Edwards, 1987). Rogers (2000) categorized the Xylariaceae by the part or position and the invasion time of a host or substrate, which they invade as shown in Table 1.

**Table 1.** The categorization of the xylariaceous fungi based on the part or position of invading host.

Position of invading host	Genera and/or species
To invade living leaves and stems, and	Many species of Anthostomella
often found fruiting on the living host	
material	
To invade living stems and remain dormant	Many species of Daldinia, Biscogniauxia,
until the host is stressed	Camillea, and Hypoxylon
To decay living roots and wood then move	Kretzschmaria clavas (Fr.) Sacc., Rosellinia
to living material from dead material	necatrix and Xylaria spp.
To form fruiting bodies on decayed	Most species of Xylaria and Nemania
material, but to be isolated as endophytes	
from living hosts	
To form fruiting bodies on seed and fruits,	Xylaria magnolia J.D. Rogers found on
and have specific and discrete	Magnolia fruits, Xylaria ianthino-velutina
relationships with their hosts	(Mont.) Fr. found on leguminous pods,
	Xylaria carpophila (Pers.) Fr. found on
	Fagus fruits, and Xylaria persicaria
	(Schwein.: Fr) Berk. & M.A. Curtis found
	on Liquidambar fruits

#### Table 1. (Continued).

Position of invading host	Genera and/or species
To inhabit dung, and found to be special	Most species of Hypocopra, Podosordaria,
relationships with animals. Many taxa	and <i>Poronia</i>
have dormant ascospores, that seem to be	
achieved via passage through a	
mammalian digestive tract.	
To associate with ant and termite nests	Most species of Xylaria including Xylaria
	melanaxis Ces. and X. nigripes (Kl.) Sacc.
To inhabit litter and organic soils	Xylariaceous anamorphs such as
	Nodulisporium and Geniculosporium
To damage host as pathogens	Camillea tinctor (Berk.) Læssøe, J.D. Rogers
	& Whalley, Biscogniauxia capnodes
	(Berk.) YM. Ju & J.D. Rogers, and B.
	mediterranea (De Not.) Kuntze

Source: Rogers (2000).

#### 2.1.2 Xylariaceous fungi as phytopathogens

Some xylariaceous fungi are considered to be weak plant pathogens causing canker disease, root rot disease, and needle blight disease (Whalley, 1996; Edwards *et al.*, 2003). Although they are not often considered to be a major cause of plant diseases, an increasing number of pathogenic species is now recognised which lead to economic loss in national ecosystems or under agricultural conditions (Rogers, 1979; Whalley, 1985; Whalley, 1996; Edwards *et al.*, 2003). Rogers (1979) and Rogers *et al.* (1997) reported that xylariaceous fungi are primarily parasites and saprophytes of angiosperm plants. Rogers believed that early angiosperms might have evolved in open areas with regular periods of drought. Therefore, one of the major factors in directing evolution of fungi associated with angiosperms might have been the capacity to survive through dry periods. If xylariaceous fungi co-evolved with

early angiosperms in exploiting the dry sites, they would have evolved to tolerate periods of drought. Rogers (1979) pointed out several properties of the Xylariaceae which might has been derived from co-evolving with their hosts on dry sites: a relatively long period of ascospore maturation and discharge, a rapid germination of ascospores in water, the discharge of ascospores when water is available, and the ability of perithecial stromata and ascospores to withstand severe desiccation (Rogers, 1979). These fungi may weaken the host by absorbing nutrients from it, blocking the vascular tissue, and preventing translocation of photosynthetic, water, and nutrients, or actually destroying cells. In some cases enzymes or toxins are produced (Alexopoulos *et al.*, 1996).

Some species of *Hypoxylon*, *Biscogniauxia*, *Camillea*, and *Xylaria* cause canker diseases (Whalley, 1996; Edwards *et al.*, 2003). Canker diseases contribute to the premature death of trees which have been stressed by drought, construction damage, or other problems. Examples of xylariaceous phytopathogens are shown in Table 2. In addition, these fungi have been investigated for phytotoxin production that may cause the disease (Bodo *et al.*, 1987; Pinon and Manion, 1991; Whalley, 1996; Edwards *et al.*, 2003).

Some species of *Rosellinia*, *Kretzschmaria*, and *Xylaria* cause root rot diseases as shown in Table 2 (Whalley, 1996; Edwards *et al.*, 2003). The symptoms of these diseases are similar to those of other root diseases, leaf yellowing, smaller leaves and premature leaf fall; some branches exhibit dieback. *Rosellinia necatrix* has been reported to produce rosellinic acid (Chen, 1964; Whalley, 1996), cytochalasin E (Aldridge, Burrows, and Turner, 1972; Whalley and Edwards, 1995; Whalley, 1996), rosellichalasin (Kimura, Nakajima, and Hamasaki, 1989; Whalley, 1996) and

rosnecatrone (Edwards *et al.*, 2001; 2003), which might have a significant role in causing the disease symptoms.

Members of xylariaceous fungi that cause needle blight diseases belong to species of *Rosellinia* (Whalley, 1996; Edwards *et al.*, 2003). Examples are noted in Table 2.

Species	Plant
Canker diseases	
Entoleuca mammata (Wahlenberg:	Acer, Alnus, Betula, Carpinus, Fagus, Picea,
F.) J.D. Rogers & YM. Ju	<i>Pyrus, Salix, Sorbus,</i> and <i>Ulnus</i> (Manion and Griffin, 1986; Whalley, 1996; Edwards <i>et al.</i> 2003)
Biscogniauxia mediterranea	Oak (Macara, 1975; Whalley, 1996)
<i>Biscogniauxia nothofagi</i> Whalley, Læssøe & Kile	Nothofagus cunninghamii (Whalley, Læssøe, and Kile, 1990; Whalley, 1996)
Camillea punctulata (Berk. & Rev.)	Quercus (Barnett, 1957; Whalley, 1996;
Læssøe, J.D. Rogers & Whalley	Edwards et al., 2003)
Root rot diseases	
<i>Rosellinia necatrix</i> Prill.	Apple, grape wive, pear, plum, sweet cherry, poplar, jasmine and scented geranium (Cellerino, 1973; Cellerino and Anselmi, 1980; Guillaumin, Mercier, and Dubois, 1982; Teixeira de Sousa, 1985; Cellerino, Anselmi, and Giorcelli, 1988; Teixeira de Sousa <i>et al.</i> , 1995; Whalley, 1996)
<i>Rosellinia bunodes</i> (Berk. & Broome) Sacc.	Cacao ( <i>Theobroma cacao</i> ), quinine ( <i>Cinchona</i> spp.), coffee ( <i>Coffea</i> spp.), rubber ( <i>Hevea brasiliensis</i> ), and tea ( <i>Camellia sinesis</i> ) (Sivanesan and Holliday, 1972; Whalley, 1996; Edwards <i>et al.</i> , 2003)
Kretzschmaria deusta (Hoffm.: Fr.)	Various tree species (Wilkins, 1934; Whalley,
P. Martin	1996; Edwards et al., 2003)
<i>Xylaria arbuscula</i> Fr.	Macadamia (Ko and Kunimoto, 1991)

**Table 2.** Examples of xylariaceous phytopathogens.

 Table 2. (Continued).

Species	Plant
Xylaria mali Fromme and Xylaria	Apple (Clayton, Julis, and Sutton, 1976;
polymorpha (Pers.: Fr.) Grev.	Whalley, 1996; Edwards et al., 2003), and
	Acer rubrum (Sivanesan and Holliday, 1972;
	Whalley, 1996)
Needle blight diseases	
Rosellinia herpotrichioides Hepting	Douglas fir (Pseudotsuga menziesii) in forest
& Davidson	nurseries (Salisbury and Long, 1956; Smith,
	1966; Whalley, 1996; Edwards et al., 2003)
Rosellinia minor (Höhn.) Francis	Young conifer seedlings (Francis, 1986)

#### 2.1.3 Xylariaceous fungi as endophytes

Endophytes are microorganisms that live inside the plant tissue for at least part of their life cycle without causing any disease symptom in the host (Petrini, 1992). Endophytes can be isolated from surface-sterilized plant tissues and cultivated on suitable nutrient agars. The grass or clavicipitaceous endophytes colonize inside of plant tissues and are believed to obtain their nutrition and some degrees of protection from the host plants. In turn, they can confer enhanced fitness to the host plants by producing certain functional metabolites. They are also implicated in improving the ecological adaptability of hosts by enhancing their tolerance to environmental stresses and resistance to phytopathogens and/or herbivores including some insects feeding on the host plant. Endophyte-infected grasses usually possess an increased tolerance to drought (Arachevaleta *et al.*, 1989; Ravel *et al.*, 1997), and aluminium toxicity (Malinowski and Belesky, 1999). Furthermore, some endophytes are able to provide the host plant with protection against some nematodes (Kimmons, Gwinn, and Bernard, 1990; Hallmann and Sikora, 1996), mammalian animals (Bacon *et al.*, 1977), and insect herbivores (Preazler, Gaylord, and Boecklen, 1996; Wilkinson *et* 

*al.*, 2000) as well as bacterial and fungal pathogens (Christensen, 1996; Sturz *et al.*, 1999). The non-clavicipitaceous endophytes occur in a wide range of non-grass hosts, are worldwide in their distribution, and are the source of many bioactive compounds (Schulz *et al.*, 2002; Strobel, 2002).

Over the past two decades, members of the Xylariaceae have been found to be widely and commonly occurring endophytic fungi being especially common and diverse in tropical plants (Petrini and Petrini, 1985; Whalley, 1996; Rodrigues and Petrini, 1997; Rogers, 2000). To date, eight genera of the Xylariaceae have been recorded as endophytes including Anthostomella, Biscogniauxia, Daldinia, Hypoxylon, Kretzschmaria, Nemania, Rosellinia, and Xylaria (Whalley, 1996). Endophytes have been widely investigated because of their ability to produce new or interesting metabolites, which can be used for natural, pharmaceutical, and biological controls of pests and diseases (Azevedo et al., 2000; Schulz et al., 2002; Strobel, 2002). An increasing numbers of studies show that individual xylariaceous species from a dominant part of the endophytes in certain tropical plant leaves (Rodrigues, 1994; Mekkamol, 1998; Photita et al., 2001). Studies on metabolites from xylariaceous fungi, including endophytic isolates, indicate that the family is a rich source of novel and often produces bioactive compounds (Whalley and Edwards, 1999; Isaka et al., 2000; Boonphong et al., 2001; Chinworrungsee et al., 2001; 2002). Xylaria cubensis (Mont.) Fr. was reported as the second most frequent species isolated from leaves of Licuala ramsayi (Muell.) Domin. (Rodrigues and Samuels, 1990) and an unidentified species of Xylaria was a frequent inhabitant of Stylosanthes guianensis Sw. leave (Pereira, Azevedo, and Petrini, 1993). Consequently, Rodrigues et al. (1993) demonstrated that xylariaceous fungi were the most frequent endophytes

isolated from *Euterpe oleracea*, especially *Xylaria cubensis*. Chapela (1989) isolated endophytic fungi from *Fagus grandifolia* and *Populus tremuloides* by using nonselective methods and found 32% and 41% to be xylariaceous fungi. In the study of leaf endophytes from a tropical palm, *Xylaria*, *Anthostomella*, *Daldinia*, and *Hypoxylon* were represented, and the most frequent species was *Xylaria cubensis* (Rodrigues, 1992). In most culture studies of leaf endophytes from tropical plants, *Xylaria* is abundant in plants tissue (Rodrigues, 1994).

Endophytic Xylaria species have been isolated from a wide range of plants including Euterpe, Trachycapus, and Livistona (Rodrigues, 1994; Taylor et al., 1999; Guo et al., 2000); Quercus and Fagus (Fagaceae); Betula, Corylus, and Alnus (Betulaceae); Acer (Sapindaceae); Fraxinus (Oleaceae); Rhizophora and Bruguiera (Rhizophoraceae); Avicennia (Avicenniaceae); Pinus and Picea (Pinaceae); and Nicotiana (Solanaceae) (Brunner and Petrini, 1992); Manilkara (Sapotaceae) (Lodge et al., 1996; Bayman et al., 1998); Tectona grandis L.f. (Mekkamol, 1998; Charesprasert 2001); Samanea saman Merr. (Charessprasert, 2001); Musa acuminate (Photita et al., 2001); Amomum siamense (Bussaban et al., 2001); bamboo (Lumyong et al., 2001); Lepanthes (Orchidaceae; Bayman et al., 1997); Casuarina (Casuarinaceae; Bayman et al., 1998); Schefflera (Araliaceae) (Læssøe and Lodge, 1994); Heisteria (Olaceae) and Ouratea (Ochnaceae) (Arnold et al., 2000); and liverworts (Davis et al., 2003). Endophytic Xylaria species have also been isolated from vascular plants in Europe (Brunner and Petrini, 1992; Taylor et al., 1999), Malaysia (Brunner and Petrini, 1992), the Brazilian Amazon (Rodrigues, 1994), Puerto Rico (Læssøe and Lodge, 1994; Lodge et al., 1996; Bayman et al., 1997; 1998), China (Taylor et al., 1999; Guo et al., 2000), Japan (Brunner and Petrini,

1992), Panama (Arnold *et al.*, 2000), and Thailand (Mekkamol, 1998; Charesprasert, 2001; Lumyong *et al.*, 2001; Ruchichakhon, 2004). In addition, there is some evidence that endophytic *Xylaria* species can be vertically transmitted through seeds of *Casuarina* as in mutualistic endophytes (Clavicipitales) (Bayman *et al.*, 1998). However, given their global range, the horizontal transmission of conidia or spores must also be very effective.

The production of secondary metabolites that are toxic to herbivores or pathogens is a common characteristic of many endophytic mutualisms and also provides the basis for selection favoring the symbiosis in the host plant (Carroll, 1988). *In vitro* studies of endophytic *Xylaria* species have shown that they actively produce secondary metabolites (Brunner and Petrini, 1992), and these may also be produced when the fungus inhabits living plant tissues. Such metabolites include antifungal and antibiotic compounds (Brunner and Petrini, 1992; Petrini *et al.*, 1995). The secondary compounds of the xylariaceous endophyte, *Muscodor albus* Worapong, Strobel & W.M. Hess, were experimentally shown to inhibit the growth of a broad range of plant and human pathogenic bacteria and fungi (Strobel *et al.*, 2001). There has been no research on how these important compounds may affect host ecology.

Accumulating evidence suggests that relationships between endophytic *Xylaria* and their hosts are complex. The further study of endophytic *Xylaria* species is needed to fully understand their ecology. Transplant and inoculation experiments are also needed to address the question of whether *Xylaria* is a mutualistic, antagonistic, or commensalistic endophyte.
# 2.2 Taxonomy of the Xylariaceae

The Xylariaceae is classified in Phylum Ascomycota, Class Pyrenomycetes, and Order Xylariales (Alexopoulos *et al.*, 1996). In the key to genera of Xylariaceae the number of genera is opened to discuss by mycologists depending on the criteria used in the taxonomy. Eriksson and Hawksworth (1993) recognised 35 genera whereas Læssøe (1994) proposed 37 genera with a few uncertain genera. Later, Whalley (1996) reviewed the family and listed 41 genera with Ju and Rogers (1996) accepting 39 genera. Recently four more new genera have been proposed. *Jumillera* J.D. Rogers, Y.-M. Ju & San Martín and *Whalleya* J.D. Rogers, Y.-M. Ju & San Martín have been separated from *Biscogniauxia* Kuntze (Rogers *et al.*, 1997). *Poroleprieuria* M.C. González, Hanlin, Ulloa et E. Aguirre, has been erected for a collection from Mexico (González *et al.*, 2004) and this is closely related to *Leprieuria* Læssøe, J.D. Rogers & Whalley. *Emarcea* Duong, R. Jeewon & K.D. Hyde has very recently been described as a new genus from Thailand containing a single species, *Emarcea castanopsidicola* (Duong *et al.*, 2004). Although there are different opinions, at least 42 genera can be assigned to the family (Table 3).

Thienhirun (1997) reported seventeen xylariaceous genera from Thailand, which were *Anthostomella*, *Astrocystis*, *Biscogniauxia*, *Camillea*, *Daldinia*, *Entonaema*, *Holttumia*, *Hypoxylon*, *Kretzschmaria*, *Kretzschmariella*, *Nemania*, *Podosordaria*, *Poronia*, *Rophalostroma*, *Rosellinia*, *Sacoxylon*, and *Xylaria*. Consequently, three more genera, *Jumillera*, *Stilbohypoxylon*, and *Whalleya* have been included (Thienhirun and Whalley, 2001) and now *Emarcea* was added (Doung *et al.*, 2004).

Eriksson and Hawksworth (1993)	Læssøe (1994)	Whalley (1996)	Ju and Rogers (1996)	Others
Anthostomella Sacc.	Anthostomella	Anthostomella	Anthostomella	
			Areolospora S.C. Jong &	
			E.E. Davis	
Ascotricha Berk.		? Ascotricha	Ascotricha	
? Ascotrichella Valldos.&Guarro		? Ascotrichella		
? Astrocystis Berk. & Broome	Astrocystis	Astrocystis		
Biscogniauxia Kuntze	Biscogniauxia	Biscogniauxia	Biscogniauxia	
Calceomyces Udagawa & S. Ueda	Calceomyces	Calceomyces	Calceomyces	
Camillea Fr.	Camillea	Camillea	Camillea	
	Chaenocarpus Fr.	? Chaenocarpus	cumie	
	<i>Collodiscula</i> I.Hino & Katum.	? Collodiscula	Collodiscula	
	Creosphaeria Theiss.	Creosphaeria	Creosphaeria	
Daldinia Ces. & De Not.	ereosphaeria meiss.	Daldinia	Daldinia	
		Daianna	Discoxylaria Lindquist & J.	
			Wright	
			Wiight	Emarcea Duong, R.
				Jeewon & K.D. Hyde
				(2004)
Engleromyces Henn.	Engleromyces	Engleromyces	Engleromyces	(2001)
	Ligier onlyces		Entoleuca Syd.	
Entonaema A. Møller	Entonaema	Entonaema	Entonaema	
	<i>Euepixylon</i> Füisting	? Euepixylon	Euepixylon	
Fassia Dennis	Linep my ton 1 disting	: <i>Duep wyten</i>	Lucputyton	
Helicogermslita Lodha & D. Hawksw.	Helicogermslita	Helicogermslita		
Theneoger mstitu Lound & D. Hawksw.	Holttumia Lloyd	? Holttumia		
Hypocopra (Fr.) J. Kickx f.	Hypocopra	Hypocopra	Hypocopra	
Hypotopia (11) 5. Henri 1. Hypoxylon Bull.	Hypoxylon	Hypoxylon	Hypoxylon	
<i>Induratia</i> Samuels, E. Mull. & Petrini	Induratia	Induratia	Induratia	
	Indui and	indun dind	Internet and a	Jumillera J.D. Rogers, Y
				M. Ju & San Martín
				(1997)
<i>Kretzschmaria</i> Fr.	Kretzschmaria	Kretzschmaria	Kretzschmaria	()
			Kretzschmariella Viégas	
Leprieuria Læssøe, J.D. Rogers&Whalley	Leprieuria	Leprieuria	Leprieuria	
Lopadostoma (Nitschke) Traverso	Lopadostoma	Lopadostoma	Lopadostoma	
	? Myconeesia Kirschst.	Lopuiosioniu	Lopuuosiomu	

**Table 3.** The fungal genera within the Xylariaceae family.

Source: Whalley (1996); Ju and Rogers (1996); Rogers, Ju and San Martín (1997); Duong et al. (2004); González et al. (2004).

# Table 3. (Continued).

Eriksson and Hawksworth (1993)	Læssøe (1994)	Whalley (1996)	Ju and Rogers (1996)	Others
	Nemania Gray emend. Pouzar	Nemania	Nemania	
	Obolarina Pouzar	Obolarina	Obolarina	
? Paucithecium Lloyd				
Penzigia Sacc.		? Penzigia		
Phaeosporis Clem.	Phaeosporis	Phaeosporis		
Phylacia Lév.	Phylacia	Phylacia	Phylacia	
Podosordaria Ellis & Holw.	Podosordaria	Podosordaria	Podosordaria	
Poroconiochaeta Udagawa & Furuya				
8				Poroleprieuria M.C.
				González, Hanlin, Ulloa
				E. Aguirre, (2004)
Poronia Willd.	Poronia	Poronia	Poronia	5 , ( )
Pulveria Malloch & Rogerson	(as Pyrenomyxa Morgan)	Pulveria	Pulveria	
Rhopalostroma D. Hawksw.	Rhopalostroma	Rhopalostroma	Rhopalostroma	
Rosellinia De Not.	Rosellinia	Rosellinia	Rosellinia	
Sarcoxylon Cooke	Sarcoxylon	Sarcoxylon	Sarcoxylon	
	? Seynesia Sacc.	2	ý	
Stilbohypoxylon Henn.		? Stilbohypoxylon	Stilbohypoxylon	
Stromatoneurospora S.C. Jong & E.E. Davis	Stromatoneurospora	Stromatoneurospora	Stromatoneurospora	
Thamnomyces Ehrenb.	Thamnomyces	Thamnomyces	Thamnomyces	
Theissenia Maubl.	Theissenia	Theissenia	Theissenia	
Thuemenella Penz. & Sacc.	Thuemenella	Thuemenella	Thuemenella	
Ustulina Tul. & C. Tul.			Ustulina	
Versiomyces Whalley & Watling		Versiomyces	Versiomvces	
			Vivantia J.D. Rogers, YM. Ju,	
			& Cand.	
Wawelia Namysl.	? Wawelia	Wawelia	Wawelia	
, ,				Whalleya J.D. Rogers, Y.
				M. Ju & San Martín
				(1997)
<i>Xylaria</i> Hill <i>ex</i> Schrank	Xvlaria	Xvlaria	Xvlaria	

Source: Whalley (1996); Ju and Rogers (1996); Rogers, Ju and San Martín (1997); Duong et al. (2004); González et al. (2004).

#### 2.2.1 Morphological taxonomy

Principally, the xylariaceous fungi have been characterised mainly on conventional methods regarding teleomorphic and anamorphic characteristics by using macroscopy and microscopy (Eriksson and Hawksworth, 1993; Læssøe, 1994; Rogers, 1994; Ju and Rogers, 1996; Whalley, 1996). Additionally, chemical characteristics have been accepted or widely used in fungal taxonomy (Whalley and Edwards, 1987; Whalley and Edwards, 1995; Stadler *et al.*, 2001; Stadler, Ju, and Rogers, 2004).

#### 2.2.1.1 Teleomorphic characteristics

#### A) Stromata

The stromatal characters of Xylariaceae are extremely variable in shape, size, and colour. They range from applanate, erumpent, effused, subglobose to globose, uniperitheciate, and upright forms. The flattened applanate and erumpent forms are found in the genera *Biscogniauxia*, *Jumillera*, *Whalleya*, and a few *Camillea* species (Figure 1). The superficial and widely effused types occur in *Nemania* and many taxa belonging to the genus *Hypoxylon*. Whereas subglobose to globose forms have been found in *Daldinia* and some species of *Hypoxylon*, and the uniperitheciate stroma is generally restricted to *Rosellinia* and *Astrocystis* (Figure 1). In *Xylaria*, *Kretzschmaria*, and *Rhopalostroma*, stromata are upright but some *Camillea* species, e.g. *C. leprieurii* Mont., have dimorphic forms, which are applanate or erect. The texture of stromata has also been emphasized being defined as hard, fairly hard, woody, and soft. However, these variations of features could result from environmental influences. In addition, moisture and light might affect pigmentation or degree of branching whilst host types or surface shapes of the substratum may

influence growth form (Miller, 1961; Rogers, 1979; Ju and Rogers, 1996; Whalley, 1996).

The colour of the stromatal surface is also an important feature in many species. Their coloration can, however, vary with age and environmental conditions (Miller, 1961). Thus, some species exhibit different stromatal colour depending on the stage and locality of the fungal growth. However, the stromatal colour has been proven to be more useful in the delimitation of taxa above species level with the application of KOH or ethyl acetate extractable pigments in *Daldinia* and *Hypoxylon* proving to be of taxonomic value (Martin, 1968; Greenhalgh and Whalley, 1970; Whalley and Greenhalgh, 1973; Whalley and Whalley, 1977; Ju and Rogers, 1996; Ju *et al.*, 1997; Stadler *et al.*, 2001; Stadler, Ju, and Rogers, 2004).

#### **B)** Perithecia

The perithecial characters of xylariaceous fungi are usually described as globose, ovoid, and obovoid to tubular (Figure 2). Their degree of protruding may be recorded as completely immersed, partially immersed or almost free. Their arrangement may be monostichous or polystichous, and they vary considerably in their dimensions (Luttrell, 1951; Rogers and Berbee, 1964; Mai, 1977; Rogers, 1967). The size of perithecia has been considered in combination with other characters. The characters of perithecia vary in detail at the species level, and might provide useful additional taxonomic information in the family (Jensen, 1985).



Figure 1. Stromata of the xylariaceous fungi; (a) *Camillea heterostromata* (Mont.) Læssøe, J.D. Rogers & Whalley (applanate form), (b) *Biscogniauxia schweinitzii* Y.-M. Ju & J.D. Rogers (applanate form), (c) *Hypoxylon bovei* Speg. (subglobose to globose form), (d) *Hypoxylon fusoideosporum* Y.-M. Ju & J.D. Rogers (subglobose to globose form), (e) *Rosellinia corticium* (Schwein.: Fr.) Sacc. (uniperitheciate form), (f) *Daldinia concentrica* (Bolt.: Fr.) Ces. & De Not. (subglobose to globose form), (g) *Kretzschmaria clavus* (Fr.: Fr.) Sacc. (upright form), (h) *Camillea leprieurii* Mont. (upright form), and (i) *Xylaria schweinitzii* (Berk. & M.A. Curtis) (upright form).

Source: Ju and Rogers (1997).



Figure 2. Perithecia of the xylariaceous fungi; (a) *Biscogniauxia schweinitzii* Y.-M. Ju & J.D. Rogers (tubular), (b) *B. dennisii* (Pouzar) Piuzar (tubular), (c) *Camillea leprieurii* Mont. (long spherical), (d) *C. bilabiata* Speg. (tubular), (e) *Hypoxylon chathamense* Y.-M. Ju & J.D. Rogers (spherical), (f) *H. hypomiltum* Mont. (obovoid to tubular), (g) *Nemania aenea* (Nitschke) Pouzar var. *macrospora* (J.H. Miller) Y.-M. Ju & J.D. Rogers (obovoid), (h) *N. serpens* (Pers.: Fr.) S.F. Gray var. *colliculosa* (Schwein.: Fr.) Y.-M. Ju & J.D. Rogers (obovoid), and (i) *H. fuscum* (Pers.: Fr.) Fr. (spherical).

Source: Ju and Rogers (1997).

#### C) Ostioles

The ostiole characters of xylariaceous fungi consist of two types, the umbilicate ostiolum and the papillate ostiolum. The umbilicate ostiolum is characterised by small circular depressions in the stroma which appear flush with the stromal surface. Umbilicate ostioles are found mainly in species belonging to Hypoxylon section Hypoxylon (Miller, 1961; Ju and Rogers, 1996) and in representatives of many other genera (Figure 3). An umbilicate ostiolum occurring sunken as in Biscogniauxia and some Camillea species is often termed punctate. In contrast, the papillate ostiolum is elevated above the surface of the stroma and, thus, appears as a small nipple-like projection. Papillate ostioles are found in most species of Nemania, Kretzschmaria, many species of Xylaria, and some species of Biscogniauxia (Figure 3). In several members of the section Annulata of Hypoxylon (Ju and Rogers, 1996) the papillate ostiolum is surrounded by a circular depression or disk which has been found to occur as the result of the sloughing off of the stromal surface in this region (Abe, 1986). Ju and Rogers (1996) recognised a bovei-type where the whole disk area is shed in one piece and the *truncatum*-type where the surface is gradually worn away to form the disk. The annulate ostiolum is also found in some species of Nemania, Kretzschmaria, and Xylaria. Therefore, the ostiolar type is an important taxonomic character in *Hypoxylon* and other xylariaceous genera.



Figure 3. Ostioles of the xylariaceous fungi; (a) *Biscogniauxia dennisii* (Pouzar)
Piuzar (papillate), (b) *B. reticulospora* Y.-M. Ju & J.D. Rogers (papillate),
(c) *Hypoxylon bovei* Speg. (papillate with disk), (d) *H. kretzschmariodes*Y.-M. Ju & J.D. Rogers (umbilicate), (e) *H. hypomiltum* Mont. (umbilicate with white substance), and (f) *Xylaria melanura* (Lév.) Sacc. (papillate).
Source: Ju and Rogers (1997).

#### **D)** Ascus and apical apparatus

Asci of most xylariaceous fungi usually contain eight spores expect *Wawelia* which has four spores (Minter and Webster, 1983; Lundqvist, 1992) and *Thuemenella* which has six spores (Samuels and Rossman, 1992). In general, the xylariaceous ascus is unitunicate, cylindrical, and terminates below in a short or long stipe. The ratio of the spore bearing part to the stipe is sometimes taxonomically useful such as in *Biscogniauxia* where the stipes are typically short. In *Xylaria*, *Kretzschmaria*, and *Nemania*, the stipes are invariably long.

The apical tip is usually rounded, and encloses an apical apparatus, which is usually amyloid stained blue in Melzer's iodine reagent (Figure 4). Some are occasionally reddish (dextrinoid) and some do not react visibly with iodine. The significance of the iodine reaction on the apical apparatus has been discussed by Eriksson (1966), Kohn and Korf (1975), and Nannfeldt (1976). The shape and size of the apical apparatus are one of the more important taxonomic features in the Xylariaceae (Munk, 1957; Carroll, 1963; Martin, 1969; Krug and Cain, 1974; Francis, 1975; Rogers, 1979; Læssøe et al., 1989; Ju and Rogers, 1996; Whalley, 1996). There are at least five types of apical apparatus which can be recognised. Firstly, an apical apparatus is constructed from stacks of smaller rings found in Hypocopra and Poronia (Krug and Cain, 1974; Jong and Rogers, 1969). Secondly, it is flattened and appears broader than high as in most species of Hypoxylon s. str. and Daldinia (Ju and Rogers, 1996; Ju, Rogers, and Martin, 1997) (Figure 4). Thirdly, it is discoid as found in *Biscogniauxia* (Ju and Rogers, 1996; Ju, Rogers, and González, 1997; Martin, 1967). Fourthly, it is rhomboid or diamondshaped but only in Camillea (Læssøe et al., 1989). Finally, it is higher than broad, often constricted sub-apically to appear uniform or inverted hat-shaped and is generally characteristic for Xylaria, Rosellinia, Kretzschmaria, and Nemania (Martin, 1967; Rogers, 1979; Van der Gucht, 1995; Whalley, 1996).



Figure 4. The apical apparatus forms of the xylariaceous fungi; (a) Nemania aenea (Nitschke) Pouzar var. macrospora (J.H. Miller) Y.-M. Ju & J.D. Rogers (higher than broad), (b) Hypoxylon rubiginosum Pers.: Fr. (broader than high), and (c) Camillea tinctor (Berk.) Læssøe, J.D. Rogers & Whalley (higher than broad).

Source: Ju and Rogers (1997).

#### E) Ascospores and germination slit

Ascospores of most xylariaceous fungi are usually described as single cell, with a smooth wall, light to dark brown in colour, with a conspicuous full-length germ slit (Rogers, 1979). In general, the ascospores are arranged in a single row within the ascus being uniseriate or obliquely uniseriate.

Most spores are subglobose, ellipsoid, oblong, fusiform, inequilaterally ellipsoid (where one side is flat to slightly concave and the other side is curved) to broadly crescentric, with ends either narrowly or broadly rounded, attenuated or apiculate. Subglobose, ellipsoid, oblong or fusiform ascospores are most common in the genera *Biscogniauxia* and *Camillea* whilst inequilateral ellipsoid spores are characteristics of the genera *Daldinia* and *Hypoxylon* s. str. Broadly crescentric spores are usual found among members of the genera *Kretzschmaria* and

#### Xylaria.

The colour of ascospores ranges from light brown to medium brown to dark brown, sometimes appearing almost black. Ascospores of *Camillea* are pale yellow or almost colourless. They lack germ slits or pores but they are characterised by ornamented spore walls readily seen by scanning electron microscope (SEM). Although most ascospores are mainly smooth, some are ornamented. The ornamentation in the genus *Camillea* varies from poroid, reticulate, ribbed, and echinulate-verrucose. Striate ascospore ornamentation has been found in members of the genus *Stromatoneurospora* (Jong and Davis, 1973), *Nemania chestersii* (Rogers & Whalley) Pöuzar (Rogers and Whalley, 1978), and *Biscogniauxia weldenii* (J.D. Rogers) Whalley & Læssøe (Rogers, 1977). There are also faint transverse striations oriented perpendicular to the long axis of the spores in some species of *Hypoxylon* s. str. section *Hypoxylon* (Rogers and Candoussau, 1982; Rogers, 1985; Van der Gucht and Van der Veken, 1992) and *Daldinia* (Van der Gucht, 1993). Thus, the spore ornamentation has been proven to be very useful in some xylariaceous species but SEM is required to observe it in most taxa.

Some species have a conspicuous hyaline outermost wall layer called the exospore (Child, 1932; Pouzar, 1979) or perispore (Rogers, 1965; 1969). These outer walls are commonly characteristic among *Hypoxylon* s. str. and *Daldinia* (Rogers, 1965; Beckett, 1976; Ju and Rogers, 1996). Whether the perispore is dehiscent or indehiscent in 10% KOH, smooth or ornamented is considered very useful taxonomic data at the species level in *Hypoxylon* s. str. (Ju and Rogers, 1996).

Most xylariaceous fungi have ascospores with germ slits, and germ slit forms have been recognised by most investigators (Vincens, 1918; Carrol, 1963; Krung and Cain, 1974; Martin, 1967; Rogers, 1979; Whalley, 1996). The germ slit is a constant and diagnostic feature of many well-circumscribed species. The important characters in describing the germ slit are shape, position on the spore, orientation along the long axis of the spore, and length. The germ slit can be straight, curved, spiraling, and even dotted. In Hypoxylon germ slits are usually straight but sometimes sigmoid (Figure 5). A few species of Hypoxylon exhibit peculiar germ slits. Some ascospores appear lacking germ slits or pores such as species of Camillea (Rogers, 1977b; Læssøe et al., 1989), Stromatoneurospora (Jong and Davis, 1978) and Nemania chestersii (Rogers and Whalley, 1978). The germ slit can be found on the ventral (concave) side such as the members of the genera Nemania, Kretzschmaria, and Xylaria, or the dorsal (convex) side such as in Daldinia and Hypoxylon s. str. The orientation can be oblique or parallel to the long axis of the spore. The length is short (less than spore length), or long (essentially the length of the spore). The germ slit, when present, is assumed to act as a site for germination providing an easy exit point for the germ tube and it may also facilitate uptake of water and nutrients. Ascospores lacking germ slits may have less elaborate wall structure or, alternatively, might have germination sites in the wall that are not obvious. The germ slit is a fissure in several of the inner wall layers which remain covered by the outermost layers until germination begins (Beckett, 1976).



Figure 5. Germ slit characters of the xylariaceous fungi. (a) *Biscogniauxia anceps* (Sacc.) J.D. Rogers, Y.-M. Ju & Cand (straight form) from Ju and Rogers (1997), (b) *Hypoxylon purpureonitens* Y.-M. Ju & J.D. Rogers (SUT004) (straight form), and (c) *Xylaria* sp. SUT155 (spiral form).

### 2.2.1.2 Anamorphic characteristics

Most species of xylariaceous fungi form anamorphs or an asexual stage. They are characterised by conidia which are holoblastically. They are usually pigmented, and have a broad, circular, flat to truncate base. The anamorphic characteristics of xylariaceous species have been proven valuable in closely related species, and were first reported by Chesters and Greenhalgh (1964). However, the major problem is the inability to obtain anamorphic cultures because the teleomorphic material might not be fresh and in good condition. The anamorphs can develop on the external surfaces of immature or maturing stromata or in close association with them. They are four major characteristics to their growth form.

Firstly, the anamorph develops on immature or mature stromata, or on the wood lying in close proximity to the stromata. The anamorph usually appears as a powdery layer, yellowish gray, gray or brown. The conidiophores develop either monematously (freely) or in a few cases they develop on synnemata. This type of growth form is commonly found in members of the genera *Biscogniauxia*, *Daldinia*, *Hypoxylon*, and *Nemania* (Chesters and Greenhalgh, 1964; Greenhalgh and Chesters, 1968; Jong and Rogers, 1972; Petrini and Müller, 1986).

Secondly, the anamorph develops on immature stromata covering the whole or a part of the surface or developing on specialized structures of the immature stromata. The conidiophores are organized in a dense regular palisade layer. This is the common form found in *Xylaria* (Rogers, 1985) and *Kretzschmaria* (Van der Gucht, 1995).

Thirdly, the anamorph develops separately from the stromata usually on distinctive structures. It is always produced earlier in the growing season than the stromata. The conidiophores form dense palisade layers. This growth form is commonly found in certain *Xylaria* species such as *X. cubensis* with its accompanying *Xylocoremium flabelliforme* (Schwein.: Fr.) J.D. Rogers state (Rogers, 1984; 1985) and *X. poitei* (Lév.) Fr. (Rogers and Callan, 1986).

Fourthly, the anamorph develops superficially on bamboo culms, and consists of a central cone of hyaline thin walled conidiophores. They are arranged in a compact palisade layer, which terminates apically in denticulate conidiogenous cells and are surrounded by sterile carbonaceous tissue. The perithcia develop beneath the conidiome and grow through it. And frequently the remnants of the conidiome persist as a rough ring on the perithecial stromata giving a stellate appearance as in *Astrocystis*. This type of anamorph also occurs in *Collodiscula* and the anamorphs have been assigned to the form-genus *Acanthodochium* Samuels, J.D. Rogers & Nagasawa (Samuels, Rogers, and Nagasawa, 1987; Ju and Rogers, 1990).

All xylariaceous anamorphs have hyaline to light brown conidiophores, and vary in the type of branching and development of the conidiogenous cells. The conidiophores are characterised by the manner of branching and the position of the conidiogenous cells. The relationship between anamorph and teleomorph is shown in Table 4.

Teleomorph	Anamorph	
Anthostomella	Geniculosporium Chesters & Greenh. (Martin 1969,=Nodulisporium	
	type 2a), Nodulisporium Preuss and Virgariella S. Hughes (Francis,	
	Minter, and Caine, 1980)	
?Ascotricha	Dicyma Boulanger (Hawksworth, 1971)	
?Ascotrichella	?Humicola-like (Valdosera and Guarro, 1988)	
Astrocystis	Acanthodochium Samuels, J.D. Rogers & Nagas. (Samuels, Rogers,	
	and Nagasawa, 1987; Ju and Rogers, 1990)	
Biscogniauxia	Geniculosporium (Eckblad and Granmo, 1978; Whalley and Edwards,	
	1985), Nodulisporium (Greenhalgh and Chesters, 1968; Callan and	
	Rogers, 1986; González and Rogers, 1993), Periconiella (Petrini and	
	Müller, 1986)	
Calceomyces	Nodulisporium (Udagawa and Ueda, 1988)	
Camillea	Xylocladium Syd. (Crane and Dumont, 1975; Læssøe, Rogers, and	
	Whalley, 1989; González and Rogers, 1993)	
?Chaeocarpus	Unknown	
Collodiscula	Acanthodocium (Samuels, Rogers, and Nagasawa, 1987)	
Daldinia	Nodulisporium (Chesters and Greenhalgh, 1964; Petrini and Müller,	
	1986)	
Engleromyces	Unknown	
Entonaema	Nodulisporium (Rogers, 1982)	
Euepixylon	Geniculosporium (Whalley, 1976)	
Helicogermslita	Unknown	
Holttumia	Unknown	
Нуросорга	Unknown	
Hypoxylon	Nodulisporium, Virgariella, Hadrotrichum Fuckel, Rhinocladiella	
	Nannf. (Martin, 1967; Greenhalgh and Chesters, 1968; Jong and	
	Rogers, 1972; Petrini and Müller, 1986)	
Induratia	Nodulisporium (Samuels, Müller, and Petrini, 1987)	
Jumillera	Unknown	

**Table 4.** The anamorph-teleomorph relationship within genera of the Xylariaceae.

Table 4. (Continued).

Teleomorph	Anamorph	
Kretzschmaria	Hadrotrichum (Petrini and Müller, 1986)	
Leprieuria	Geniculosporium (Samuels and Müller, 1980)	
Lopadostroma	Scolecosporous anamorph, Libertella-like (Ju, González, and	
	Rogers, 1993)	
Nemania	Geniculosporium (Chesters and Greenhalgh, 1964; Petrini and	
	Müller, 1986)	
Obolarina	Rhinocladiella-like (Candoussau and Rogers, 1990)	
?Penzigia	Unknown	
Phaeosporis	Sporothrix Hektoen & C.F. Perkins (Jong and Davis, 1974)	
Phaeosporis	Sporothrix Hektoen & C.F. Perkins (Jong and Davis, 1974)	
Phylacia	Geniculosporium (Rodrigues and Samuels, 1989)	
Podosordaria	Lindquistia Subram. & Chandrash.(Subramanian and	
	Chandrashekara, 1977; Rogers and Læssøe, 1992)	
Poroleprieuria	Unknown	
Poronia	Lindquistia (Subramanian and Chandrashekara, 1977; Stiers,	
	Rogers, and Russell, 1973)	
Pulveria	Unknown	
Rhopalostroma	Nodulisporium (Hawksworth and Whalley, 1985)	
Rosellinia	Geniculosporium, Dematophora R. Hartig, Nodulisporium (Petrini,	
	1992)	
Sarcoxylon	Unknown	
Seynesia	Acanthodochium (Hyde, 1995)	
Stilbohypoxylon	Unknown	
Stromatoneurospora	Unknown	
Thamnomyces	Nodulisporium (Samuels and Müller, 1980)	
Theissenia	Unknown	
Theumenella	Nodulisporium (Samuels, 1989; Samuels and Rossman, 1992)	
Theumenella	Nodulisporium (Samuels, 1989; Samuels and Rossman, 1992)	
Versiomyces	Unknown	
Wawelia	Anamorph described by Minter & Webster (1983) as being	
	geniculate but not assigned to a form genus	
Whalleya	Unknown	
Xylaria	Typically produced on developing stromata but no form genus yet	
	assigned Xylocoremium flabelliforme (Schwein .: Fr.) J.D. Rogers is	
	associated with X. cubensis (Rogers, 1984, 1985)	

Source: Whalley (1996); Ju and Rogers (1996); Rogers, Ju, and San Martín (1997); Duong *et al.* (2004); González *et al.* (2004).

Conidia of most xylariaceous fungi are all morphologically similar, and exhibit little variation except for moderate differences in overall shape and size. They are unicellular, subglobose, obovoid to ellipsoid, hyaline to light brown, and usually smooth. Since they are produced holoblastically, they all possess a basal scar indicating the former site of attachment to the conidiogenous cell (Greenhalgh, 1967; Stiers *et al.*, 1973; Koehn and Cole, 1975).

#### 2.2.2 Chemical taxonomy

Although secondary metabolites have not been accepted or widely used in fungal taxonomy, they are now known to be useful in the taxonomy of *Penicillium* (Frisvad and Samson, 1991; Lund and Frisvad, 1994; Frisvad *et al.*, 1998), *Aspergillus* (Kozakiewicz, 1994), *Fusarium* (Onji, Aoki, and Tani, 1994), and lichens (Culberson and Culberson, 1994). Fungal secondary metabolites have a great diversity of molecular structures, and frequently show taxonomic specificity in their production which usually occurs during the stationary phase of growth or the idiophase (Bulock, 1980; Whalley and Edwards, 1999).

In the Xylariaceae, there are several genera reported to produce pigments or other secondary metabolites in their stromata and cultures. Ju and Rogers (1996) characterised many species of genera of Xylariaceae with *Nodulisporium*-like anamorphs by conspicuous colours of their fruit bodies such as in *Hypoxylon* and *Daldinia* which extracted stromatal pigment colours in 10% KOH and employed as key features. Van der Gucht (1994) also used colours of organic extracts made with solvents such as acetone to include in species descriptions. These colours of extracted pigments are determined by comparison with a standard chart (Rayner, 1970). The concentration of colour-extracted pigments may vary with age and stages of preservation, however, they usually still contain the same metabolites found in young and fresh specimens, albeit at lower concentrations. In any case, the Xylariaceae is quite creative when it comes to the production of chemical diversity, hence their stromatal pigment colours usually result from the presence of a mixture of several metabolites. Pigments and other secondary metabolites can be separated according to their polarity, and detected as single component. The profile of secondary metabolites can be investigated using chromatographic methods such as thin layer chromatography (TLC) on *Hypoxylon* (Whalley and Whalley, 1977) and ultra-violet light or high performance liquid chromatography and diode array detection (HPLC-DAD) on *Daldinia, Entonaema, Rhopalostroma*, and other xylariaceous fungi (Andersen *et al.*, 2001; Stadler *et al.*, 2001; Mühlbauer *et al.*, 2002; Quang *et al.*, 2002; Stadler *et al.*, 2004).

The Xylariaceae has been shown to produce a large number of secondary metabolites which can be grouped as butyrolactones, dihydroisocoumarins, succinic acid, cytochalasins, and other compounds. These metabolites have been used to demonstrate the possible phylogenetic relationships (Whalley and Edwards, 1987). *Daldinia concentrica* was found to contain 4, 9-dihydroxyperylene quinone in its ascocarps (Allport and Bu'lock, 1958) whilst 1, 8-dimethyoxynaphthalene and its corresponding ether were produced in culture broth (Allport and Bu'lock, 1960). During the same period, Chen (1960; 1964) isolated rosellinic acid and diketopiperazine from cultures of phytopathogenic *Rosellinia necatrix*, and subsequently it was found to produce cytochalasin E (Aldridge *et al.*, 1972). Engleromycin, an epoxide of cytochalasin D, was later isolated from the xylariaceous

taxon, Engleromyces goetzii P. Henn. (Pedersen et al., 1980). Hypoxylon fragiforme was found to owe its orange to brick red stromatal colour to mitorubrin and its derivatives (Steglich et al., 1974) whilst Xylaria polymorpha Pers. produces a hydroxyphthalide derivative, xylaral, which develops a violet purple colour reaction with aqueous ammonia (Gunawan et al., 1990). Extensive studies have resulted in the characterisation of many secondary metabolites from a range of representatives of the family, and have demonstrated a remarkable diversity of chemical compounds produced. A considerable number of these metabolites have proven to be new (Whalley and Edwards, 1995). Most of metabolites produced by the representatives investigated can be grouped as dihydroisocoumarins and derivatives (Anderson et al., 1983), succinic acid and derivatives (Anderson et al., 1985), butyrolactones (Edwards and Whalley, 1979; Anderson et al., 1982), cytochalasins (Edwards et al., 1989), sesquiterpene alcohols (punctaporonins) (Edwards et al., 1988; Edwards et al., 1989), griseofulvin and griseofulvin derivatives (Whalley and Edwards, 1995), naphthalene derivatives (Whalley and Edwards, 1995), and long chain fatty acids (Adeboya et al., 1995).

Generally, the presence of these compounds can be seen to be closely related to systematic position, and the chemical data has proved invaluable in recognising associations between species and genera (Whalley and Edwards, 1995; Whalley, 1996). The dihydroisocoumarins are widely distributed throughout the family but they are probably more representatives of *Hypoxylon*, *Biscogniauxia* and *Camillea* (Whalley and Edwards, 1995). Butyrolactones, so far, appear to be restricted to *Nemania serpens* (Pers.: Fr.) Pouzar whilst cytochalasins are frequently encountered in species of *Xylaria*, *Rosellinia*, and members of the defunct section Primocinerea of Hypoxylon (Whalley, 1996).

Dreyfuss (1986) reported new cytochalasins in endophytic *Xylaria* species from tropic plants. A relationship between the production of some secondary metabolites, e.g. cytochalasins, and the phytopathogenicity of the isolates cannot be excluded (Whalley and Edwards, 1999). On the other hand, the production of secondary metabolites is increasingly used to clarify the taxonomic position of fungal taxa.

#### 2.2.3 Molecular taxonomy

Since the morphological characteristics of several fungal genera are frequently too limited to allow its identification, the molecular techniques are applied. The group of genes, which is most frequently targeted for phylogenetic analysis, is the ribosomal RNA genes (or rDNA). In addition, introns of several protein-encoding genes, such as the  $\beta$ -tubulin (O'Donnell, 1992; Tsai *et al.*, 1994), actin (Cox *et al.*, 1995), chitin synthase (Bowen *et al.*, 1992; Szaniszlo and Momany, 1993), acetyl coenzyme A synthase (Birch, Sims, and Broda, 1992), glyceraldehydes-3-phosphate dehydrogenase (Harmsen *et al.*, 1992), or orotidine 5'-monophosphate decarboxylase genes (Radford, 1993), can also be applied, and can provide the valuable information of molecular taxonomy.

#### 2.2.3.1 Ribosomal DNA

Ribosomal DNA (rDNA) is widely used for the inference of phylogenetic relationships because it is present in all living organisms, and different rates of evolution in different regions. Therefore, it makes rDNA useful for studies at different taxonomic levels (Bruns *et al.*, 1991). The sequences coding for nuclear ribosomal RNA (rDNA) have been chosen in many studies of phylogenetic systematics and evolutionary patterns of fungi (Okada et al., 1997). The fungi and most eukaryotes contain 80S ribosomes, which consist of two subunits, the large (60S) and small (40S) subunits. Each subunit consists of rRNA as a structural molecule and a number of associated proteins. The large subunit contains 28S, 5.8S and 5S rRNA molecules and the small subunit contains 18S rRNA molecule (Figure 6). Genes coding for rRNA are suitable signal molecules as the synthesis of ribosomes has been strongly conserved over evolution, due to the central role of ribosomes in gene expression. The rRNA genes for the rRNA subunits, although not varying greatly in length, contain both strongly conserved and variable regions within their sequences (Van de Peer, Chapelle, and Wachter, 1996). The genes for these rRNA molecules are also separated by the two external transcribed spacers (ETS) and the nontranscribed spacer regions (NTS), which contain the signals for rDNA expression (Figure 6). Both spacers are mainly called the intergenic spacer (IGS). The regions that lie between these RNAs are the two noncoding internal transcribed spacers (ITS1 and ITS2) (Hwang and Kim, 1999). The nucleotide sequences of the rDNA repeat unit have been detected by designed primers according to the highly conserved 18S and 28S regions (White et al., 1990). The most detailed information can be obtained by direct sequencing of the PCR products, which detect every single base-pair difference of the amplified fragment between different samples. Phylogenetic analysis using sequence data combined with mating compatibility studies has shown more promise for resolving phylogenetic relationships and understanding speciation for problematic species complexes in fungi (Bruns et al., 1991).



**Figure 6.** Schematic diagram of a tandem repeat unit of rDNA. Source: Hwang and Kim (1999).

#### A) Small-subunit ribosomal DNA (SSU rDNA)

The nuclear SSU rDNA (18S rRNA gene in eukaryote) is one of the most highly conserved DNA regions, and the size is approximately 1,800 bp (White *et al.*, 1990). The sequence analysis of 18S in most filamentous fungi has been used completely or over 600 bp in subunit. In particularly, the SSU has been studied to reconstruct deep phylogenetic branches that include kingdoms, phyla, classes, or orders (Field *et al.*, 1988; Abele *et al.*, 1989; Friedrich and Tautz, 1995; Aguinaldo *et al.*, 1997; Whiting, 1998).

#### B) 5.8S ribosomal DNA

The degree of nucleotide conservation of 5.8S rDNA, which is the smallest nuclear rDNA of the cluster, is similar to that of SSU rDNA, but its length (approximately 150 bp) is too short to contain enough phylogenetic information. Due to the short length in DNA sequence, it is not advisable to use the 5.8S rDNA region for phylogenetic reconstruction (Hwang and Kim, 1999).

#### C) Large-subunit ribosomal DNA (LSU rDNA)

Nuclear LSU rDNA is much larger than SSU rDNA approximately > 4,000 bp, and shows more variation in the rate of evolution of its different domains compared to the SSU rDNA. It has many divergent domains or expansion segments, so the size of the gene varies considerably among phyla. Nuclear LSU rDNA is known to be useful for examining phylogenetic relationships in slightly low categorical levels such as orders or families (Friedrich and Tautz, 1997; Hwang *et al.*, 1998; Whiting, 1998).

# D) The intergenic sequence (IGS) and the internal transcribed spacer (ITS) regions

Ribosomal DNA spacer regions, IGS and ITS, have been employed to resolve phylogenetic problems in lower categorical levels among genera, species, or populations (Morgen and Blair, 1998; Navajas *et al.*, 1998; Perera *et al.*, 1998). The size of IGS (approximately 4-5 kb) is far larger than those of ITS region (approximately 1 kb). In fungi, the ITS region is often between 600 and 800 bp in length. The ITS region, as well as the intergenic NTS repeat, shows much evolutionary change. Differences in these regions occur between species within a genus (Goosen and Debets, 1996). Several studies have demonstrated that the ITS region is often highly variable among morphologically distinct fungal species, but the intraspecific variation is low in most cases (Gardes and Bruns, 1991; Lee and Taylor, 1992). Due to the large size of the IGS, the ITS regions have been preferred to IGS in phylogenetic approach. However, the IGS has been used in restriction fragment length polymorphism (RFLP) of entire rDNA arrays (Wheeler, 1989). In ITS region from several distantly related evolutionary groups, the variation often consists of tandem arrays of repeat motifs of up to 10-bp length (Gonzalez *et al.*, 1990; Lee and Taylor, 1992; Vogler and DeSalle, 1994). These short repeat motifs are believed to be caused by slipped-strand mispairing or replication slippage (Levinson and Gutman, 1987; Li and Graur, 1991). The processes involve intra-helical mispairing during DNA replication, which results insertion or deletion of bases. The short repeat motifs derived from this process have also been observed in rpoC2, a plastid gene encoding the  $\beta$ " subunit of RNA polymerase in grasses (Cummings *et al.*, 1994). Once an array of repeat motifs has been established, it becomes increasingly prone to additional slipped-strand mispairing events and, thus, accumulation of repeats.

The appropriate region for phylogenetic analysis is very important. Most of such misuses are caused by the lack of understanding of properties of molecular markers or gene regions by the negligence in the categorical levels examined. The selection of molecular markers or gene regions is necessary because the selection of inappropriate molecular makers or gene regions can not explain the correctly phylogenetic relationships. For instance, for studies of relationships among closely related species, the use of nuclear rRNA coding regions (such as nuclear SSU, LSU, 5.8S rDNA) can be problematic, whereas nuclear rDNA spacers such as IGS or ITS appear to have fewer problems because of their higher variation. On the other hand, for deep levels of divergence, the proteins coding genes are saturated at the amino acid level, and highly conserved regions of rDNA are useful. Hwang and Kim (1999) summarized the appropriate categorical levels of commonly used molecular markers or gene regions in rDNA (Table 5). **Table 5.** The applicable categorical levels of each molecular marker or gene region

 in molecular taxonomic study. The bold lines indicate mainly applicable

 categorical levels of each molecular marker or gene region while the dot

 lines indicate less frequently applicable categorical levels



Source: Hwang and Kim (1999).

#### 2.2.3.2 Phylogenetic study

Phylogenetics is the area of research concerned with finding the genetic relationships between species (Baldauf, 2003). The basic idea is to compare specific features of the species, under the natural assumption that similar species (i.e. species with similar characters) are genetically close. The classic phylogenetics used mainly with physical or morphological features, whilst the modern phylogeny uses information extracted from genetic material mainly DNA and protein sequences. Phylogenetics is sometimes called cladistics because the word "clade", a set of descendants from a single ancestor, is derived from the Greek word for branch. Intuitively, the phylogenetic trees are drawn from the ground up like real trees (Figure 7a). However, as these trees get larger and more complex, they can become cluttered and difficult to read. As an alternative, the nodes can be expanded (Figure 7b), and turned the tree on its side (Figure 7c). Then, the tree grows left to right, and all the labels are horizontal. This makes the tree easier to read and to annotate. Thus, the widths of the nodes have no meaning. They are simply adjusted to give even spacing to the branches. To make things slightly more complicated, all branches can rotate freely about the plane of their nodes, then, all trees in Figure 7 are identical (Baldauf, 2003). Molecular phylogenetic trees are usually drawn with proportional branch lengths, which is the lengths of the branches correspond to the amount of evolution (roughly, percent sequence difference) between the two nodes they connect (Figure 7a-f). Thus, the long branches are more divergent than the one attached to short branches. Alternatively, trees can be drawn to display branching patterns only (cladograms), in which case the lengths of the branches have no meaning (Figure 7g), but this is rarely done with molecular sequence trees (Baldauf, 2003).

In the tree construction from nucleotide sequences, the first step is building the dataset. This means finding and retrieving sequences from the public domain. The main repository for these data is the public nucleotide database such as GenBank (U.S.A.), EMBL (Europe), and DDBJ (Japan) (Baldauf, 2003). Then, the next step is sequence alignment, which is the heart of the matter. The role of sequence alignment is to organize sequences so that homologous residues appear in the same column of the alignment. This is a relatively straightforward task for regions that have a highly conserved sequence. Regions of sequences that cannot be unambiguously aligned are normally not included in phylogenetic analyses.



**Figure 7.** Phylogenetic tree styles. All these trees have identical branching patterns. Source: Baldauf (2003).

The common program that has been widely used in multiple sequence alignment is CLUSTAL, which is freely available for use on all major computer platforms (Higgins *et al.*, 1998). This program takes an input set of sequences, and calculates a series of pairwise alignments, comparing each sequence to every other sequence, one at a time.

For phylogenetic tree construction, methods for calculating the trees fall into two general categories (Page and Holmes, 1998). These are distance-matrix methods, also known as clustering or algorithmic methods (e.g. the unweighted pair group method using arithmetric averages (UPGMA), neighbourjoining, Fitch-Margoliash), and discrete data methods, also known as tree searching methods (e.g. parsimony, maximum likelihood, Bayesian methods) (Page and Holmes, 1998; Graur and Li, 1999; Nei and Kumar, 2000; Baldauf, 2003). Distance is relatively simple and straightforward. The distance (roughly, the percent sequence difference) is calculated for all pairwise combinations of OTUs (operational taxonomic units), and then the distances are assembled into a tree. Discrete data methods examine each column of the alignment separately and look for the tree that best accommodates all of this information. The programs for phylogenetic construction are examples of PHYLIP, Mega, and PAUP\*, which are the most comprehensive and widely used (Felsenstein, 1985; Hall, 2000; Swofford, 1999).

However, the methods of phylogenetic tree construction may assign organisms incorrectly to positions along a phylogenetic tree as a result of "false identity" in sequence positions. The extent of this problem varies from one method to another. Thus, the next step in constructing a sequence phylogeny is to assess the reliability of the inferred branching pattern. This is often accomplished by a bootstrap analysis (Felsenstein, 1985). Bootstrap procedures involve construction of new sequence sets by resampling with replacement sites (columns) of the original set, building a tree for each new set, and calculating the percentage of times. A cluster reappears in the bootstrap replications. This percentage is called the bootstrap value, and clusters with a bootstrap value >95% are widely considered to reflect correct relationships (Felsenstein, 1985).

#### 2.2.3.3 Molecular studies of the Xylariaceae

Since the morphological and biochemical characteristics of the Xylariaceae are frequently too limited, molecular techniques have, therefore, been applied.

Lee *et al.* (2000) analyzed 18 species of *Xylaria* and related genera by using nuclear ribosomal ITS1-5.8S-ITS2 sequences. Species of selected *Xylaria* were divided into three groups, and phylogenetic analysis of these was also supported by a set of signature nucleotides of ITS1-5.8S-ITS2 sequences. Group A consisted of *Xylaria arbuscula*, *Xylaria mali*, and *Xylaria apiculata* Cooke, whereas group B consisted of *Xylaria cornu-damae* (Schw.) Fr., *Xylaria longipes* Nitschke, *Xylaria acuta* Peck, *Xylaria castorea* Berkeley, *Xylaria enteroleuca* (Spegazzini) Martin, and *Xylaria fioriana* Saccardo. Group C included *Xylaria polymorpha* and *Xylaria hypoxylon* (L.: Fr.) Greville. In contrast, *Xylaria cubensis* appeared to be separated from other *Xylaria* species. The results showed that a few characteristics based on ascospores, perithecia and stromata, support grouping of *Xylaria* inferred from the molecular data. But there seems to be no character of universal significance that can justify the present phylogenetic results. It may indicate that convergent evolution of characters occurred many times within *Xylaria* species. Such possible changes in convergent evolution along with variations associated with developmental stages of stromata, might have caused confusions in identifying and classifying *Xylaria* species. Phylogenetic analysis based on molecular data such as ITS sequences of the present study proved to be very practical for taxonomic investigations at specific or generic levels in identification or classification of fungi of highly variable morphology like *Xylaria*.

Molecular and morphological investigations of Daldinia in Northern Europe have also been undertaken by Johannesson et al. (2000). Since the study of Daldinia was undertaken by Ju et al. (1997), which was based on morphological and cultural characteristics, it has proven difficult to name collections from Northern Europe. The confusion over the typification of especially the type species of the genus has also created problems. Therefore, five taxonomic entities of Daldinia concentrica, Daldinia cf. fissa, Daldinia grandis, Daldinia loculata, and Daldinia cf. petriniae, that were found exclusively on burnt wood, were defined based on both morphotaxonomical and ITS-sequence criteria. The results showed that at least five different taxa of Daldinia are present in Northern Europe, and the preference for burnt hosts has either been gained or lost more than once in the history of the genus. Later, Stadler et al. (2001) studied the secondary metabolite profiles coupled with DNA fingerprints of Daldinia. They selected 18S rDNA to amplify and digested DNA fragments with three different restriction enzymes, HpaII, HaeIII, and TaqI. Then the DNA restriction patterns were used to construct the phylogenetic tree according to the unweighted pair group method using arithmetric averages (UPGMA). The results showed clearly within species.

# For the genus Hypoxylon, Sanchez-Ballesteros et al. (2000)

studied the phylogenetic relationships of Hypoxylon and its allies, the complete DNA sequences of the ITS regions (including the 5.8S rRNA gene) from 41 isolates were determined, then aligned and processed for phylogenetic reconstruction, and critically compared to the available taxonomic information. Their results generally agreed with the current concepts and limits established for the genus by Ju and Rogers (1996). The species and varieties of Hypoxylon in the sense of modern authors appear to be a monophyletic group within the Xylariaceae. However, the recent infrageneric division of Hypoxylon into sections Hypoxylon and Annulata (Ju and Rogers, 1996) was not supported by this limited molecular phylogenetic analysis. In another study, Mazzaglia et al. (2001b) confirmed the efficacy of the 5.8S-ITS2 sequence analysis in phylogenetic studies of Hypoxylon fragiforme, Hypoxylon multiforme and related genera. The analysis confirmed that Hypoxylon is a taxonomically and phylogenetically separated taxon from Biscogniauxia and Entoleuca. Moreover, Hypoxylon fragiforme isolates formed a group separated from the single isolate of Hypoxylon multiforme. Although clearly belonging to the same genus, they were once recognised as being very closely related (Miller, 1961). However in the revision of Ju and Rogers (1996), these two species were separated with H. fragiforme being placed in section Hypoxylon and H. multiforme in section Annulata. This was on the basis of absence (Hypoxylon) or presence (Annulata) of a layer of carbonaceous stromatal tissue enclosing the perithecia (Ju and Rogers, 1996).

For *Biscogniauxia*, Mazzaglia *et al.* (2001a) developed a polymerase chain reaction (PCR) assay to detect *B. mediterranea* in asymptomatic tissues of *Quercus cerris*. They designed two specific primers (MED1 and MED2) by

comparison of sequences of ITS1 and ITS4 of 21 isolates of *B. mediterranea* and related species. Both primers were able to detect *B. mediterranea* DNA in the host tissues at picogram quantity of target DNA. The reliability of the results was confirmed by Southern blot analysis.

In addition, Platas *et al.* (2001) found a simple tandem repeat sequence in the ITS1 region of the rDNA of members of order Xylariales. The number of repetitions detected ranged from one to six, and they could be found in pure tandem or interspersed. These replications could have been generated by slipped strand mispairing. The presence of this sequence increases the normal rate of divergence in the ITS1 of the Xylariales.

On the basic of published data to date, molecular taxonomy may be applied and prove to be valuable as a standard technique for identification of members of the Xylariaceae. Therefore, ribosomal DNA subunit sequence analysis of selected xylariaceous fungi and their comparison with the available sequences on databases will greatly help in their identification especially in the absence of a teleomorph or where morphological characteristics are insufficient to clearly separate closely related species. However, it will be necessary to greatly enlarge the available data by including more genera and by increasing the number of isolates examined for each species. The reliable identification of teleomorphic materials using the conventional taxonomy will be an important prerequisite to ensure validity of molecular data deposited in databases.

# 2.3 Problematic groups in the systematic of the xylariaceous fungi

Since the xylariaceous fungi are cosmopolitan fungi and often exhibit high variation in morphology depending on localities of collection, stage of development, and criteria of identification, there are problems in recognizing and delimiting some of the genera and species.

#### 2.3.1 Group I: Astrocystis and Rosellinia

According to the broadly accepted current concept of the genus, *Rosellinia* is delimited within the Xylariaceae by five main characters: the stromata are uniperitheciate (rosellinioid), superficial, subglobose, associated with a hyphal mat usually called subiculum, and associated with a *Geniculosporium*-like anamorph (including *Dematophora* R. Hartig and *Geniculosporium* Chesters & Greenhalgh). The delimitation of *Rosellinia* led L. Petrini (1992) to move taxa excluded from this genus to *Amphisphaerella*, *Anthostromella*, *Astrocystis*, *Coniochaeta*, *Xylaria*, and other sordariaceous or xylariaceous genera.

Astrocystis Berk. & Broome is based on Astrocystis mirabilis Berk. & Broome as a type species, which occurs on bamboo and features a skirt or volva on the perithecial stroma (Berkeley and Broom, 1875). The stellate aspect of the volva led the authors of the species name to provide a somewhat fanciful illustration that gives the impression that *A. mirabilis* looks exactly like a minute earth star (Geaster) (Berkeley and Broome, 1875). Penzig and Saccardo (1904) recognised the strong relationship of *Astrocystis* with *Rosellinia* De Not., and noted that the illustrations provided by Berkeley and Broome are "strongly fictitious". Diehl (1925) published a detailed account of *A. mirabilis*, including its nomenclatural and taxonomic history. He had a broad concept of ascospores as "…acuminate to rounded, elliptical, narrow to broad, light brown becoming dark brown and subopaque when mature 10-21 x 4-2  $\mu$ m, chiefly 11-13 x 5-7  $\mu$ m..." Diehl's expanded concept of the species resulted, in part, from his acceptance of *Rosellinia bambusae* P. Henn. as a synonym of *A. mirabilis*.

Some disagreement over the status of the genus *Astrocystis* Berk. & Br., which accommodates *Rosellinia*-like fungi devoid of subiculum but with stromata splitting the host surface or with a carbonaceous extension at the base, associated with an *Acanthodochium* anamorph, persists between different authors. *Astrocystis* is recognised by Petrini (1993), and Læssøe and Spooner (1994), but synonymized with *Rosellinia* by Ju and Rogers (1990) and San Martín and Rogers (1994). Ju and Rogers (1990) have examined type and other materials identified as *Astrocystis mirabilis*. Using ascospore and stromatal features, these collections can mostly be divided into two distinct groups corresponding to *A. mirabilis* in the original sense and *R. bambusae*. The division is strongly reinforced by data from cultures obtained from recent field collections. Cultural characters differ between representatives of these groups. Each produces a distinctive anamorph in nature and in culture that is referable to *Acanthodochium* Samuels. They, thus, consider *R. bambusae* and *A. mirabilis* (as *Rosellinia*) to be distinct species.

The genus *Rosellinia* appears to be poorly represented in Thailand. Only two species, *R. necatrix* and *R.* cf. *procera*, were reported by Thienhirun (1997) and for *Astrocystis* only one species, *A. mirabilis*, has been reported (Thienhirun, 1997). It appears to be more common in peninsular Malaysia (Whalley, 2001). Since there are not clear separation of these genera and since their anamorphs are entirely different the molecular examination could determine their true relationship.

#### 2.3.2 Group II: Camillea

The genus *Camillea* was erected from other xylariaceous fungi, which possess erect cylindrical or short discoid black stromata and have a hard carbonaceous crust (Fries, 1849). Camillea was reviewed by Læssøe et al. (1989) when the genus was considerably enlarged with many species formerly placed in the genera Nummularia and Hypoxylon section Applanata (Miller, 1961). Camillea is characterised by applanate or cylindric stroma, erumpent through bark, and perithecia completely immersed. The apical apparatus of the ascus is vase- to urn-shaped, domeshaped or somewhat diamond-shaped. Ascospores are light-coloured, ornamented, lacking germ slits, and without a loosening perispore (Læssøe et al., 1989). The ascospores of most *Camillea* species appear smooth by light microscopy but they appear characteristically ornamented by SEM with warts, spines, pits, reticulations or to be longitudinally ribbed (Læssøe et al., 1989; Rogers et al., 1991; San Martín, Gonzales, and Rogers, 1993; Whalley, 1995; Whalley, 1996; Whalley et al., 1999). Læssøe et al. (1989) recognised 28 species and varieties of Camillea of which C. obularia (Fr.) Læssøe, J.D. Rogers & Lodge (as C. broomeiana and C. tinctor were the only species known from outside the New World, with C. tinctor exhibiting a widespread distribution. The discovery of three new species of Camillea from Mexico (San Martín, González, and Rogers, 1993) maintained this pattern until the recent discoveries of C. selangorensis (Whalley et al., 1996) and C. malaysianensis described from Kuala Selangor, Malaysia. Camillea selangorensis was later reported from Thailand (Whalley et al., 1999).

*Camillea leprieurii* Mont. has a dimorphic form, erect (camilleoid) form and applanate (expanded, "hypoxyloid" form), which had been recognised by
Patouillard (1888). He stated that the applanate *H. melanaspis* Mont. was the "forme étalée" or expanded state of C. leprieurii (Mont.) Mont. The examination of ascospores of C. leprieurii and H. melanaspis by SEM revealed an intricate ornamented ascospore wall composed of anastomosing ridges overlying a regular ribbed substructure in which the ribs are orientated perpendicular to the ridges (Rogers, 1977; 1979). Although he was not convinced that these two forms were the same species, he concluded that they have a close relationship (Rogers, 1979). Læssøe et al. (1989) explained the characteristics of both dimorphic forms of C. leprieurii. In erect form, the stromata are erumpent through bark, cylindrical, seated on slightly broader disc, which remains after broken off stromata. The stromata are also apex discoid-depressed with narrow rounded margin, brittle, black or with thin, flaky white ectostroma, initially with brown fungus and host covering, sometimes with felt-like dark brown subiculum of old *Xylocladium* anamorph. For applanate form, the stromata is erumpent through bark, plano-convex with plane ostiolar part, margin without rim, circular, orbicular or confluent, shiny black ('polished') or with flaky white ectostroma, initially with brown fungus (Læssøe et al., 1989). Both forms of C. leprieurii are distributed in Bolivia, Brazil, Colombia, Ecuador, Franch Guiana, Guyana, Nicaragua, Panama, Peru, Puerto Rico, Surinam, and Venezuela, but they have not been recorded in Thailand or Southeast Asia.

*Camillea tinctor* is characterised by stromata which is orbicular to elongate, and applanate with a slightly convex centre. Ascospores are smooth by light microscopy with distinct poroid ornamentation by SEM (Læssøe *et al.*, 1989; Rogers *et al.*, 1991; San Martín, Gonzales, and Rogers, 1993; Whalley, 1995; Whalley, 1996; Whalley *et al.*, 1999). The stromata of *C. tinctor* is usually accompanied by yellow

staining of the wood immediately beneath. In Thailand, *C. tinctor* has been first recorded by Thienhirun (1997), and exhibits a wide distribution from North to South. In addition, *Camillea tinctor* occurs in neighbouring countries.

*Camillea selangorensis* is characterised by stromata which is circular to orbicular, slightly elevated, and 2-3 thick with a slightly raised rim. Ascospores are minutely warted by light microscopy, strongly verrucose by scanning electron microscopy, and the type locality is lowland forest bordering on mangrove (Whalley, 1995; Whalley *et al.*, 1999). The discovery of *C. selangorensis* (Whalley *et al.*, 1995) provided clear evidence that *Camillea*, once considered to be a New World genus, has greater world wide representation than is generally believed. In Thailand, *C. selangorensis* was discovered from a similar ecological situation in Phuket Island (Whalley *et al.*, 1999). This suggested that this species might be expected in similar areas elsewhere in the region.

#### 2.3.3 Group III: Daldinia

The genus *Daldinia* is characterised by conspicuous internal alternating ring zones, which presently comprises of about 25 species (Stadler *et al.*, 2004). The type of the genus is *D. concentrica* which was firstly described by Bolton (1789) in Great Britain. Lloyd (1924) and Child (1932) were among the first to study the biology of *Daldinia*, and gave evidence on the existence of several species within the genus. Tropical species of the genus were studied as well. The surveys of *Daldinia* are now available from countries such as Papua New Guinea (Van der Gucht, 1994; 1995), Mexico (San Martín, 1992), and Thailand (Thienhirun, 1997). The current taxonomy of *Daldinia* is outlined in the latter monograph and subsequent additions by

Rogers *et al.* (1999), Ju, Vasilyeva, and Rogers (1999), Stadler *et al.* (2001) and Stadler, Baumgartner, and Wollweber (2001). Species of *Daldinia* are segregated by the combination of anamorphic and teleomorphic characters and by their colours of stromatal pigments in 10% KOH. The anamorphic states of *Daldinia* spp. and allies are morphologically rather similar, ranging from *Nodulisporium*-like to *Sporothrix* and *Virgariella*-like and further branching patterns (Stadler *et al.*, 2001).

Daldinia concentrica (Rogers et al., 1999; Johannesson, Læssøe, and Stenlid, 2000; Stadler et al., 2001) is now generally accepted to occur primarily in Western and Northern Europe in temperate regions, and its stromata is preferentially encountered on *Fraxinus* whereas *Daldinia eschscholzii* (Ehrenb.: Fr.) Rehm is a widely distributed species of subtropical and tropical climates (Van der Gucht, 1994; Ju et al., 1997). The stromata of *D. eschscholzii* has extractable pigments in KOH, whose colours and intensities were weaker than of *D. loculata* and *D. fissa*, but similar to those observed in *D. concentrica*. According to the current definition of *D. concentrica*, this species cannot be easily distinguished from *D. eschscholzii* by the colour of stromatal pigments alone. However, both species differ in several morphological features. Culture of *D. eschscholzii* generally produces rather small conidia, never exceeding 6  $\mu$ m in length and 3.5  $\mu$ m in width. Moreover, *D. eschscholzii* is characterised by having smaller ascospores, and its stromatal surface is not crackled into a fine network in mature specimens (Ju et al., 1997; Stadler et al., 2004).

Recently, Stadler *et al.* (2004) proposed five new species separated from *D. concentrica* and *D. eschscholzii*. The new species are *D. macaronesica* M. Stadler, Wollweber & J. Castro, *D. martinii* M. Stadler, Venturella & Wollweber, D. raimundi M. Stadler, Venturella & Wollweber, D. palmensis M. Stadler,Wollweber & H-.V. Tichy, and D. vanderguchtiae M. Stadler, Wollweber & Briegert.

*Daldinia macaronesica* differs from *D. eschscholzii*, *D. palmensis*, and *D. vanderguchtiae* in size, morphology and ornamentation of its ascospores and in its anamorphic characters. Stadler *et al.*, (2004) reported *D. macaronesica* is a close relative of *D. concentrica* but it differs in ascospores. They are more slender, show a wider range in size and bear a more conspicuous ornamentation than those of *D. concentrica*. In addition, *D. macaronesica* shows apparent host specificity for *Ocotea foetens*, a plant endemic to the Macaronesian Islands (Kunkel, 1993).

Daldinia martinii differs from D. concentrica and D. eschscholzii in anamorph form. Daldinia martinii has Sporothrix to Virgariella and Nodulisporiumlike forms.

*Daldinia raimundi*, which was originally reported as *D. concentrica pro parte* (Venturella *et al.*, 2001), differs in more conspicuous ornamentation of perispore by SEM. Moreover, the ascospore size of *D. raimundi* is in the range of *D. eschscholzii* (Ju *et al.*, 1997) rather than in the one typically found in *D. concentrica*.

Daldinia palmensis was originally identified as *D. eschscholzii*. The SEM characteristics of *D. palmemsis* ascospores were found in agreement with *D. eschscholzii* found from around the world. Notably, in contrast to the former species, the germ slit of the ascospores of *D. palmensis* may either be straight or slightly undulate. However, only a detailed study of the anamorph revealed significant differences to the former species (Stadler *et al.*, 2004). The conidiophores of *D. palmensis* are *Nodulisporium*-like or *Sporothrix*-like forms whilst those of *D. eschscholzii* are *Nodulisporium*-like in form.

*Daldinia vanderguchtiae* is peculiar among the concentricol-containing taxa of *Daldinia* in having smooth ascospores by SEM. Otherwise its ascospores resemble those of *D. eschscholzii* in size range as well as in shape. However, conidiophores approaching a *Virgariella*-like branching pattern as defined in Ju and Rogers (1996) were hitherto only seen in a culture of *D. grandis* Child 11932 from New Zealand (Ju *et al.*, 1997), and in *D. martini*, but they have never seen in *D. eschscholzii*.

In Thailand, three species of *Daldinia* had been reported since 1963. Carrol (1963) recorded *D. eschscholzii* from Chiang Mai Province and *D. concentrica* was recorded by Phanichapol (1968), Cansrikul (1977) and Schumacher (1982) whilst Ju, Rogers and San Martin (1997) described *D. bambusicola* for a distinctive taxon associated with bamboo and having a Southeast Asian distribution. Although intensive collection has been undertaken by Thienhirun (1997) in the Doi Chiang Dao area and similar forests in Chiang Mai Province, where Schumacher (1982) reported *D. concentrica* as occurring, it was *D. eschscholzii* not *D. concentrica* which was found there. Carroll's record of *D. eschscholzii* is from the nearby Doi Suthep (Carroll, 1963). Thienhirun (1997) believed that the former recorded of *D. concentrica* in fact represent *D. eschscholzii*. Certainly *D. concentrica* is more frequently associated with temperate regions and *D. eschscholzii* with tropics and subtropics (Ju *et al.*, 1997). Thienhirun (1997) reported that five species, *D. cf. caldariorum, D. loculata, D. eschscholzii, D. bambusicola*, and *Daldinia* taxonomic species 1, were found in Thailand.

#### 2.3.4 Group IV: Hypoxylon

The genus *Hypoxylon* delimitation has been rearranged several times among mycologists (Miller, 1961; Martin, 1968; Ju and Rogers, 1996). Miller's monograph of Hypoxylon was divided into four sections, Hypoxylon, Annulata, Applanata and Papillata (Miller, 1961). His monograph was strongly relied on stromatal form, texture, and nature of the ostiole. Consequently, this monograph failed to recognise the relationships between groups of species. The section Applanata sensu Miller has since been redistributed between Camillea and Biscogniauxia (Læssøe, Rogers, and Whalley, 1989; Whalley, Læssøe, and Kile 1990; González and Rogers, 1993) whereas member of the section Papillata subsection Primocinerea (Miller, 1961) have been allocated to a range of genera including Nemania (Pouzar, 1985), Rosellinia (Petrini, 1992), and Euepixylon (Læssøe and Spooner, 1994). The genus Hypoxylon was revised by Ju & Rogers (1996) using four major criteria to define the genus; Nodulisporium-like anamorphs having stromata unipartite, never erect, with a solid and homogenous basal tissue below the perithecial layer. They divided Hypoxylon in two sections, section Hypoxylon and section Annulata, containing at least 130 accepted species and varieties. Ju and Rogers (1996) were able to utilize data, which absent from the monograph of Miller (1961) such as ascospore ornamentation using SEM, form of the apical apparatus of the ascus, germination slit morphology and the colour of stromatal pigments extracted with 10% potassium hydroxide (10% KOH). However, the revision of the genus identification of certain Hypoxylon species remains problematic resulting from considerable variation in species characteristics. This has proved to be most pronounced when considering tropical species of the Annulata especially Hypoxylon nitens, H. moriforme, H. bovei

var. *microspora*, *H. purpureonitens*, *H. stygium*, and *H. stygium* var. *annulata*, when there are variations and overlap in their morphological features.

#### 2.3.5 Group V: Xylariaceous endophytes

There are eight xylariaceous genera recorded as endophytes, Anthostomella (Petrini and Petrini, 1985; Petrini et al., 1987), Biscogniauxia (Petrini and Müller, 1986), Daldinia (Petrini and Petrini, 1985; Petrini and Müller, 1986), Hypoxylon (Petrini and Müller, 1986), Kretzschmaria (Petrini and Petrini, 1985; Petrini and Müller, 1986), Nemania (Petrini and Petrini, 1985; Petrini and Rogers, 1986), Rosellinia (Petrini and Petrini, 1985; Petrini, 1992), and Xylaria (Petrini and Petrini, 1985). The identification of xylariaceous endophytes is often difficult since they fail to produce suitable diagnostic features. And it is very infrequently to form their teleomorph in culture. The situation regarding tropical endophytes is much more complex as a result of their abundance and impressive diversity (Rodrigues and Samuels, 1990; Whalley, 1993; Whalley, 1996). It is doubtful whether differentiation of species on the basis of cultural and anamorphic features alone will ever be possible since differences between individual species are often insufficient to allow for absolute identifications to be made (Petrini, Petrini and Rodrigues, 1995). However, studies of Xvlaria indicate that a combination of morphological characters and biochemical analyses might enable satisfactory identifications to be made (Brunner and Petrini, 1992; Rodrigues, 1992; Rodrigues, Leuchtmann, and Petrini, 1993). There are also indications that secondary metabolite profiles from endophytic isolates might be matched with those obtained from cultures derived from teleomorphic material thus enabling identity to be established (Whalley and Edwards, 1995; 1999).

A preliminary study of *X. cubensis* comparing secondary metabolites e.g. cubensic acid (Adeboya *et al.*, 1995) obtained from teleomorphic derived cultures with those produced by endophytic *Xylaria* isolates from *Euterpe oleracea* leaves confirmed the finding of Rodrigues that they belonged to *X. cubensis* (Whalley, 1996; Rodrigues, 1992). Ongoing research on secondary metabolites of the Xylariaceae was included endophytic isolates to determine the suitability of this approach for the identification of endophytic members of the family.

# **CHAPTER III**

# **MATERIALS AND METHODS**

#### 3.1 Chemicals, reagents, and media

#### 3.1.1 Morphological taxonomic study

Reagents used for teleomorphic characteristics of xylariaceous fungi were Melzer's solution (Appendix 1.2A) to determine whether or not the ascus apical ring blued (the amyloid iodine reaction), and 10% potassium hydroxide (KOH) (Fluka, Sigma-Aldrich Chemical Company, U.S.A.) to determine whether or not the perispore, and to extract colour pigments of stromata.

The medium used for cultural characteristic study was potato dextrose agar (PDA) (Appendix 1.1A).

#### 3.1.2 Chemical taxonomic study

The medium used for fungal growth in metabolite extraction was 2% malt extract broth (MEB) (Difco, Difco Laboratories, U.S.A.) containing 6% glucose (Merck, Merck KGaA, Germany).

Reagent used for fungal metabolite extraction was ethyl acetate (Sigma, Aldrich Chemical Company, U.S.A.). The components of mobile phase in thin layer chromatography (TLC) were toluene, ethyl acetate, and acetic acid (Sigma). Reagents used for chemical detection of TLC plate were p-nitroaniline and anisaldehyde (Sigma).

#### 3.1.3 Nucleic acid study

Reagents used for genomic DNA extraction were lysis buffer (Appendix 3.1A); phenol, chloroform, isoamyl alcohol (Merck) to purify genomic DNA; isopropyl alcohol (Merck) to precipitate genomic DNA; 70% ethanol to wash genomic DNA pellet. Agarose (Promega, Promega Coporation, U.S.A.) was used to detect DNA by agarose gel electrophoresis.

Reagents used for Polymerase Chain Reaction (PCR) amplification were the 10X PCR buffer (Sigma), dNTPs (dATP, dCTP, dGTP, and dTTP) (Invitrogen, Invitrogen life technologies, U.S.A.), and *Taq* DNA polymerase (Sigma). The oligonucleotide primers were ordered from the Science Pacific Company, Ltd., Thailand. The QIA-quick PCR purification kit (Qiagen, Qiagen Coporation, U.S.A.) was used in PCR purification. The BigDye Terminator Ready Reaction kit (Perkin Elmer, Applied Biosystems Inc., U.S.A.) was used for the nucleotide sequencing reaction.

#### 3.2 Instrumentation

Instruments required for morphological taxonomic and nucleic acid studies of xylariaceous fungi were located at the Instrument Buildings of the Centre for Scientific and Technological Equipment, Suranaree University of Technology, Nakhon Ratchasima, and specimen comparison of collected fungi with reference collections were performed at the Royal Forest Department, Bangkok, Thailand.

Instruments required for chemical taxonomic studies were located at the School of Biomolecular Sciences, Liverpool John Moores University, Liverpool, U.K.

Instruments required for DNA sequencing were located at the Biotechnology

and Development Office, Department of Agriculture, Pathumthani, Thailand.

## 3.3 Collection of xylariaceous fungi for taxonomic studies

The teleomorph stage of xylariaceous fungi were collected during rainy season, June to December in years 2002 and 2003, from 14 different locations in Thailand as described in Table 6. All specimens were recorded for their collection dates, locations, and habitats. The collections were kept as herbarium by freezing at -20°C for one week to destroy insects and/or mites and then drying at 37°C for approximately 7 days before keeping in sealed plastic bags.

Year	Location
2002	Chiang Mai Province
	Nakhon Ratchasima Province
2003	Phu Luang, Nakhon Ratchasima Province
	Nong Rawieng, Nakhon Ratchasima Province
	Burirum Province
	Chaiyaphum Province
	Plant Nursery of the Royal Forest Department, Ratchaburi Province
	Suranaree University of Technology, Nakhon Ratchasima Province
	Kanchanaburi Province
	Petchaboon Province
	Songkhla Province
	Trad Province
	Yasothon Province
	Chiang Rai Province

**Table 6.** Locations and time of specimen collection in this study.

# 3.4 Morphological taxonomic studies of the problematic groups in xylariaceous fungi

#### 3.4.1 Macroscopic study

The teleomorph of the collected xylariaceous specimens were observed for shape, size, colour of their stromatal surface, perithecia, and ostioles using the Olympus Stereomicroscope SZX fitted with the Olympus Digital Camera DP11 (Olympus, Olympus Optical Co., Ltd. Japan). Colours of stromatal surface were determined comparing to the Rayner Mycological Colour Chart (Rayner, 1970). The stromatal pigments of *Hypoxylon* and *Daldinia* were extracted in 10% potassium hydroxide (KOH), leaved for one minute, and observed the colour compared to the colour chart (Ju and Rogers, 1996; Ju, Rogers, and San Martín, 1997).

#### 3.4.2 Microscopic study

The collected xylariaceous fungi were observed for colour, shape, and size of ascospores by mounting with distilled water and using the Olympus Compound Microscope BX51 fitted with the Olympus Digital Camera DP11 (Olympus). The ornamentation of perispore and epispore were observed in 10% KOH to determine dehiscence or indehiscence. The apical apparatus of ascus was examined for amyloid reaction including shape and sized by using Melzer's iodine reagent. The type of germ slit was included.

Ascospore ornamentation of some xylariaceous isolates was also observed using SEM. Dried xylariaceous stromata were attached to aluminium stubs with Dag metallic paint, coated with gold, and examined using JEOL-6400 SEM (JEOL, Japan).

#### **3.5** Isolation and cultivation of the selected xylariaceous fungi

The ascospores of selected xylariaceous specimens were isolated for culture by the method as described by Ju and Rogers (1996) and Thienhirun (1997). A portion of the stromatal surface including the upper parts of perithecia was removed with a sterile razor blade. The contents of the exposed perithecia were scooped out and spotted with a fine-tipped sterile needle in Petri dishes containing PDA medium (Appendix 1.1A). Hyphal tips emerging from the perithecial contents were then cut and transferred to fresh media. All isolates were routinely incubated at 25°C, and subcultured every two months. The anamorph form was also observed. Their mycelia were maintained in 15% glycerol at -20°C as stock cultures.

#### **3.6** Chemotaxonomic study of the selected xylariaceous fungi

Since *Xylaria* species are common endophytes isolated from several plants as described previously and most of them could not form mature teleomorph stage in their cultures, the representatives of *Xylaria* isolates and xylariaceous endophytes were selected to study on secondary metabolite profiles by using TLC technique.

#### 3.6.1 TLC analysis of secondary metabolites from agar plugs

Selected *Xylaria* isolates were grown on yeast extract sucrose (YES) agar (Appendix 2.1A) in 9-cm Petri dishes at 25°C for 4 weeks. The extracellular metabolite analysis was performed according to the standard method (Lund and Frisvad, 1994). Small agar plugs were cut from the fungal colony using a 4-mm flamed cork borer. The plugs were wetted by a drop of chloroform : methanol (2 : 1, v/v) and immediately applied onto a TLC plate (Silica gel 60, Merk Kieselgel

GF254), 2.5 cm from the bottom line. The eluent system composed of toluene : ethyl acetate : 90% formic acid, 5 : 4 : 1, v/v/v). The TLC plate was inspected in daylight and under ultra-violet (UV) transilluminator (366 nm and 265 nm), and all spots were noted. Each detected spot was calculated for retention factor ( $R_f$ ) as follows:

$$R_{f} = \frac{\text{Distance of each compound}}{\text{Distance of solvent}}$$

#### **3.6.2** TLC analysis of secondary metabolites from cultural broth

# 3.6.2.1 Secondary metabolite extraction from 100 mL of cultural broth

Selected *Xylaria* isolates were grown in 100 mL of 2% malt extract broth (Difco) containing 6% glucose for 8 weeks (Pittayakhajonwut, 2000). The broth medium was filtrated from fungal mycelium, and extracted with equal volume of ethyl acetate (Sigma). After extraction, the ethyl acetate layer was transferred to a volume metric flask and the extracted solution was concentrated by evaporating until the solution was changed to powder. Then, the extracted powder was dissolved with 5 mL ethyl acetate. The extracted solution was spotted onto the TLC plate (Silica gel 60, Merk Kieselgel GF254) using capillary tube. The eluent system was toluene : ethyl acetate : acetic acid; 50 : 49 : 1 (v/v/v). The detection systems were p-nitroaniline (Sigma) spray agent and anisaldehyde (Sigma) spray agent.

#### **3.6.2.2** Secondary metabolite extraction from 1 L of cultural broth

An isolate of *Xylaria* was selected to culture in 1 L of 2% malt extract broth (Difco) containing 6% glucose for 8 weeks. The cultural broth was separated and extracted for secondary metabolites using the same procedures as described in section 3.6.2.1.

#### **3.7** Nucleic acid studies of the selected xylariaceous fungi

DNA of the selected xylariaceous fungi and the reference strains were studied.

#### 3.7.1 Extraction of genomic DNA

Genomic DNA of the xylariaceous specimens selected as representatives was extracted from their cultural mycelia and stromatal herbarium, in case of uncultured specimens, using the method of Lee and Taylor (1990) with some modifications. The fungal mycelium was harvested and rinsed with TE buffer (Appendix 3.2A). The washed mycelium was squeezed, placed in a microcentrifuge tube, and stored at -20°C overnight. The frozen mycelium was ground, added lysis buffer (Appendix 3.1A), and incubated at 65°C for an hour. The equal volume of phenol : chloroform : isoamyl alcohol (25 : 24 : 1, v/v/v) was added, gently mixed, and centrifuged at 12,000 rpm (Labofuge 400R, Heraeus Instruments, Heraeus Instruments GmbH, Germany) at 4°C for 20 min. The top supernatant was transferred to a fresh microcentrifuge tube. An equal volume of isopropanol (Merck) and onetenth volume of 3 M ammonium acetate (pH 5.2, Appendix 3.3A) (BDH, BDH Laboratory Supplies Poole, England) were then added, gently mixed, and placed in an icebox for 10-30 min to precipitate genomic DNA. The tube was centrifuged at 12,000 rpm for 30 min at 4°C. The DNA pellet was washed with 400 µL of 70%

ethanol (Merck), air dried, and then resuspended in 50 µL TE buffer. RNA was removed by adding Ribonuclease A (1 mg/mL) (Invitrogen) (Appendix 3.4A) to give a concentration of 10 µg RNase/mL sample and the tube was incubated at 37°C for 30 min. Genomic DNA was detected in 1% agarose gel electrophoresis, stained with ethidium bromide (1 mg/mL) (BioRad, BioRad Laboratories, Italy), and examined under UV transilluminator (BioRad). The concentration of DNA was measured by SmartSpec <sup>TM</sup> 3000 spectrophotometer at 260 nm (BioRad) and the purity of DNA was calculated from the ratio of optical density at 260/280. The conversion factor for determination of DNA concentration is 1 OD<sub>260</sub> = 50 µg/mL of double stranded DNA. Then, DNA solution was maintained at -20°C until use.

#### 3.7.2 Amplification of the ribosomal RNA genes

The 18S ribosomal RNA gene and the internal transcribed spacer (ITS) regions 1 and 2 including 5.8S ribosomal RNA gene of xylariaceous fungi were amplified using PCR.

#### 3.7.2.1 Amplification of 18S ribosomal RNA gene

The 18S ribosomal RNA gene amplification was performed using NS1 and NS8 primers as forward and reverse to obtain the whole gene (Figure 8 and Table 7). The PCR amplification reaction was performed in 50  $\mu$ L mixture containing 50 ng of fungal DNA, 5  $\mu$ L of 10X reaction buffer (10 mM KCL, 20 mM Tris-HCl pH 8.8, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgSO<sub>4</sub>, 0.1% Triton X-100), 200  $\mu$ M of each dATP, dCTP, dGTP, and dTTP, 1  $\mu$ M of each primer, 1.0 unit of *Taq* DNA polymerase (Sigma), and adjusted volume to 50  $\mu$ L with deionized water. The program of amplification consisted of 1 cycle of 95°C for 5 min; 35 cycles of 95°C for 1 min, 53°C for 2 min, 72°C for 2 min; and the final cycle of 72°C for 10 min. The PCR reactions were carried out in the automated thermal cycle (i-cycle, BioRad, U.S.A.).





 Table 7. Nucleotide sequences of PCR primers used in this study.

Name	Sequence (5'- 3')	Target region <sup>a</sup>	Reference
NS1	GTAGTCATATGCTTGTCTC	SSU 20-38	White et al. (1990)
NS8	TCCGCAGGTTCACCTACGGA	SSU 1788-1769	White et al. (1990)
ITS4	TCCTCCGCTTATTGATATGC	LSU 60-41	White et al. (1990)
ITS5	GGAAGTAAAAGTCGTAACAAGG	SSU 1744-1763	White <i>et al.</i> (1990)

<sup>a</sup> Saccharomyces cerevisiae numbering

#### 3.7.2.2 Amplification of internal transcribed spacer (ITS) 1 and 2

#### including 5.8S ribosomal RNA gene

The ITS1-5.8S-ITS2 region was amplified using ITS4 and

ITS5 primers (Table 7). The PCR amplification reaction was performed in 50  $\mu$ L mixture containing 10 ng of fungal DNA, 5  $\mu$ L of 10X reaction buffer (10 mM KCL,

20 mM Tris-HCl pH 8.8, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgSO<sub>4</sub>, 0.1% Triton X-100), 200  $\mu$ M of each dATP, dCTP, dGTP, dTTP, 2.5  $\mu$ M of each primer, 1.0 unit of *Taq* DNA polymerase, and adjusted volume to 50  $\mu$ L with deionized water. The program of ITS1-5.8S-ITS2 region amplification consisted of 1 cycle of 95°C for 5 min; 35 cycles of 95°C for 30 sec, 53°C for 1 min, 72°C for 1 min; and final cycle of 72°C for 10 min. The PCR reactions were carried out in the automated thermal cycle (BioRad).

#### **3.7.3 Detection of PCR-amplified products by agarose gel electrophoresis**

Agarose gel electrophoresis is a standard method used to separate, identify, and purify DNA fraction. Agarose gel was prepared at a concentration of 1.5% (w/v) in 1X TBE buffer (Appendix 3.5A), melted in microwave oven until completely dissolved, and then poured into gel box with an appropriate comb.

Five microliters of PCR-amplified product was thoroughly mixed with 6X loading buffer (Appendix 3.6A). The mixture was loaded into the submarine 1.5% agarose gel, and electrophoresis was carried out at constant 100 volts until the bromphenol blue dye reached about 2 cm from the lower edge of the gel, then the electrophoresis was stopped. One hundred base pair DNA ladder (Invitrogen) was used as standard markers to determine the molecular size of DNA fragments.

After electrophoresis, the agarose gel was stained with ethidium bromide by soaking the gel in a solution containing 10  $\mu$ g/mL of ethidium bromide, and visualized under UV transilluminator (BioRad). The agarose gel was photographed for being reference.

#### **3.7.4** Purification of DNA-amplified products

The single band of the DNA-amplified product as estimated size was purified throughout the QIAquick purification kit (Qiagen) according the manufacturer's instruction. The DNA-amplified product that contained primer dimer band, approximately 50 bp, was purified by low melting point (LMP) agarose gel purification (BIO 101, Inc., U.S.A.). The DNA was mixed with 6X loading buffer and then loaded into the 1.5% LMP agarose gel. Electrophoresis was carried out at the constant 100 volts until the bromophenol blue dye reached about 2 cm from the lower edge of the gel then electrophoresis was stopped. The gel was stained and viewed as previously described in section 3.7.3.

The DNA band of the expected size visualized under the UV light was cut from the gel by a clean blade and placed into a new 1.5 mL microcentrifuge tube. The gel matrix that did not contain DNA material was trimmed off to obtain the minimum volume of the gel. DNA was eluted from a slice of gel using QIAquick gel purification kit (Qiagen) according the manufacturer's instruction.

#### 3.7.5 Sequencing of ribosomal DNA

#### **3.7.5.1** Preparation of DNA for sequencing

The purified ribosomal DNA amplicons were sequenced using the BigDye Terminator Ready Reaction kit version 2.0 (Perkin Elmer) according to the manufacturer's protocol. The 10  $\mu$ L cycle sequencing reaction mixture contained 80-200 ng DNA, 4  $\mu$ L BigDye, and 5 pM primer. Primers used for the sequencing of 18S rDNA and ITS fragments, were the same as in PCR amplification of each PCR fragment. The thermal profile consisted of 25 cycles of 10 sec at 96°C, 5 sec at 50°C, and 4 min at 60°C. The cycle sequencing was performed in the thermal cycle (BioRad). The PCR mixtures were spun down briefly before DNA precipitation. The sequencing DNA fragments were precipitated by adding 16  $\mu$ L of deionized water and 64  $\mu$ L of 95% ethanol. The tube was vortexed briefly, incubated at 4°C for 15 min, and then spun at 12,000 rpm for 20 min at 4°C. The DNA pellet was washed with 300  $\mu$ L of 70% ethanol, centrifuged at 12,000 rpm for 20 min at 4°C, and discarded the supernatant. The DNA pellet was dried at room temperature in the dark.

#### 3.7.5.2 DNA sequencing

The sequencing gel used for an ABI 377 automated DNA sequencer (Perkin Elmer) was prepared as described in the manufacturer's protocol. The 6% polyacrylamide gel was casted in slab gel glass plates. The sequencing pellet was dissolved in 3  $\mu$ L of loading buffer, and loaded onto the gel. Electrophoresis was carried out at constant 750 volts for 8 h. Fluorescent signals were detected with ABI Collection software. Base calling was performed using sequencing analysis software, and nucleotide sequence determination was performed using sequence navigator software. The resulting sequences were assembled and manually corrected by using Chromas 1.56 program (Technelysium Pty. Ltd).

#### 3.7.6 Alignment of DNA sequences

Completed DNA sequences were aligned using Clustual X software package (Thompson *et al.*, 1994). All alignments were examined and manually optimized with the BioEdit program (North Carolina State University, U.S.A.). In addition, the available xylariaceous sequences of 18S rDNA and ITS1-5.8S-ITS2 from GenBank database were downloaded, and imported to xylariaceous database examined for DNA sequence alignment.

#### **3.7.7** Construction of phylogenetic tree

Phylogenetic trees were constructed with different methods and software packages.

#### 3.7.7.1 Neighbour-joining (NJ) method

Phylogenetic trees were constructed based on genetic distances using neighbour-joining method. The conditional clustering, Kimura 2 parameter distances (Kimura, 1980), was computed with the Dnadist module of the PHYLIP software package version 3.6 (Felsenstein, 1995). Strengths of internal branches of resulting trees were statistically tested by the bootstrap analysis of 1,000 replications.

#### 3.7.7.2 Maximum parsimony (MP) method

Phylogenetic trees were constructed using PAUP\* version 4.0b10 (Swofford, 2000) for the maximum parsimony method. They were analyzed by heuristic searches. The MAXTREES set to 10,000 and TBR branch swapping. All characters were assessed as independent, unordered and equally weighted. Bootstrapping in these analyses was performed using 1,000 replicates.

### **CHAPTER IV**

# **RESULTS AND DISCUSSION**

#### 4.1 Collection of the xylariaceous fungi for taxonomic studies

Three hundreds and thirty eight xylariaceous specimens were collected from 14 different locations in Thailand. The different locations, period of collection, number and type of specimens were recorded. The number of collected specimens is shown in Table 8. The collection locations were selected following consultation with Dr. Surang Thienhirun as being proven as good forest sites for the Xylariaceae. Although most of the collections are from the Northeastern Thailand sites from the North (Chiang Mai Province) to the South (Songkhla Province) were also surveyed. Thus, a range of different forest types was included in this study.

Year	Location	No. of
		specimens
2002	Chiang Mai Province	11
	Nakhon Ratchasima Province	7
2003	Phu Luang, Nakhon Ratchasima Province	7
	Nong Rawieng, Nakhon Ratchasima Province	11
	Burirum Province	9
	Chaiyaphum Province	4

**Table 8.** Xylariaceous collections from 14 locations of Thailand in years 2002 and2003.

Table 8. (Continued).

Year	Location	No. of
		specimens
2003	Ratchaburi Province	51
	Suranaree University of Technology, Nakhon Ratchasima Province	51
	Kanchanaburi Province	49
	Petchaboon Province	9
	Songkhla Province	30
	Trad Province	77
	Yasothon Province	19
	Chiang Rai Province	3

The majority of collections were from Trad Province where the forest was classified as the mixed forest (Thienhirun, 1997).

# 4.2 Morphological taxonomic studies of the problematic groups in xylariaceous fungi

All collected xylariaceous specimens were identified and classified into nine genera and 59 species according to their morphological characteristics as shown in Table 9.

The high numbers of collected specimens belonged to *Hypoxylon* and *Xylaria* respectively. There was also wide distribution and variation of both genera whereas the other xylariaceous genera were rarely represented especially *Astrocystis*, which occurs only on bamboo. Unfortunately *Daldinia bambusicola* occurring on bamboo was not found during the two years of survey. The details for each genus and species are described as follows:

Genus	No. of specimens	No. of species	
Astrocystis Berk. & Broome	8	1	
Biscogniauxia Kuntze	6	2	
<i>Camillea</i> Fr.	5	1	
Daldinia Ces. & De Not.	13	1	
Hypoxylon Bull.	196	29	
Kretzschmaria Fr.	2	1	
Nemania S.F. Gray	2	1	
Rosellinia De Not.	4	1	
Xylaria Hill ex Schrank	102	22	

**Table 9.** Numbers of genera and species of xylariaceous collections in this study inyears 2002 and 2003.

#### 4.2.1 Group I: Astrocystis and Rosellinia

Since the genera *Astrocystis* and *Rosellinia* are poorly represented in Thailand, which agreed with Thienhirun (1997), with only two species having been found in this study, *A. mirabilis* Berk. & Broome and *R. procera* Syd. Comparison of the two species is given in Table 10.

Astrocystis mirabilis SUT047, SUT048, SUT049, SUT051, SUT052, SUT054, SUT055, and SUT056 (Figure 9), which was reported as the type species of the genus (Berkeley and Broom, 1875), were found to be very similar to specimens previously described by Thienhirun (1997) collected from Surat Thani Province except for ascospore size, 10-13.8 x 3.8-5  $\mu$ m cf. (10-)10.6-12.5 x 5.6-6.3  $\mu$ m (Thienhirun, 1997). However other characters were well matched *A. mirabilis* as described by Ju and Rogers (1990).

Character	A. mirabilis*	R. procera*
Stromata		
Shape	Subglobose to hemispherical,	Subglobose to hemispherical,
	blackish, each stroma encircled	blackish, embedded on a brown
	with a more or less stellate to	cottony subiculum
	irregular ring of mixed host and	
	stromatic material at the base to	
	midportion	
Color	Black	Black
Perithecia		
Shape	Obovoid	Obovoid
Size	1-1.5 mm diameter	1-1.5 mm diameter
Ostiole	Papillate	Conico-papillate
Asci	Cylindrical	Not observed
Ascospores		
Color	Brown to dark brown	Brown to dark brown
Shape	Ellipsoid	Fusiform with tapering ends
Size	10-13.8 x 3.8-5 μm	(65-)95-125 x 10-15 μm
Apical apparatus	Inverted hat, 2-3(-4) µm high x	Inverted hat, 2-3 µm high x 3.5-
	3-4 μm broad	5 μm broad
Germ slit	Straight slightly less than spore	Straight longitudinal germ slit
	length	spore length
Culture	White radiate strands with	Uncultured
	fimbriate margins, velutinous or	
	floccose, and faintly zonate	
Habitat	On bamboo	On wood
Location	Ratchaburi	Ratchaburi, Nakhon Ratchasima
Specimen	SUT047, SUT048, SUT049,	SUT102, SUT109, SUT113,
examined	SUT051, SUT052, SUT054,	SUT114
	SUT055, SUT056	

**Table 10.** Species comparison of Astrocystis and Rosellinia found in this study.

\* More details on collections are given in Appendix B.



**Figure 9.** Astrocystis mirabilis Berk. & Broome (SUT051); (a) stromatal form (Bar = 1 mm), (b) ascospores with ellipsoid equilateral (Bar = 10  $\mu$ m), (c) apical apparatus bluing in Melzer's iodine reagent (Bar = 4  $\mu$ m), (d) germ slit straight nearly spore length (arrowed) (Bar = 2  $\mu$ m), and (e) cultural characteristics on PDA cultured at 25°C after 3 weeks (Bar = 1 cm).

Four collections of the *Rosellinia* (SUT102, SUT109, SUT113, and SUT114) examined were in close agreement with *R. procera* Syd. (Figure 10) as described by Petrini (1990) except for small differences in ascospore size (65-)95-125 x 10-15  $\mu$ m cf. 75-130 x 15-18  $\mu$ m (Petrini, 1990). These might be because of variation within the species and the different collection areas. Unfortunately, the *Astrocystis* found in this study did not form its anamorph in culture, and also the *Rosellinia* examined could not be cultured. Therefore, there was no information of anamorph characteristics for both taxa. The genus *Astrocystis* has been separated from *Rosellinia* on the basis of host specificity on bamboo and also on features of the stromata splitting the host surface or the presence of a carbonaceous extension at the base (Berkeley and Broome, 1887; Petrini, 1993; 2003; Læssøe and Spooner, 1994). However, there is some disagreement with Ju and Rogers (1990; 1995) and San Martín and Rogers (1994) considering *Astrocystis* to be congeneric with *Rosellinia*.



Figure 10. *Rosellinia procera* Syd. (SUT113); (a) stromatal form with conico-papillate of ostiole (arrowed) (Bar = 2 mm), (b) perithecia (Bar = 3 mm), (c) ascospore (Bar = 10 μm), and (d) apical apparatus (Bar = 5 μm).

#### 4.2.2 Group II: Camillea

One species of *C. tinctor* (Berk.) Læssøe, J.D. Rogers & Whalley (Figure 11) was recorded from this study. *Camillea selangorensis* M.A. Whalley, A.J.S. Whalley & E.B.G. Jones., which is another tropical species firstly described in Malaysia by Whalley (1995), has since been reported to be found in Thailand (Whalley *et al.*, 1999). Unfortunately, it was not found during this study. Therefore, *C. selangorensis* and *C. leprieurii* (Figure 12), provided by Dr. Margaret A. Whalley were used to compare with the *C. tinctor* collected in Thailand, and their morphological characteristics are described in Table 11.

Four collections (SUT099, SUT161, SUT211, and SUT260) examined collected from different localities in Chiang Mai, Trad, Songkhla, and Yasothorn Provinces closely matched *C. tinctor* (Berk.) Læssøe, J.D. Rogers & Whalley as described by Læssøe *et al.* (1989), and also the previously described specimens from Thailand by Thienhirun (1997). The comparison of all collections, which was collected from different locations, suggested that they were identical. The present of orange color staining on the substratum was also observed from some specimens. Since the ascospores of most *Camillea* species appear smooth by light microscopy but appear characteristically ornamented by SEM (Læssøe *et al.*, 1989; Rogers *et al.*, 1991; San Matín, Gonzáles, and Rogers, 1993; Whalley, 1995; Whalley, 1996; Whalley *et al.*, 1999). The collected *C. tinctor* specimens were, therefore, observed by SEM, and found to exhibit the reticulate ornamentation which was the distinctive character of this species as shown in Figure 11f. However, *C. selangorensis* and *C. leprieurii* revealed strongly verrucose or intricately ornamented ascospore walls as described by Whalley *et al.* (1996) and Læssøe *et al.* (1989) respectively.

Character	C. tinctor (Berk.) Læssøe, J.D. Rogers &	C. selangorensis M.A. Whalley, A.J.S. Whalley	C. leprieurii Mont.
	Whalley *	& E.B.G. Jones.	
Stromata			
Shape	Applanate with a slightly raised center,	Circular to orbicular, or elongated, with applanate	Erumpent through bark, cylindrical, seated on
	elongate elliptic	to convex apex, surrounded by a slightly raised rim	slightly broader disc
Color	Externally black, mat or shiny, internally dark	Black	Black
	brown, surface smooth		
Perithecia			
Shape	Deeply immersed, cylindrical to slightly	Deeply immersed, brittle entostroma, basally	Elongate ovoid
	elongate	seated, cylindrical, individually erumpent	
Size	0.3-1 mm high x 0.2-0.5 mm diameter	0.5-0.8 mm diameter	0.2-0.4 mm diameter
Ostiole	Punctiform, slightly raised	Finely papillate becoming punctate in age	
Asci	Cylindrical	Cylindrical	Cylindrical
Ascospores			
Color	Pale yellow	Pale yellow	Colorless to dilute yellow
Shape	Ellipsoid to fusiform, reticulate-poroid by	Ellipsoid inequilateral, minutely warted by light	Elongate with upper end acute wedge shaped and
	SEM	microscopy, strongly verrucose by SEM	lower end draw into very long tail, wavy
			longitudinal rib-structure with ladder-like
			transverse substructure by SEM
Size	(12.5-)13.8-21.3 x (5.6-)6.3-8.8 µm	10.0-13.8 x 3.8-6.3 μm	(26.3-)29.1-37.6(-38.5) x (5.3-)6.1-7.5 μm
Apical apparatus	Urniform, 2-3 (-4) µm high x 3-4 µm broad	Rhomboid, 2.5-3.8 µm high x 3-3.8 µm broad	Dome or thimble-shaped, 3.3-8.5 (-9.5) high x
			4.4-7.3 μm broad
Germ slit	No	No	No
Habitat	On wood	On wood	On wood
Location	Songkhla, Yasothorn, Trad	Malaysia (provided by M.A. Whalley)	Malaysia (M.A. Whalley)
Specimen examined	SUT099, SUT161, SUT211, and SUT260	KS15	

**Table 11.** Morphological characteristics of *Camillea tinctor* found in this study, *C. selangorensis*, and *C. leprieurii*.

\* More details on collections are given in Appendix B.



Figure 11. *Camillea tinctor* (Berk.) Læssøe, J.D. Rogers & Whalley (SUT260); (a) stromatal form (Bar = 1 mm), (b) perithecia (Bar = 0.5 mm), (c) ascospores (Bar = 15  $\mu$ m), (d) apical apparatus bluing in Melzer's iodine reagent (Bar = 4  $\mu$ m), (e) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm), and (f) the reticulate ornamentation of ascospore by SEM (Bar = 2  $\mu$ m).



Figure 12. Camillea selangorensis M.A. Whalley, A.J.S. Whalley & E.B.G. Jones (KS15), and C. leprieurii (Mont.) Mont; (a) and (b) stromatal form of C. leprieurii (Bar = 0.5 cm and 0.2 cm respectively), (c) ascospores (Bar = 6 μm), and (d) stromatal form of C. selangorensis (Bar = 1 cm).

#### 4.2.3 Group III: Daldinia

One species of *D. eschscholzii* (Ehrenb.: Fr.) Rehm was recorded from thirteen collections (SUT013, SUT037, SUT038, SUT039, SUT084, SUT085, SUT086, SUT168, SUT169, SUT178, SUT209, SUT268, and SUT278) of the genus (Figure 13). The type species, *D. concentrica* (Bolton: Fr.) Ces. & De Not. which is frequently found in temperate region, was provided by Prof. Anthony J.S Whalley to compare with collected *D. eschscholzii* specimens. Unfortunately, *D. bambusicola*, which occurs on bamboo, has only been found twice in Thailand and it was not found during this study. The description of the *D. eschscholzii* examined is given in Table 12.

Thirteen collections collected from eight provinces were matched *D.* eschscholzii (Ehrenb.: Fr.) Rehm as described by Ju, Rogers, and San Martín (1997). This is a widely distributed species, and it is the most common xylariaceous species found throughout Thailand as previously reported by Whalley (1996), Thienhirun (1997), and Thienhirun and Whalley (2004). From SEM observation, the ascospore wall of *D. eschscholzii* was ornamented with conspicuous transversely oriented fibrils (Figure 13). However, only teleomorphic characteristics, including perispore ornamentation by SEM, were insufficient to identify species of *D. eschscholzii* or *D. concentrica.* Recently Stadler *et al.* (2004) rearranged the two species of *Daldinia* into five new species according to their anamorph characteristics, shape of ascospores, and chemical characteristics. One out of five species belonging to *D. palmensis* M. Stadler, Wollweber & H-.V. Tichy. is closed to *D. eschscholzii*. The conidiophore of *D. palmensis* is *Nodulisporium*-like or *Sporothrix*-like forms whilst *D. eschscholzii* is a *Nodulisporium*-like form. Unfortunately, only four of the collected *D. eschscholzii*  isolates could be cultured, and all of them were *Nodulisporium*-like in form. The remainder of collections was still unable to confirm to be either *D. eschscholzii* or *D. palmensis*.

 Table 12. Morphological characteristics of Daldinia eschscholzii found in this study

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Character	D. eschscholzii *	D. concentrica (Ju, Rogers, and San Martín, 1997)**
Stromata		
Shape	Turbinate to placentiform, sessile or with short, stout stipe, solitary to infrequently aggregated, smooth	Spherical, sessile, solitary to aggregated, smooth or with inconspicuous perithecial mounds
Color	Surface brown vinaceous, dark brick, sepia, grayish sepia, or vinaceous grey, blackened and varnished in age	Surface brown vinaceous, chestnut, or sepia, blackened and varnished in age
KOH-extractable	Livid purple, dark livid, or vinaceous purple	Livid purple or dark purple
pigments Perithecia		
Shape	Tubular	Tubular
Size	0.8-1.5 mm x 0.3-0.4 mm diameter	0.3-0.5 mm diameter x 1-2 mm high
Ostiole	Obsolete or slightly papillate	Slightly papillate
Asci	Cylindrical	Cylindrical
Ascospores		
Color	Brown to dark brown	Brown to dark brown
Shape	Ellipsoid-inequilateral with narrowly rounded ends	Ellipsoid-inequilateral with narrowly rounded ends
Size	11.3-13.8 x 5-6.3 μm	13-17 x 6-7.5 μm
Apical apparatus	Discoid, 0.5 µm high x 2-2.5 µm broad	Discoid, 0.5-1 µm high x 3-3.5 µm broad
Germ slit	Straight full spore-length on convex side	Slightly sigmoid germ slit spore- length on convex side
Perispore	Dehiscent, conspicuous coil-like ornamentation	Dehiscent, smooth
Culture	White at first, becoming brownish grey, fluffy, rapidly grow	-
Location	Bangkok, Burirum, Chiang Rai, Nakhon Ratchasima, Ratchaburi, Trad, and Yasothorn	-
Specimen examined	SUT013, SUT037, SUT038, SUT039, SUT084, SUT085, SUT086, SUT168, SUT169, SUT178, SUT209, SUT268, SUT278	L1 and L2

and the reference specimen of *D. concentrica*.

\* More details on collections are given in Appendix B.

\*\* Typical specimens with anamorphic culture from the U.K.



Figure 13. *Daldinia eschscholzii* (Ehrenb.: Fr.) Rehm (SUT039); (a) stromatal form (Bar = 1 cm), (b) perithecia (Bar = 0.5 mm), (c) ascus containing eight ascospores (Bar = 10  $\mu$ m), (d) apical apparatus bluing in Melzer's iodine reagent (arrowed), (e) germ slit straight nearly spore length (arrowed) (Bar = 2  $\mu$ m), (f) ascospore dehiscent in 10% KOH (arrowed), (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm), and (h) the perispore with conspicuous coil-like ornamentation by SEM (Bar = 2  $\mu$ m).

#### 4.2.4 Group IV: Hypoxylon

Twenty nine species of *Hypoxylon* were recorded including 4 new species. Although *Hypoxylon* sect. *Annulata* was focused in this study, other species of *Hypoxylon* sect. *Hypoxylon* also were examined for species differentiation and DNA database construction. The *Hypoxylon* species found and investigated are listed in Table 13.

 Table 13. Species of Hypoxylon sect. Annulata, and sect. Hypoxylon found in this study.

Species	No.*	Remark
Hypoxylon sect. Annulata		
H. cf. archeri	6	Hazel in KOH-extracted pigments,
		white substance on the stromatal
		surface
H. atroroseum J.D. Rogers	10	Ju and Rogers (1996)
H. bovei Speg. var. microspora J.H. Miller	1	Ju and Rogers (1996)
H. moriforme Henn.	5	Ju and Rogers (1996)
H. purpureonitens YM. Ju & J.D., Rogers	12	Ju and Rogers (1996)
H. stygium (Lév.) Sacc.	13	Ju and Rogers (1996)
H. urceolatum (Rehm) YM. Ju & J.D. Rogers	1	Ju and Rogers (1996)
Hypoxylon taxonomic species 1 sp. nov.	15	Green in KOH-extracted pigments
		truncatum-type in ostiolar form,
		0.3-0.4 mm diameter of ostiolar di
Hypoxylon sect. Hypoxylon		
H. anthochroum Berk. & Broome	7	Ju and Rogers (1996)
H. brevisporum YM. Ju & J.D. Rogers	1	Ju and Rogers (1996)
H. duranii J.D. Rogers	11	Ju and Rogers (1996)
H. fendleri Berk. ex Cooke	20	Ju and Rogers (1996)
H. cf. ferrugineum (SUT017)	1	Small in ascospore size
H. cf. ferrugineum (SUT070)	1	Brown vinaceous in stromatal
		surface colour, orange in granule
		colour
H. cf. ferrugineum (SUT237)	4	Close to H. ferrugineum except for
		stromatal surface color, KOH-
		extractable pigment, and ascospore
		size

\* Number of *Hypoxylon* collections.
Table 13. (Continued)

Species	No.*	Remark
Hypoxylon sect. Hypoxylon		
H. haematostroma Mont. apud Sagra	7	Ju and Rogers (1996)
H. hypomiltum Mont.	1	Ju and Rogers (1996)
H. investiens (Schwein.) M.A. Curtis	7	Ju and Rogers (1996)
H. lenormandii Berk. & M.A. Curtis apud Berk	11	Ju and Rogers (1996)
H. lenormandii var. microspora	1	Thienhirun (1997)
H. macrocarpum Pouzar	1	Ju and Rogers (1996)
H. monticulosum Mont.	28	Ju and Rogers (1996)
H. cf. perforatum (SUT020)	1	Grayish sepia in stromatal surface colour, dark brown to black in granule colour, straight full length germ slit
H. cf. perforatum (SUT224)	1	Brown vinaceous in stromatal surface colour, brown vinaceous in granule colour, straight full length germ slit
H. cf. perforatum (SUT294)	1	Reddish brown in stromatal surfac colour, reddish brown to black in granule colour, straight full length germ slit
H. rubiginosum (Pers.: Fr.) Fr., Summa Veg	4	Ju and Rogers (1996)
H. subgilvum Berk. & Broome var. microsporum (Abe) YM. Ju & J.D. Rogers	3	Ju and Rogers (1996)
H. trugodes Berk. & Broome	6	Ju and Rogers (1996)
H. sublenormandii sp. nov.	3	Closed to <i>H. lenormandii</i> except for reddish brown in stromatal colour, smaller in ascospore size, and straight germ slit
H. kanchanapisekii sp. nov.	5	Close to <i>H. lenormandii</i> except for stromatal surface color of dull reddish brown not grayish sepia, small ascospores, and having a straight germ slit
H. suranareei sp. nov.	5	Conspicuous perithecial mounds, orange brown in stromatal surface colour, yellowish orange in KOH- extractable pigments, ostioles same or lower than the stromatal surface with white substance

\* Number of *Hypoxylon* collections.

 Table 13. (Continued)

Species	No.*	Remark
Hypoxylon sect. Hypoxylon		
Hypoxylon taxonomic species 2 (SUT082)	1	Brownish yellow in KOH- extractable pigment, ascospore size, and inconspicuous coil-like ornamentation
<i>Hypoxylon</i> taxonomic species 3 (SUT158)	1	Dark brick or brown vinaceous in stromatal surface colour, brown vinaceous in granule colour, amber or yellowish brown in KOH- extracted pigments

\* Number of *Hypoxylon* collections.

#### 4.2.4.1 Hypoxylon section Annulata

Eight species of *Hypoxylon* sect. *Annulata* were observed including a new species, *Hypoxylon* taxonomic species 1 sp. nov. The results are given in Table 14. *Hypoxylon* cf. *archeri* (SUT079, SUT103, SUT105, and SUT112) (Figure 14) closely agreed with *H. archeri* Berk. *apud* J.D. Hook. as described by Ju and Rogers (1996) except for its KOH-extractable pigments having greenish olivaceous according to Ju and Rogers (1996) but they were hazel in the Thai collections. In addition, two specimens related to this taxon provided by Dr. Surang Thienhirun (ST2333 and ST2527) were used as the reference strains. Initially, the specimens examined looked like *H. michelianum* in having a layer of white substance on the stromatal surface, which was striking and has not been observed in other taxa of section *Annulata* (Ju and Rogers, 1996) but the other characters were different.

Character	H. cf. archeri*	H. atroroseum J.D. Rogers*	H. bovei Speg. var. microspora J.H. Miller*
Stromata			
Shape	Effused-pulvinate	Effused-pulvinate	Hemispherical to effused-pulvinate
Color	Blackish brown	Vinaceous gray	Black
Granules beneath surface	Black	Dull reddish brown	Black
KOH pigments	Hazel	Greenish olivaceous	Greenish olivaceous
Perithecia			
Shape	Spherical	Obovoid	Spherical
Size	0.3-0.4 mm diameter	0.2-0.3 mm diameter x 0.3-0.5 mm high	0.6-1 mm diameter
Ostiole	Coarsely papillate	Papillate	Papillate
Disc			
Туре	Truncatum-type	Truncatum-type	Bovei-type
Size	0.1-0.2 mm diameter	0.1-0.2 mm diameter	0.3-0.7 mm diameter
Apical apparatus	Discoid, 0.5 µm high x 1-1.5 µm broad	Discoid, 0.5 µm high x 1 µm broad	Discoid, 1-1.5 µm high x 2 µm broad
Ascospores			
Color	Brown to dark brown	Light brown	Brown to dark brown
Shape	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-equilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends
Size	8.8-10(-11.5) x 3.8-5 μm	6.3-8.8 x 2.5-3.8 μm	7.5-10 x 3.8-5 μm
Germ slit	Straight full length	Straight full length	Straight full length
Perispore	Smooth	Smooth	Smooth
Habitat	On wood	On wood	On wood
Location	Songkhla Province	Nakhon Ratchasima, Trad	Chaiyaphum
Specimen examined	SUT079, SUT103, SUT105, and SUT112	SUT009, SUT010, SUT214, and SUT219	SUT025 and SUT242

**Table 14.** Morphological characteristics of *Hypoxylon* sect. *Annulata* found in this study.

 Table 14. (Continued).

Character	H. moriforme Henn.*	H. purpureonitens YM. Ju & J.D., Rogers*	H. stygium (Lév.) Sacc.*
Stromata			
Shape	Glomerate, hemispherical to effused- pulvinate	Glomerate, hemispherical to effused-pulvinate	Effused-pulvinate
Color	Blackish with reddish brown tone, some shiny black	Blackish with reddish brown tone, some shiny black	Blackish with reddish brown tone
Granules beneath surface	Black	Black	Dull reddish brown
KOH pigments	Greenish olivaceous	Vinaceous purple	Greenish olivaceous
Perithecia			
Shape	Spherical	Spherical	Obovoid
Size	(0.4-)0.5-1(-1.2) mm diameter	(0.3-)0.5-1 mm diameter	0.2-0.3 mm diameter x 0.3-0.5 mm high
Ostiole	Conical-papillate	Conical-papillate	Papillate
Disc			
Type	Bovei-type	Bovei-type	Truncatum-type
Size	0.2-0.3 mm diameter	0.2-0.3 mm diameter	0.1-0.2 mm diameter
Apical apparatus	Discoid, 0.5 µm high x 1-1.5 µm broad	Discoid, 0.5 µm high x 1-1.5 µm broad	Discoid, 0.5 µm high x 1µm broad
Ascospores			
Color	Brown	Brown	Light brown
Shape	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-equilateral, with narrowly rounded ends
Size	7.5-9 x 2.8-4.2 μm	7.5-10 x 3.8-5 μm	3.8-6.3 x 2.5-3.8 μm
Germ slit	Straight full length	Straight full length	Straight full length
Perispore	Smooth	Smooth	Smooth
Habitat	On wood	On wood	On wood
Location	Chaiyaphum, Kanchanaburi, Nakhon Ratchasima, Trad	Nakhon Ratchasima, Songkhla, Trad, Yasothorn	Ratchaburi, Trad
Specimen examined	SUT216, SUT220, SUT231, SUT249, SUT285, and SUT288	SUT001, SUT004, SUT005, SUT100, SUT160, SUT167, and SUT262	SUT058, SUT222, SUT226, SUT229, SUT230, SUT243, SUT245, SUT247, SUT253, and SUT257

Table 14.	(Continued).
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Character	H. urceolatum (Rehm) YM. Ju & J.D. Rogers *	Hypoxylon taxonomic species 1 sp. nov.*
Stromata		
Shape	Effused-pulvinate	Glomerate, hemispherical to effused-pulvinate
Color	Black	Blackish with reddish brown tone, some shiny black
Granules beneath surface	Black	Black
KOH pigments	Vinaceous purple	Green
Perithecia		
Shape	Obovoid to tubular	Spherical
Size	0.2-0.4 mm diameter x 0.4-1 mm high	0.5-0.8 mm diameter
Ostiole	Conical-papillate	Conical-papillate
Disc		
Туре	Truncatum-type	Truncatum-type
Size	0.2-0.3 mm diameter	0.2-0.3 mm diameter
Apical apparatus	Not observed	Discoid, 0.5 µm high x 1-1.5 µm broad
Ascospores		
Color	Pale brown	Brown
Shape	Ellipsoid to fusoid, slightly inequilateral to equilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends
Size	10-12.5 x 2.5-5 μm	7.5-9 x 2.8-4.2 μm
Germ slit	Straight less than spore-length and originating from one end	Straight full length
Perispore	Smooth	Smooth
Habitat	On wood	On wood
Location	Songkhla	Chaiyaphum, Nakhon Ratchasima, Trad, Kanchanaburi
Specimen examined	SUT098	SUT081, SUT238, SUT241, SUT244, SUT246, SUT251, and SUT255

*Hypoxylon atroroseum* SUT009, SUT010, SUT214, and SUT219 (Figure 15) examined were similar to *Hypoxylon atroroseum* J.D. Rogers as described by Ju and Rogers (1996) except for its ascospore size, which were 6.3-8.8 x 2.5-3.8  $\mu$ m and 5-7 x 2-3  $\mu$ m respectively. The teleomorphic characteristics of *H. atroroseum* were similar to *H. stygium* except the stromata of *H. atroroseum* often have rosy surface tones.

*Hypoxylon bovei* var. *microspora* SUT242 (Figure 16) examined was similar to *Hypoxylon bovei* Speg. var. *microspora* J.H. Miller. as described by Ju and Rogers (1996).

*Hypoxylon moriforme* SUT216, SUT220, SUT231, SUT249, SUT285, and SUT288 (Figure 17) were similar to *Hypoxylon moriforme* Henn. (Ju and Rogers, 1996). This taxon closely resembles *H. nitens* and *H. bovei* var. *microspora* in KOH-extractable pigments and size of perithecia, disc, and ascospores but they are different in ostiolar disc type. *Hypoxylon moriforme* has a *truncatum*-type disc whilst the other species have a *bovei*-type disc (Ju and Rogers, 1996). For the *truncatum*-type, the outermost layer of stroma around ostioles is flaked off gradually from the ostiole outwards, whereas in the *bovei*-type the outermost layer of stroma dehisces abruptly. However, in the case of mature specimens lacking these outer layers, the ostiolar discs of *bovei*-type look like the *truncatum*-type disc and as a result were difficult to identify. The collected specimens examined were placed in this taxon because of the lack of these outer layers and as their ostiolar discs were identified as belonging to the *truncatum*-type. They also mainly formed glomerate stromata.



Figure 14. *Hypoxylon* cf. *archeri* (SUT105); (a) stromatal form when immature (Bar = 0.2 mm), (b) KOH-extractable pigment, (c) stromata with white fringe surrounding ostiolar disc (Bar = 0.1 mm), (d) ascospores (Bar = 10 μm), (e) straight germ slit spore length (arrowed) (Bar = 2 μm), (f) the thickening on perispore (arrowed) (Bar = 1 μm), (g) SEM micrograph of ascospore (Bar = 1 μm), and (h) cultural characteristics on PDA cultured at 25°C after 3 weeks (Bar = 1 cm).



**Figure 15.** *Hypoxylon atroroseum* J.D. Rogers (SUT009); (a) stromatal form (Bar = 0.1 mm), (b) ascospores (Bar = 10  $\mu$ m), (c) KOH-extractable pigment greenish olivaceous, (d) straight germ slit spore length (arrowed) (Bar = 2  $\mu$ m), (e) the thickening on perispore (arrowed) (Bar = 2  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 16.** *Hypoxylon bovei* Speg. var. *microspora* J.H. Miller (SUT025); (a) and (c) stromatal form (Bars = 1 cm and 0.5 mm respectively), (b) KOH-extractable pigment, (d) ascospores (Bar =  $10 \ \mu m$ ), (e) the thickening on perispore (arrowed) (Bar =  $5 \ \mu m$ ), and (f) cultural characteristics on PDA cultured at  $25^{\circ}$ C after 3 weeks (Bar = 1 cm).



**Figure 17.** *Hypoxylon moriforme* Henn. (SUT220); (a) and (c) stromatal form (Bars = 1 cm and 0.3 mm respectively), (b) KOH-extractable pigment greenish olivaceous, (d) ascospores (Bar = 10  $\mu$ m), (e) straight germ slit spore length (arrowed) (Bar = 2  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).

*Hypoxylon purpureonitens* SUT001, SUT004, SUT005, SUT100, SUT160, SUT167, and SUT262 (Figure 18) matched *Hypoxylon purpureonitens* Y.-M. Ju & J.D. Rogers (Ju and Rogers, 1996). This taxon is similar to *H. nitens* except its KOH-extractable pigments are purplish (Ju and Rogers, 1996).

*Hypoxylon stygium* SUT058, SUT222, SUT226, SUT229, SUT230, SUT243, SUT245, SUT247, SUT253, and SUT257 (Figure 19) from Thailand fitted *Hypoxylon stygium* (Lév.) Sacc. (Ju and Rogers, 1996). The ascospore size of collected specimens was 3.8-6.3 x 2.5-3.8 μm but *Hypoxylon stygium* (Lév.) Sacc. was 5-7 x 2-3 μm.

*Hypoxylon urceolatum* SUT098 (Figure 20) matched *Hypoxylon urceolatum* (Rehm) Y.-M. Ju & J.D. Rogers as described by Ju and Rogers (1996) except the ascospore size (10-12.5 x 2.5-5  $\mu$ m), which was smaller than specimens recorded by Ju and Rogers (1996) (9-14(-17) x 3.5-4.5  $\mu$ m) but it was close to Thai specimens reported by Thienhirun (1997) (8.8-10 x 3-3.8  $\mu$ m). The cultural characteristics of this taxon have never been observed. In this study, the specimen was cultured on PDA and hypha covered a 9-cm Petri dish in 3 weeks at 25°C. At first, the mycelium was white. Then, it became dull green, floccose, azonate, with diffuse margins, with scattered black patches as shown in Figure 20f. No anamorph was observed.

*Hypoxylon* taxonomic species 1 sp. nov. (Figure 21). Characteristics of this taxon are as follows: stromata glomerate, hemispherical to effused-pulvinate, with perithecial mounds; surface blackish, with reddish brown tone; blackish granules beneath surface, with KOH-extractable pigments greenish olivaceous (90); perithecia spherical, 0.5-0.8 mm diameter, ostioles papillate, encircled with a flattened *truncatum*-type disc 0.3-0.5 mm diameter; asci 100-130  $\mu$ m total length x 3.8-5  $\mu$ m broad, the spore bearing parts 40-65  $\mu$ m long with stipes 30-55  $\mu$ m; ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 7.5-9 x 2.8-4.2  $\mu$ m, with straight-germ slit spore length; perispore dehiscent in 10% KOH, smooth; epispore smooth.

Specimens examined: Thailand, Trad Province, 14 December 2003, Suwannasai, N. (Holotype SUT236), SUT238, SUT241, SUT244, SUT246, SUT251, and SUT255; Chaiyaphum Province (SUT025); Nakhon Ratchasima Province (SUT081).

Colonies on PDA covering 9 cm Petri dish in two weeks at room temperature, 23-28°C, at first white then dull green, floccose, azonate, with diffuse margins, with scattered black patches. Anamorph not formed.

This species was close to *Hypoxylon nitens* (Ces.) Y.-M. Ju & J.D. Rogers. (Ju and Rogers, 1996). Some specimens examined however were shiny black but some were matt. The type of ostiolar disc was *truncatum*-type but *Hypoxylon nitens* (Ces.) Y.-M. Ju & J.D. Rogers was *bovei*-type.



**Figure 18.** *Hypoxylon purpureonitens* Y.-M. Ju & J.D. Rogers (SUT004); (a) stromatal form (Bar = 0.3 mm), (b) ascospores (Bar = 5  $\mu$ m), (c) KOH-extractable pigment vinaceous purple, (d) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), (e) straight germ slit spore length (arrowed) (Bar = 2  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 19.** *Hypoxylon stygium* (Lév.) Sacc. (SUT058); (a) stromatal form (Bar = 0.2 mm), (b) KOH-extractable pigment greenish olivaceous, (c) ascospores dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), (d) ascospores (Bar = 5  $\mu$ m), and (e) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



Figure 20. Hypoxylon urceolatum (Rehm) Y.-M. Ju & J.D. Rogers (SUT098); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment vinaceous purple, (c) stromatal form (Bar = 0.3 mm), (d) perithecia (Bar = 0.4 mm), (e) ascospores (Bar = 10 μm), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 21.** *Hypoxylon* taxonomic species 1 sp. nov. (SUT236); (a) stromatal form (Bar = 0.4 mm), (b) KOH-extractable pigment greenish olivaceous, (c) apical apparatus (arrowed) (Bar = 1  $\mu$ m), (d) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), (e) straight germ slit spore length (arrowed) (Bar = 2  $\mu$ m), (f) ascospores (Bar = 10  $\mu$ m), and (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).

#### 4.2.4.2 Hypoxylon section Hypoxylon

Twenty one species of *Hypoxylon* sect. *Hypoxylon* (Table 15) were observed. Three of them were described as new species, *Hypoxylon* sublenormandii sp. nov., *Hypoxylon kanchanapisekii* sp. nov., and *Hypoxylon* suranareei sp. nov.

*Hypoxylon anthochroum* SUT233, SUT240, and SUT263 (Figure 22) examined were virtually identical to *Hypoxylon anthochroum* Berk. & Broome as described by Ju and Rogers (1996).

*Hypoxylon brevisporum* SUT256 (Figure 23) examined closely resembled the species *Hypoxylon brevisporum* Y.-M. Ju & J.D. Rogers as described by Ju and Rogers (1996) except for the KOH-extractable pigments, which were hazel or blackish brown and olivaceous grey or greenish olivaceous respectively.

*Hypoxylon duranii* SUT223, SUT239, SUT248, SUT252, SUT254, SUT259, and SUT284 (Figure 24) examined fitted *Hypoxylon duranii* J.D. Rogers (Ju and Rogers, 1996) except the ascospores which were 8.8-10(-11.3) x 2.8-5  $\mu$ m and 9.5-13(-14.5) x 4.5-6.5  $\mu$ m respectively. However, the ascospore size of these collections was similar to those of specimens found in Thailand (Thienhirun, 1997).

*Hypoxylon fendleri* SUT040, SUT061, SUT120, SUT145, SUT159, SUT162, SUT163, SUT165, and SUT280 (Figure 25) examined closely resembled *Hypoxylon fendleri* Berk. *ex* Cooke as described by Ju and Rogers (1996) except the germ slit form which was sigmoid. Initially, one of specimens, SUT120, was placed to *H. retpela* because the germ slit form was straight to slightly sigmoid. After observing perispore ornamentation by SEM, the specimen exhibited inconspicuous coil-like ornamentation, which was a character of *H. fendleri*. Therefore, the specimen SUT120 was considered to be *H. fendleri*. This taxon is similar to *H. retpela*, and these are the only two *Hypoxylon* taxa with a vinaceous stromatal surface among the *Hypoxylon* taxa with orange or orange red granules inside the stromata. These two species differ mainly in the conspicuousness of the ornamentation on the perispore (Ju and Rogers, 1996).

*Hypoxylon* cf. *ferrugineum* SUT017 (Figure 26), *H*. cf. *ferrugineum* SUT070 (Figure 27), and *H*. cf. *ferrugineum* SUT237 (Figure 28) were similar to *H. ferrugineum* Otth. (Ju and Rogers, 1996). *Hypoxylon* cf. *ferrugineum* SUT070 differed in stromatal surface color (dark brick or hazel), and in granule color (rusty brown or ochraceous brown) whereas *H.* cf. *ferrugineum* SUT017 differed in ascospore size 12.5-15(-17.5) x 5-7.5  $\mu$ m cf. (13.5-)14-17 x 6.5-8(-8.5)  $\mu$ m (Ju and Rogers, 1996). *Hypoxylon* cf. *ferrugineum* (SUT237) differed in stromatal surface color. In addition, this taxon was different from *H.* cf. *ferrugineum* (SUT070) in stromatal form and KOH-extractable pigment colour. Although, *H. ferrugineum* was placed as a variety of *H. rubiginosum* by Miller (1961), it was recognised as a different species based on habitat of stromata, distribution of granules, colors of the tissue below the perithecial layer, and ascospore size range, (8-)9-12 x 4-5.5  $\mu$ m (Ju and Rogers, 1996). Nevertheless, *H. ferrugineum* has been found in Swiss and U.S.A. It has never been reported in Southeast Asia.

Hypoxylon haematostroma SUT062, SUT064, SUT164, SUT292, and SUT293 (Figure 29) examined fitted Hypoxylon haematostroma Mont. apud Sagra as described by Ju and Rogers (1996) but they differed from H. haematostroma as reported by Thienhirun (1997), which had smaller ascospores 13-17.9 x 6.3-8.6  $\mu$ m cf. 12.5-13.8 x 6.3-7.5  $\mu$ m (Thienhirun, 1997).

Character	H. anthochroum Berk. & Broome*	H. brevisporum YM. Ju & J.D. Rogers*	H. duranii J.D. Rogers*
Stromata			
Shape	Effused-pulvinate	Effused-pulvinate	Glomerate, restricted-pulvinate to effused- pulvinate
Color	Chestnut or brown vinaceous	Brown vinaceous	Brown vinaceous
Granules beneath surface	Brown to blackish	Black	Reddish brown
KOH pigments	Olivaceous	Hazel or blackish brown	Isabelline or yellowish brown
Perithecia			-
Shape	Obovoid	Obovoid to tubular	Spherical to obovoid
Size	0.2-0.3(-0.4) mm diameter x 0.3-0.6 mm high	0.2 mm diameter x 0.3-0.7 mm high	0.1-0.3 mm diameter x 0.2-0.5 mm high
Ostiole	Lower than the stromatal surface	Lower than the stromatal surface, with white substance	Lower than the stromatal surface
Apical apparatus	Discoid, 0.5 µm high x 2-2.5 µm broad	Not observed	Discoid, 0.8-1.5 µm high x 2-3µm broad
Ascospores			
Color	Brown to dark brown	Light brown to brown	Brown to dark brown
Shape	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-equilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends
Size	10.8-13(-14) x 4-6 μm	6.1-7.2 x 2.7-3.7 μm	8.8-10(-11.3) x 3.8-5 μm
Germ slit	Straight full length	Straight full length	Straight full length
Perispore	Dehiscent, with inconspicuous coil-like ornamentation	Smooth	Dehiscent, with very conspicuous coil-like ornamentation
Habitat	On wood	On wood	On wood
Location	Trad	Nakhon Ratchasima, Trad	Kanchanaburi, Trad
Specimen examined	SUT233, SUT240, and SUT263	SUT256	SUT223, SUT239, SUT248, SUT252, SUT254, SUT259, and SUT284

**Table 15.** Morphological characteristics of *Hypoxylon* sect. *Hypoxylon* found in this study.

#### Table 15. (Continued).

Character	H. fendleri Berk. ex Cooke*	H. cf. ferrugineum (SUT017)*	H. cf. ferrugineum (SUT070)*
Stromata			
Shape	Effused-pulvinate	Hemispherical, pulvinate to effused-	Effused-pulvinate
		pulvinate	
Color	Brown vinaceous or dark brick	Hazel	Brown vinaceous
Granules beneath surface	Orange red	Yellowish orange	Brown vinaceous
KOH pigments	Orange	Orange	Orange
Perithecia		-	
Shape	Obovoid	Obovoid	Obovoid
Size	0.2-0.4 mm diameter x 0.3-0.6 mm high	0.2-0.4 mm diameter x 0.3-0.5 mm high	0.2-0.4 mm diameter x 0.3-0.5 mm high
Ostiole	Lower than the stromatal surface	Lower than the stromatal surface, usually with white substance	Lower than the stromatal surface
Apical apparatus	Discoid, 0.5-1.2 µm high x 1.8-2.5 µm broad	Not observed	Not observed
Ascospores			
Color	Brown to dark brown	Brown to dark brown	Dark brown
Shape	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends, infrequently with one or two ends pinched
Size	8.75-11.25(-12.5) x 3.75-5 μm	12.5-15(-17.5) x 5-7.5 μm	(15-)16.3-17.5 x 7.5 μm
Germ slit	Slightly sigmoid full length	Straight full length	Straight full length
Perispore	Dehiscent, with inconspicuous coil-like ornamentation	Dehiscent, with inconspicuous coil-like ornamentation	Dehiscent, with conspicuous coil-like ornamentation
Habitat	On wood	On wood	On wood
Location	Kanchanaburi, Nakhon Ratchasima, Ratchaburi, Yasothorn	Burirum	Ratchaburi
Specimen examined	SUT040, SUT061, SUT120, SUT145, SUT159, SUT162, SUT163, SUT165, and SUT280	SUT017	SUT070

Table 15. (Con
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Character	H. cf. ferrugineum (SUT237)*	H. haematostroma Mont. apud Sagra*	H. hypomiltum Mont.*
Stromata			
Shape	Glomerate	Hemispherical to effused-pulvinate	Effused-pulvinate
Color	Brown vinaceous or rusty brown	Orange red or rust	Dark brick
Granules beneath	Brown vinaceous	Reddish brown	Dull rusty brown
surface			
KOH pigments	Orange	Orange red	Amber or yellowish brown
Perithecia			
Shape	Obovoid	Long tubular	Obovoid
Size	0.2-0.4 mm diameter x 0.3-0.5 mm	0.2-0.5 mm diameter x 1.8-2.2 mm high	0.3-0.5 mm diameter x 0.5-0.7 mm high
	high		
Ostiole	Lower than the stromatal surface	Lower than the stromatal surface	Lower than the stromatal surface
Apical apparatus	Discoid, 0.5 µm high x 2.7-3.4 µm	Discoid, 2.5-3 µm high x 1.3-1.5 µm broad	Discoid, 0.3-0.6 µm high x 1.2-1.5 µm broad
	broad		
Ascospores			
Color	Brown to dark brown	Brown to dark brown	Light brown to brown
Shape	Ellipsoid-inequilateral, with narrowly	Ellipsoid-inequilateral, with narrowly	Nearly equilateral, with nearly acute ends
	rounded ends	rounded ends	
Size	(12.2)-13.4-17.8 x 5.3-8.3 μm	13-17.9 x 6.3-8.6 μm	7.5-8 x 2.5-3.8 μm
Germ slit	Straight full length	Slightly sigmoid full length	Straight full length
Perispore	Dehiscent, with conspicuous coil-like	Dehiscent, smooth	Dehiscent, smooth
	ornamentation		
Habitat	On wood	On wood	On wood
Location	Trad	Kanchanaburi, Ratchaburi, Yasothorn	Yasothorn
Specimen examined	SUT237	SUT062, SUT064, SUT164, SUT292, and	SUT166
		SUT293	

Character	<i>H. investiens</i> (Schwein.) M.A. Curtis*	H. lenormandii Berk. & M.A. Curtis apud Berk.*	H. lenormandii var. microspora (Thienhirun, 1997)*
Stromata			
Shape	Effused-pulvinate	Glomerate to effused-pulvinate with the tendency to be perithecioid	Effused-pulvinate, with the tendency to be perithecioid
Color	Brown vinaceous or chestnut	Grayish sepia	Blackish brown
Granules beneath surface	Black	Dull orange brown to dark brown	Black
KOH pigments	Dull green	Red	Reddish brown
Perithecia			
Shape	Obovoid to tubular	Spherical	Spherical
Size	0.3-0.4 mm diameter x 0.5-1 mm high	0.3-0.5 (-0.6) mm diameter	0.5-0.8 mm diameter
Ostiole	Lower than the stromatal surface	Slightly higher than the stromatal surface	Coarsely papillate
Apical apparatus	Not observed	Discoid, 0.7-1.5 µm high x 2-3 µm broad	Discoid, 0.5 µm high x 1-1.5 µm broad.
Ascospores		· - · ·	· - · ·
Color	Light brown to brown	Brown to dark brown	Brown to dark brown
Shape	Ellipsoid, nearly equilateral with broadly rounded ends	Ellipsoid-inequilateral to equilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends
Size	7.3-8.8 x 2.5-3.8 μm	10-12.5 x 3.8-5 μm	5-6.3 x 2.5-3.8 μm
Germ slit	Straight less than length	Slightly sigmoid full length	Straight full length
Perispore	Indehiscent	Dehiscent, with inconspicuous coil-like ornamentation	Dehiscent, smooth
Habitat	On wood	On wood	On wood
Location	Nakhon Ratchasima, Ratchasima	Burirum, Kanchanaburi, Nakhon Ratchasima	Chaiyaphum
Specimen examined	SUT041 and SUT063	SUT016, SUT065, SUT144, SUT147, SUT151, SUT180, SUT181, and SUT283	SUT022

 Table 15. (Continued).

# Table 15. (Continued).

Character	H. macrocarpum Pouzar*	H. monticulosum Mont.*	H. cf. perforatum (SUT020)*
Stromata			
Shape	Effused-pulvinate	Pulvinate to effused-pulvinate	Hemispherical, pulvinate to effused- pulvinate
Color	Brown vinaceous	Rust, brown vinaceous then blackish when mature	Grayish sepia
Granules	Brown vinaceous	Black	Dark brown or black
KOH pigments	Hazel or yellowish brown	Colorless or purple	Amber or yellowish brown
Perithecia			
Shape	Obovoid	Obovoid	Spherical
Size	0.17-0.2 mm diameter x 0.6-0.9 mm high	0.2-0.5 mm diameter x 0.3-0.5 mm high	0.1-0.3 mm diameter
Ostiole	Slightly higher than the stromatal surface	Higher than the stromatal surface and minutely papillate	Lower than the stromatal surface
Apical apparatus	Not observed	Discoid, 1 $\mu$ m high x 2 $\mu$ m broad	Discoid, 0.5-1.8 µm high x 2-2.8 µm broad
Ascospores			
Color	Brown to dark brown	Brown to dark brown	Brown to dark brown
Shape	Ellipsoid-inequilateral to equilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-inequilateral to equilateral, with narrowly rounded to end pinched
Size	8.8-11.3 x 3.8-5 μm	(6.3-)7.5-8.8(-11.3) x 3.8-5µm	(7.5-)8.8-10 x 5-6.3 μm
Germ slit	Straight full length	Slightly sigmoid full length	Straight full length
Perispore	Dehiscent, with inconspicuous coil-like ornamentation	Dehiscent, with smooth to inconspicuous coil-like ornamentation	Dehiscent, with inconspicuous coil-like ornamentation
Habitat	On wood	On wood	On wood
Location	Ratchaburi	Kanchanaburi, Nakhon Ratchasima, Songkhla, Trad	Burirum
Specimen examined	SUT045	SUT042, SUT059, SUT060, SUT073, SUT080, SUT094, SUT106, SUT115, SUT116, SUT179, SUT185, SUT189, SUT225, SUT227, SUT232, SUT235, SUT264, SUT265, SUT266, SUT287, and SUT295	SUT020

Table 15.	(Continued).
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Character	H. cf. perforatum (SUT224)*	H. cf. perforatum (SUT294)*	H. rubiginosum (Pers.: Fr.) Fr., Summa Veg*
Stromata			ž
Shape	Hemispherical, pulvinate to effused- pulvinate	Pulvinate to effused-pulvinate	Effused-pulvinate and sometimes pulvinate or even hemispherical
Color	Brown vinaceous	Reddish brown	Brown vinaceous
Granules beneath surface	Brown vinaceous	Reddish brown	Dark brown
KOH pigments	Amber or yellowish brown	Amber or honey	Rust
Perithecia			
Shape	Obovoid	Spherical	Obovoid
Size	0.1-0.3 mm diameter x 0.3-0.5 mm high	0.1-0.3 mm diameter	0.2-0.5 mm diameter x 0.3-0.6 mm high
Ostiole	Lower than the stromatal surface, usually overlay with conspicuous white substance	Lower than the stromatal surface	Lower than the stromatal surface
Apical apparatus	Discoid, 0.5 μm high x 1-1.5 μm broad.	Discoid, 0.5-1 $\mu$ m high x 2.5 $\mu$ m broad	Discoid, 0.8-1.5 $\mu m$ high x 2-3 $\mu m$ broad
Ascospores			
Color	Brown to dark brown	Brown to dark brown	Brown to dark brown
Shape	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-inequilateral to equilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends
Size	8.8-10 x 3.8-5(-6.3) μm	8.8-11.3 x 3.8-5 μm	(7.5-)8.8-10 x 3.8-5 μm
Germ slit	Straight full length	Straight full length	Straight full length
Perispore	Dehiscent, with conspicuous coil-like ornamentation	Dehiscent, with inconspicuous coil-like ornamentation	Dehiscent, with smooth to inconspicuous coil-like ornamentation
Habitat	On wood	On wood	On wood
Location	Trad	Kanchanaburi	Trad
Specimen examined	SUT224	SUT294	SUT215 and SUT221

Character	H. subgilvum Berk. & Broome var. microsporum (Abe) YM. Ju & J.D. Rogers *	H. trugodes (SUT154)*	H. trogodes (SUT187)*
Stromata			
Shape	Effused-pulvinate	Effused-pulvinate	Effused-pulvinate
Color	Hazel or dark brick	Brown vinaceous	Sepia
Granules beneath surface	Yellowish orange	Brown vinaceous	Brownish yellow
KOH pigments	Orange	Amber or yellowish brown	Amber or yellow
Perithecia			
Shape	Obovoid	Obovoid	Obovoid
Size	0.2-0.5 mm diameter x 0.3-0.6 mm high	0.2-0.4 mm diameter x 0.3-1.2 mm high	0.2-0.4 mm diameter x 0.3-1.2 mm high
Ostiole	Lower than the stromatal surface	Lower than the stromatal surface, inconspicuous, sometimes on flattened area	Lower than the stromatal surface
Apical apparatus	Not observed	Discoid, 0.3-0.8 µm high x 1.5-2 µm broad	Discoid, 0.3-0.8 µm high x 1.5-2 µm broad
Ascospores			
Color	Brown to dark brown	Dark brown	Brown to dark brown
Shape	Ellipsoid-inequilateral to equilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends
Size	(3.8-)5-7.5 x 2.5-3.8 μm	10-12.5 x 3.8-5 μm	10-11.3(-12.5) x 3.8-5(-6.3) μm
Germ slit	Straight to slightly sigmoid full spore- length	Straight full length	Straight full length
Perispore	Dehiscent, with inconspicuous coil-like	Dehiscent, with inconspicuous coil-like	Dehiscent, with inconspicuous coil-like
*	ornamentation	ornamentation	ornamentation
Location	Songkhla	Nakhon Ratchasima	Nakhon Ratchasima, Trad
Specimen examined	SUT095, SUT104, and SUT108	SUT154	SUT187

# Table 15. (Continued).

<b>Table 15.</b> (	Continued).
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Character	H. kanchanapisekii N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S.	H. sublenormandii N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S.	H. suranareei N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley. sp. nov. *
<u>Q</u> (	Whalley. sp. nov. *	Whalley. sp. nov. *	nov. *
Stromata Shape	Glomerate to pulvinate	Effused-pulvinate	Glomerate to effused-pulvinate with the tendency to be perithecioid
Color	Dull reddish brown	Dark brick or brown vinaceous	Orange brown
Granules beneath surface	Reddish brown	Brown vinaceous	Orange
KOH pigments Perithecia	Reddish brown	Amber or yellowish brown	Yellowish orange
Shape	Spherical	Spherical	Obovoid
Size	0.1-0.2 mm diameter	0.3-0.5 (-0.6) mm diameter	0.2-0.4 mm diameter x 0.3-0.5 mm high
Ostiole	Slightly higher or the same as the stromatal surface	Higher than the stromatal surface	Same or lower than the stromatal surface, with white substance
Apical apparatus	Discoid, 1.25 μm high x 2.5 μm broad	Discoid, 0.7-1.5 $\mu$ m high x 2-3 $\mu$ m broad	Discoid, 0.7-1.5 $\mu$ m high x 2-3 $\mu$ m broad
Ascospores			
Color	Brown to dark brown	Brown to dark brown	Brown to dark brown
Shape	Ellipsoid-inequilateral to equilateral, with narrowly rounded ends	Ellipsoid-inequilateral to equilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends
Size	(7.5-)10-11.3(-12.5) x 3.8-5 μm	9-12 x 3.8-5 μm	(10-)12.5-13.8 x 5-6.3 μm
Germ slit	Straight full length	Straight full length	Straight full length
Perispore	Indehiscent, smooth	Dehiscent, with inconspicuous coil-like ornamentation	Dehiscent, with inconspicuous coil-like ornamentation
Location	Ratchaburi	Kanchanaburi, Nakhon Ratchasima, Trad	Nakhon Ratchasima
Specimen examined	SUT066, SUT067, SUT068, and SUT069	SUT250 and SUT282	SUT182, SUT183, and SUT184

# Table 15. (Continued).

Character	Hypoxylon taxonomic species 2 *	Hypoxylon taxonomic species 3 *
Stromata		
Shape	Effused-pulvinate and sometimes pulvinate or even hemispherical	Effused-pulvinate
Color	Brown vinaceous	Dark brick or brown vinaceous
Granule beneath surface	Brown vinaceous	Brown vinaceous
KOH pigments	Yellowish brown	Amber or yellowish brown
Perithecia		
Shape	Obovoid	Obovoid
Size	0.2-0.4 mm diameter x 0.3-0.5 mm high	0.2-0.5 mm diameter x 0.3-0.6 mm high
Ostiole	Lower than the stromatal surface	Lower than the stromatal surface, overlay with white substance
Apical apparatus	Discoid, 0.5-1.5 µm high x 2-3 µm broad	Discoid, 0.8-1.5 µm high x 2-3 µm broad
Ascospores		
Color	Brown to dark brown	Brown to dark brown
Shape	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends
Size	(8.8)11.3-12.5(17.5) x 5-7.5 μm	10-11.3 x 3.8-5 μm
Germ slit	Straight full length	Straight full length
Perispore	Dehiscent, smooth	Dehiscent, smooth
Habitat	On wood	On wood
Location	Nakhon Ratchasima	Yasothorn
Specimen examined	SUT082	SUT158



**Figure 22.** *Hypoxylon anthochroum* Berk. & Broome (SUT233); (a) stromatal form (Bar = 0.3 mm), (b) ascospores (Bar = 12  $\mu$ m), (c) KOH-extractable pigment olivaceous, (d) ascospore dehiscent in 10% KOH (arrowed) (Bar = 4  $\mu$ m), (e) straight germ slit spore length (Bar = 2  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 23.** *Hypoxylon brevisporum* Y.-M. Ju & J.D. Rogers (SUT256); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment hazel, (c) stromatal with white substance on the ostioles (Bar = 0.2 mm), (d) ascospores (Bar =  $6 \mu \text{m}$ ), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar =  $4 \mu \text{m}$ ), and (f) straight germ slit spore length (Bar =  $2 \mu \text{m}$ ).



**Figure 24.** *Hypoxylon duranii* J.D. Rogers (SUT223); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment yellowish brown, (c) stromatal form (Bar = 0.3 mm), (d) ascospores (Bar =  $10 \mu \text{m}$ ), (e) apical apparatus (Bar =  $2 \mu \text{m}$ ), (f) ascospore dehiscent in 10% KOH (arrowed) (Bar =  $2 \mu \text{m}$ ), and (g) straight germ slit spore length (Bar =  $2 \mu \text{m}$ ).



**Figure 25.** *Hypoxylon fendleri* Berk. *ex* Cooke (SUT162); (a) stromatal form (Bar = 0.3 mm), (b) ascospores (Bar = 10  $\mu$ m), (c) KOH-extractable pigment orange, (d) slightly sigmoid germ slit spore length (arrowed) (Bar = 1  $\mu$ m), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 1  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 26.** *Hypoxylon* cf. *ferrugineum* (SUT017); (a) and (c) stromatal form (Bars = 0.5 cm and 0.3 mm respectively), (b) KOH-extractable pigment orange, and (d) ascospores (Bar =  $10 \mu \text{m}$ ).



**Figure 27.** *Hypoxylon* cf. *ferrugineum* (SUT070); (a) stromatal form (Bar = 0.2 mm), (b) perithecia (Bar = 0.4  $\mu$ m), (c) KOH-extractable pigment yellowish orange, (d) ascospores (Bar = 10  $\mu$ m), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 2 weeks (Bar = 1 cm).



**Figure 28.** *Hypoxylon* cf. *ferrugineum* (SUT237); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment brownish yellow, (c) stromatal form (Bar =  $0.4 \mu m$ ), (d) ascospores (Bar =  $10 \mu m$ ), (e) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm), (f) ascospore dehiscent in 10% KOH (arrowed) (Bar =  $5 \mu m$ ), and (g) straight germ slit spore length (arrowed) (Bar =  $2 \mu m$ ).



**Figure 29.** *Hypoxylon haematostroma* Mont. (SUT164); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment orange, (c) stromatal form (Bar = 0.3 mm), (d) ascospores (Bar =  $10 \ \mu$ m), (e) apical apparatus (arrowed) (Bar =  $3 \ \mu$ m), (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm), and (g) ascospore dehiscent in 10% KOH (arrowed) (Bar =  $2 \ \mu$ m).

*Hypoxylon hypomiltum* SUT166 (Figure 30) examined was similar to *Hypoxylon hypomiltum* Mont. described by Ju and Rogers (1996).

*Hypoxylon investiens* SUT041 and SUT063 (Figure 31) matched *Hypoxylon investiens* (Schwein.) M.A. Curtis described by Ju and Rogers (1996) except for a small difference in ascospore size (7.3-8.8 x 2.5-3.8  $\mu$ m cf. (6-)6.5-9.5(-10) x 3-4.5  $\mu$ m. (Ju and Rogers, 1996)).

*Hypoxylon lenormandii* SUT016, SUT065, SUT144, SUT147, SUT151, SUT180, SUT181, and SUT283 (Figure 32) examined were very similar to *Hypoxylon lenormandii* Berk. & M.A. Curtis *apud* Berk. described by Ju and Rogers (1996) except for slightly smaller ascospores (10-12.5 x 3.8-5 μm cf. 9.5-15(-16) x 4-6.5(-7) μm (Ju and Rogers, 1996)).

*Hypoxylon lenormandii* var. *microspora* SUT022 (Figure 33) examined was similar to the species firstly reported by Thienhirun (1997) except for their ascospores, which were 5-6.3 x 2.5-3.8  $\mu$ m and 3.8-5 x 2.5-3  $\mu$ m respectively. This taxon was different from *H. lenormandii* in ascospore size, germ slit form, and its smooth perispore.

*Hypoxylon macrocarpum* SUT045 (Figure 34) closely fitted *Hypoxylon macrocarpum* Pouzar (Ju and Rogers, 1996) except for slightly differences in ascospore size, 8.8-11.3 x 3.8-5  $\mu$ m cf. 9-12.5(-13) x 4-5.5  $\mu$ m, and type of perithecia, obovoid cf. obovoid to tubular (Ju and Rogers, 1996). Although *H. macrocapum* is similar to *H. rubiginosum* but they differ in stromatal pigments (Ju and Rogers, 1996).


**Figure 30.** *Hypoxylon hypomiltum* Mont. (SUT166); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment amber, (c) stromatal form (Bar = 0.2 mm), (d) ascospore (Bar = 8  $\mu$ m), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), (f) straight germ slit spore length (arrowed) (Bar = 2  $\mu$ m), and (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



Figure 31. *Hypoxylon investiens* (Schwein.) M.A. Curtis. (SUT063); (a) stromatal form (Bar = 0.4 mm), (b) Perithecia (Bar = 0.2 mm), (c) KOH-extractable pigment dull green, (d) ascospore dehiscent in 10% KOH (arrowed) (Bar =  $2 \mu m$ ), (e) ascospore (Bar =  $8 \mu m$ ), (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm), and (g) straight germ slit spore length (arrowed) (Bar =  $2 \mu m$ ).



**Figure 32.** *Hypoxylon lenormandii* Berk. & M.A. Curtis. (SUT065); (a) stromatal form (Bar =  $0.5 \ \mu$ m), (b) ascospores (Bar =  $10 \ \mu$ m), (c) ascospore dehiscent in 10% KOH (arrowed) (Bar =  $2 \ \mu$ m), (d) KOH-extractable pigment of red, (e) slightly sigmoid germ slit spore length (arrowed) (Bar =  $2 \ \mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 33.** *Hypoxylon lenormandii* var. *microspora* (SUT022) (Thienhirun, 1997); (a) stromatal form (Bar = 0.5  $\mu$ m), (b) perithecia (Bar = 0.5  $\mu$ m), (c) KOH-extractable pigment of red, (d) apical apparatus (arrowed) (Bar = 1  $\mu$ m), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), (f) ascospores (Bar = 5  $\mu$ m), and (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 34.** *Hypoxylon macrocarpum* Pouzar (SUT045); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment hazel, (c) stromatal form (Bar = 0.2 mm), (d) perithecia (Bar = 0.2 mm), (e) straight germ slit spore length (Bar = 2  $\mu$ m), (f) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), (g) ascospores (Bar = 10  $\mu$ m), and (h) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).

## Hypoxylon monticulum SUT042, SUT059, SUT060, SUT073,

SUT080, SUT094, SUT106, SUT115, SUT116, SUT179, SUT185, SUT189, SUT225, SUT227, SUT232, SUT235, SUT264, SUT265, SUT266, SUT287, and SUT295 (Figure 35) closely fitted *Hypoxylon monticulum* Mont. (Ju and Rogers, 1996). The KOH-extractable pigments of the examined samples varied from colorless to purplish in color.

*Hypoxylon* cf. *perforatum* SUT020 (Figure 36), *H*. cf. *perforatum* SUT224 (Figure 37), and *H*. cf. *perforatum* SUT294 (Figure 38) collected from different locations were closed to *H. perforatum* (Schwein.: Fr.) Fr. as reported by Ju and Rogers (1996). All of specimens differed from *H. perforatum* in KOHextractable pigments, ascospore size, (8-)9-12(-13) x 4-6 µm, and germ slit form (slightly sigmoid) as shown in Table 15. *Hypoxylon perforatum* was considered to be a variety of *H. rubiginosum* by Petrini and Müller (1986). However, they were separated from each other by color of stromatal granules, color of KOH-extractable pigments, color of their stromata, and their anamorph (Ju and Rogers, 1996).

*Hypoxylon rubiginosum* SUT215 and SUT221 (Figure 39) examined matched *Hypoxylon rubiginosum* (Pers.: Fr.) Fr. as described by Ju and Rogers (1996).



**Figure 35.** *Hypoxylon monticulosum* Mont. (SUT116); (a), (b), and (c) stromatal form (Bars = 1 cm, 1 cm, and 0.5 mm respectively), (d) ascospore (Bar = 10  $\mu$ m), (e) KOH-extract colorless, (f) slightly sigmoid curve germ slit spore length (Bar = 2  $\mu$ m), (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm), and (h) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m).



**Figure 36.** *Hypoxylon* cf. *perforatum* (SUT020); (a) stromatal form (Bar = 0.5 cm), (b) KOH-extractable pigment amber, (c) stromatal form (Bar = 0.3 mm), (d) ascospores (Bar = 5  $\mu$ m), (e) straight germ slit spore length (Bar = 2  $\mu$ m), (f) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), and (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 37.** *Hypoxylon* cf. *perforatum* (SUT224); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment amber, (c) stromatal form (Bar = 0.4 mm), (d) perithecia (Bar = 0.2 mm), (e) ascospores (Bar = 10  $\mu$ m), (f) straight germ slit spore length (Bar = 2  $\mu$ m), and (g) ascospore dehiscent in 10% KOH (arrowed) (Bar = 5  $\mu$ m).



**Figure 38.** *Hypoxylon* cf. *perforatum* (SUT294); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment amber, (c) stromatal form (Bar = 0.3 mm), (d) ascospores (Bar = 10  $\mu$ m), (e) straight germ slit spore length (Bar = 2  $\mu$ m), (f) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), and (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 39.** *Hypoxylon rubiginosum* (SUT215); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment rust, (c) stromatal form (Bar = 0.2 mm), (d) ascospores (Bar = 10  $\mu$ m), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).

*Hypoxylon subgilvum* var. *microsporum* SUT095, SUT104, and SUT108 (Figure 40) examined fitted *Hypoxylon subgilvum* Berk. & Broome var. *microsporum* (Abe) Y.-M. Ju & J.D. Rogers as described by Ju and Rogers (1996).

*Hypoxylon trogodes* SUT187 and SUT154 (Figures 41 and 42) fitted *Hypoxylon trogodes* Berk. & Broome as described by Ju and Rogers (1996). *Hypoxylon trogodes* SUT187 and SUT154 differed slightly from *H. trogodes* in ascospore size.

*Hypoxylon kanchanapisekii* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley, sp. nov. (Figure 43). Characteristics of this taxon are as follows: stromata glomerate to pulvinate, restricted and usually containing less than 20 perithecia, perithecia occasionally almost free, 0.5-2 mm x 0.1-0.2 mm thick, with perithecial mounds inconspicuous to 1/3 exposed, surface dull reddish brown with KOH extractable pigments brown vinaceous (84), umber (9); perithecia spherical 0.1-0.2 mm diameter, ostioles slightly higher or the same as the stromatal surface; asci 105-120  $\mu$ m total length x 3.8-5  $\mu$ m broad, the spore bearing parts 75-85  $\mu$ m long with stipes 12.5-45  $\mu$ m; ascospores brown, unicellular, equilateral, with narrowly rounded ends, 10-11.25(-12.5) x (0.5-)3.75-5  $\mu$ m, with straight-germ slit less than to nearly spore length; perispore indehiscent in 10% KOH, smooth, epispore smooth.

Specimens examined: Thailand, Plant Nursery of the Royal Forest Department, Ratchaburi Province, the branch of the Royal Forest Department, on bamboo, 28 August 2003, Suwannasai, N. (Holotype SUT069); SUT066; SUT068.

Colonies on PDA covering 9-cm Petri dish in two weeks at room temperature, 23-28°C, at first creamy white then buff, velvety to felty, with concentric zones where aerial hyphal tufts develop. Anamorph not formed. This taxon was close to *H. lenormandii* but it differed in stromatal surface color of dull reddish brown not grayish sepia, in small ascospores  $(7.5-)10-11.3(-12.5) \ge 3.8-5 \ \mu\text{m}$  cf.  $9.5-15(-16) \ge 4-6.5(-7) \ \mu\text{m}$ , Ju and Rogers (1996), and in having a straight rather than slightly sigmoid germ slit. This taxon was only found on bamboo.

*Hypoxylon sublenormandii* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley, sp. nov. (Figure 44). Characteristics of this taxon are as follows: stromata glomerate to effused-pulvinate, often appearing almost rosellinioid but joined by thin stromal tissue, conspicuous perithecial mounds, surface reddish brown; reddish brown granules immediately beneath surface and between perithecia, with KOH-extractable pigments brown vinaceous (84), umber (9); perithecia spherical, 0.2-0.4 mm diameter, ostioles slightly higher than the stromatal surface; asci 95-110  $\mu$ m total length x 3.8-5  $\mu$ m broad, the spore bearing parts 65-75  $\mu$ m long with stipes 30-42.5  $\mu$ m; ascospores brown, unicellular, equilateral, with narrowly rounded ends, 9-12 x 4-5  $\mu$ m, with straight-germ slit spore length; perispore dehiscent in 10% KOH, with inconspicuous coil-like ornamentation; epispore smooth.

Specimens examined: Thailand, Kanchanaburi Province, Chong Kho Neab Forest, on bamboo, 14 December 2003, Suwannasai, N. (Holotype SUT282); Trad Province, Ta Gum Forest, on bamboo, 19 September 2003, Phosri, C. SUT250.

Colonies on PDA covering 9-cm Petri dish in two weeks at room temperature, 23-28°C, at first creamy white then brown, felty, azonate, with diffuse margins. Anamorph not formed. This species was similar to *H. lenormandii* and differed mainly in its ascospore size 8-10 x 3.8-5  $\mu$ m cf. 9.5-15(-16) x 4-6.5(-7)  $\mu$ m, Ju and Rogers (1996) and in its straight germ slit of spore length.

*Hypoxylon suranareei* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley, sp. nov. (Figure 45). Characteristics of this taxon are as follows: stromata glomerate to effused-pulvinate, often appearing almost rosellinioid but joined by thin stromal tissue, conspicuous perithecial mounds, surface orange brown; orange granules immediately beneath surface and between perithecia, with KOH-extractable pigments yellowish orange; perithecia obovoid, 0.2-0.4 mm diameter, ostioles same or lower than the stromatal surface, with white substance; asci 90-120  $\mu$ m total length x 3.8-5  $\mu$ m broad, the spore bearing parts 70-85  $\mu$ m long with stipes 30-50  $\mu$ m; ascospores brown to dark brown, unicellular, equilateral, with narrowly rounded ends (10-)12.5-13.8 x 5-6.3  $\mu$ m, with straight-germ slit spore length; perispore dehiscent in 10% KOH, with inconspicuous coil-like ornamentation; epispore smooth.

Specimens examined: Thailand, Suranaree University of Technology, Nakhon Ratchasima, on wood, 17 November 2003, Suwannasai, N. (Holotype SUT182), SUT183, and SUT184.

Colonies on PDA covering 9-cm Petri dish in two weeks at room temperature, 25°C, at first creamy white then brown, felty, azonate, with diffuse margins. Anamorph not formed.

This taxon was similar to *H. lenormandii* Berk. & M.A. Curtis *apund* Berk. in stromatal form but it was different in stromatal surface color, ascospore size  $(9.5-15(-16) \times 4-6.5(-7) \mu m)$ , germ slit form, and KOH pigment.



**Figure 40.** *Hypoxylon subgilvum* Berk. & Broome var. *microsporum* (Abe) Y.-M. Ju & J.D. Rogers. (SUT234); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment orange, (c) stromatal form (Bar = 0.5 mm), (d) perithecia (Bar =  $0.5 \ \mu m$ ), (e) ascospores (Bar =  $7 \ \mu m$ ), (f) straight to slightly sigmoid germ slit spore length (arrowed) (Bar =  $2 \ \mu m$ ), (g) apical apparatus (arrowed) (Bar =  $1 \ \mu m$ ), (h) ascospore dehiscent in 10% KOH (arrowed) (Bar =  $1 \ \mu m$ ), and (i) cultural characteristics on PDA cultured at  $25^{\circ}$ C after 4 weeks (Bar =  $1 \ cm$ ).



**Figure 41.** *Hypoxylon trogodes* Berk. & Broome (SUT187); (a) stromatal form (Bar = 1 cm), (b) perithecia (Bar = 0.2  $\mu$ m), (c) KOH-extractable pigment yellow, (d) straight germ slit spore length (arrowed) (Bar = 2  $\mu$ m), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), (f) ascospores (Bar = 10  $\mu$ m), and (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 42.** *Hypoxylon trugodes* Berk. & Broome (SUT154); (a) stromatal form (Bar = 1 cm), (b) perithecia (Bar = 0.4  $\mu$ m), (c) KOH-extractable pigment yellowish brown, (d) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), (e) ascospores (Bar = 10  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 43.** *Hypoxylon kanchanapisekii* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley, sp. nov. (SUT069); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment reddish brown, (c) stromatal form (Bar = 0.2 mm), (d) perithecia (Bar =  $0.2 \ \mu m$ ), (e) ascospores (Bar =  $10 \ \mu m$ ), (f) apical apparatus (arrowed) (Bar =  $1 \ \mu m$ ), (g) straight germ slit less than spore length (arrowed) (Bar =  $2 \ \mu m$ ), and (h) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar =  $1 \ cm$ ).



**Figure 44.** *Hypoxylon sublenormandii* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley, sp. nov. (SUT282); (a) stromatal form (Bar = 0.5 cm), (b) ascospores (Bar = 10  $\mu$ m), (c) KOH-extractable pigment of reddish brown, (d) apical apparatus (arrowed) (Bar = 1  $\mu$ m), (e) straight germ slit spore length (arrowed) (Bar = 2  $\mu$ m), (f) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm), and (h) SEM micrograph of coil-like ornamentation of perispore (Bar = 1  $\mu$ m).



Figure 45. *Hypoxylon suranareei* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley, sp. nov. (SUT182); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment yellowish orange, (c) stromatal form (Bar = 0.5 μm), (d) straight germ slit spore length (arrowed) (Bar = 5 μm), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 5 μm), (f) ascospores (Bar = 10 μm), and (g) cultural characteristics on PDA cultured at 25°C after 2 weeks (Bar = 1 cm).

*Hypoxylon* taxonomic species 2 (SUT082) (Figure 46) was close to *Hypoxylon rubiginosum* (Pers.: Fr.) Fr. as described by Ju and Rogers (1996) except that KOH-extractable pigment colour was yellowish brown and the range of ascospore size was broader ((8.8-)11.3-12.5(-17.5) x 5-7.5  $\mu$ m) than *Hypoxylon rubiginosum* (Pers.: Fr.) Fr. ((8-)9-12 x 4-4.5  $\mu$ m). In addition, perispore of this taxon was inconspicuous coil-like ornamentation whereas *H. rubiginosum* was smooth (Ju and Rogers, 1996).

*Hypoxylon* taxonomic species 3 (SUT158) (Figure 47) was close to *Hypoxylon rubiginosum* (Pers.: Fr.) Fr. as described by Ju and Rogers (1996) except for KOH-extractable pigment colour. *Hypoxylon* taxonomic species 3 had the same colour of KOH-extractable pigment as *Hypoxylon* taxonomic species 2 but ascospore size of *Hypoxylon* taxonomic species 2 was slightly smaller than *Hypoxylon* taxonomic species 3, (8.8-)11.3-12.5(-17.5) x 5-7.5  $\mu$ m and 10-11.3 x 3.8-5  $\mu$ m respectively.



**Figure 46.** *Hypoxylon* taxonomic species 2 (SUT082); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment rust, (c) stromatal form (Bar = 0.2 mm), (d) ascospores (Bar = 10  $\mu$ m), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 47.** *Hypoxylon* taxonomic species 3 (SUT158); (a) stromatal form (Bar = 0.5 cm), (b) ascospores (Bar = 10  $\mu$ m), (c) straight germ slit spore length (arrowed) (Bar = 2  $\mu$ m), (d) KOH-extractable pigment amber, (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).

## 4.2.5 Group V: Xylariaceous endophytes

Although eight xylariaceous genera have been reported as endophytes, Anthostomella, Biscogniauxia, Daldinia, Hypoxylon, Kretzschmaria, Nemania, Rosellinia, and Xylaria, the common endophytic genus is Xylaria. In this study, twenty two species of Xylaria were recorded (Table 16). Other xylariaceous genera, Kretzschmaria, Nemania, and Biscogniauxia, were also included (Table 16).

 Table 16.
 Species of Xylaria, Kretzschmaria, Nemania, and Biscogniauxia found in this study.

Species	No.*	Remark
<i>Xylaria</i>		
X. anisopleura (Mont.) Fr.	3	According to Rogers and Samuels
		(1986), Rogers (1988), González
		and Rogers (1989), and Thienhirur
		(1997)
X. badia Pat.	15	According to Van der Gucht
		(1995), and Thienhirun (1997)
X. beccari Lloyd	1	Lloyd (1924)
X. brachiata Sacc.	1	Lloyd (1919)
X. cubensis (Mont.) Fr.	6	According to Rogers and Samuels
		(1986), Rogers et al. (1988),
		González and Rogers (1989), and
		Thienhirun (1997)
X. ianthino-velutina (Mont.) Fr.	2	According to Dennis (1957),
		González and Rogers (1989), and
		Thienhirun (1997)
X. cf. juruensis (SUT035)	1	Ascospore size overlaps between
		X. juruensis and X multiplex.
X. juruensis var. microspora	8	Thienhirun, 1997
X. maitlandii (Dennis) D. Hawksw.	2	According to González and Rogers
		(1989)
X. mellisii (Berk.) Cooke	2	Van der Gucht (1995)
X. multiplex (Kunze) Fr.	1	Dennis (1957; 1961), González
		and Rogers (1989), and Thienhirur
		(1997)

\* Number of collections

Table 16.(Continued).

Species	No.*	Remark
Xylaria		
X. muscula Lloyd	2	Dennis (1957)
X. psidii J.D. Rogers & Hemmes	5	According to Rogers, Ju and
		Hemmes (1992), and Thienhirun (1997)
X. schweinitzii Berk. & M.A. Curtis	1	According to Rogers <i>et al.</i> (1988),
A. Schweinulzu Derk. & M.A. Curus	1	González and Rogers (1989), and Thienhirun (1997)
X. scruposa (Fr.) Fr.	1	Van der Gucht (1995)
X. telfairii (Berk.) Fr.	1	According to Dennis (1961),
A. tegunti (Dork.) 11.	1	Rogers <i>et al.</i> (1987 and 1988),
		Callan and Rogers (1990), and
		Thienhirun (1997)
<i>Xylaria</i> species 2	17	Thienhirun, 1997
<i>Xylaria</i> sp. nov.	27	Rough stromatal surface, finely
		reticulately cracked into small
		angular closely spaced scales so as
		to outline the individual perithecia
<i>Xylaria</i> taxonomic species 1 (SUT075)	1	Smooth stromatal surface except for
	-	peeling layer, externally blackish
		with dark brown outer peeling laye
		internally creamy white
<i>Xylaria</i> taxonomic species 2 (SUT203)	1	Smooth stromatal surface except for
		peeling layer, externally blackish
		brown to black with dull black
		peeling of outer later, internally white
<i>Xylaria</i> taxonomic species 3 (SUT204)	1	Rugose and usually roughened
Ayunu uxonomie species 5 (501201)	1	stromatal surface by warts, external
		black and internally white
<i>Xylaria</i> taxonomic species 4 (SUT207)	1	Smooth stromatal surface except fo
		ostiolar slightly raised, externally
		copper- to bronze-colored to brown
		with black of ostioles, internally
		creamy white
Kretzschmaria		
Kretzschmaria species (SUT101)	2	Stromata clustered subglobose ferti
		head with short stalk, not branched
		externally blackish, internally white
Nemania		
Nemania species (SUT258)	2	Erumpent to superficial stromata,
		smooth with slightly papillate surfa

\* Number of collections

Table 16.(Continued).

Species	No.*	Remark
Biscogniauxia		
Biscogniauxia capnodes (Berk.) YM.	4	Ju and Rogers (1998)
Ju & J.D. Rogers		
Biscogniauxia sp. nov. (SUT290)	2	Applanate stromata, smooth
		surface, externally black and
		internally yellow which
		distinguished from the other known
		species

\* Number of collections

The main characteristics for each species examined are described in Table 17. *Xylaria anisopleura* SUT196, SUT205, and SUT208 (Figure 48) matched *Xylaria anisopleura* (Mont.) Fr. as described by Rogers and Samuels (1986), Rogers (1988), and González and Rogers (1989), but the collections from this study were different from the specimens described by Thienhirun (1997) in ascospore size, (20-)23.8-25(-27.5)  $\mu$ m cf. 17.5-22.5 x 10-11.3  $\mu$ m (Thienhirun, 1997). In addition, the specimens examined had two different stromatal types. The stromata of SUT208 and SUT209 were boarder than high but SUT196 was higher than board. However, both of them had the same cultural characteristics on PDA. The species that is related to *X. anisopleura* is *X. polymorpha* (Pers.: Fr.) Grev., but they are different in the smaller size and moriform shape of stromata and the short, oblique to somewhat spiraling ascospore germ slit (Rogers and Samuels, 1986).

*Xylaria badia* SUT026, SUT032, SUT076, SUT142, SUT309, and SUT310 (Figure 49) were close to *Xylaria badia* Pat. as described by Van der Gucht (1995), and Thienhirun (1997). This taxon was specific to bamboo, and was widely distributed throughout Thailand.

Character	X. anisopleura (Mont.) Fr.*	X. badia Pat.*	X. beccari Lloyd. *
Stromata			
Shape	Clavate to cylindrical, or subglobose, with rounded fertile apices	Short cylindrical to clavate, with rounded fertile apices	Cylindrical to clavate from short concolorous stipe
Color	Externally dark brown to dull black, internally white	Externally silvery brown and became to grayish brown with age, internally brownish orange	Externally brownish black, internally white
Texture	Woody	Hard	Woody
Surface	Cracked into minute angular scales, rough due to wrinkles, warts and tomentum	Smooth and shinning	Roughened due to little cracks and small excrescences
Perithecia			
Shape	Partially immersed, subglobose	Completely immersed, subglobose	Subglobose
Size	0.5-1 mm diameter	0.2-0.3 mm diameter	0.2-0.3 mm diameter
Ostiole	Inconspicuous, papillate, appearing as small hemispherical black discs	Finely papillate and black	Slightly papillate
Apical apparatus	Rectangular, constricted sub-apically, 7- 8(-9) μm high x 4-5 μm broad	Discoid, 0.6 µm high x 1.2 µm broad	Rectangular, 1-1.5 µm high x 1-1.5µm broad
Ascospores			
Color	Brown to dark brown	Light brown	Brown
Shape	Ellipsoid-inequilateral to crescentic, with narrowly rounded apiculate ends	Ellipsoid, with narrowly rounded ends	Ellipsoid-inequilateral with broadly rounded ends
Size	(20-)23.8-25(-27.5) x 7.5-8.8μm	7.5-8.8 x 3.8-4.4 μm	(5-)6.3-7.5 x 2.5-3 μm
Germ slit	Straight to curving, oriented obliquely to the long axis of the spore	Straight less than spore length	Straight full length
Habitat	On wood	On wood	On wood
Location	Trad	Kanchanaburi, Nakhon Ratchasima, Ratchaburi	Songkhla
Specimen examined	SUT196, SUT205, and SUT208	SUT026, SUT032, SUT076, SUT142, SUT309, and SUT310	SUT092

**Table 17.** Morphological characteristics of *Xylaria*, *Kretzschmaria*, *Nemania*, and *Biscogniauxia* found in this study.

 Table 17. (Continued).

Character	X. brachiata Sacc.*	X. cubensis (Mont.) Fr.*	X. ianthino-velutina (Mont.) Fr.*
Stromata			
Shape	Upright or prostrate, the fertile part short cylindrical to fusoid with perithecia immersed or with evident perithecial contours, with acute sterile apices	Cylindric-allantoid to clavate, occasionally flattened, with rounded fertile apices, short stipes, arising from tomentose discoid bases	Cylindrical, long conical, or flattened, the fertile parts bearing more or less naked perithecia, grading into ill-defined stipes
Color	Externally brown outer peeling layer, internally white	Externally bronze, becoming dark with age, internally white to cream	Externally blackish, internally white
Texture	Fairly hard	Hard	Soft
Surface	Smooth to roughened with perithecial contours	Smooth, sometimes very faintly, reticulately cracked around the ostioles, or surface conspicuously cracked into small polygonal surface scales	Roughened with perithecia and tomentose except the stromatal apices
Perithecia			
Shape	Subglobose	Completely immersed, subglobose	Subglobose
Size	0.3-0.5 mm diameter	0.3-0.8 mm diameter	0.2-0.3 mm diameter
Ostiole	Inconspicuous to papillate	Finely papillate to annulate	Minutely papillate
Apical apparatus	Rectangular, 3.5-4 µm high x 1.5-2.5 µm broad	Cubic to rectangular, 1.6-2.4 µm high x 1.4- 1.8(-2) µm broad	Cubic to rectangular, 1.3 µm high x 1.3 µm broad
Ascospores			
Color	Brown to dark brown	Light brown	Brown
Shape	Ellipsoid-inequilateral, with broadly to narrowly rounded ends	Ellipsoid-inequilateral, with rounded ends	Ellipsoid-inequilateral with rounded ends
Size	(8-)10-11.3(-12.5) x 3.8-5 μm	(6.3-)7.5-8.8 x 3.8-5 μm	(7.5-)8.8-10(-12.5) x 3.8-4 μm
Germ slit	Straight full length	Straight less than spore length	Straight less than spore length
Habitat	On wood	On wood	On legume pod
Location	Nakhon Ratchasima	Ratchaburi, Trad	Songkhla
Specimen examined	SUT078 and SUT175	SUT089, SUT090, SUT193, SUT194, and SUT199	SUT091 and SUT123

 Table 17. (Continued).

Character	X. cf. juruensis (SUT035)*	X. cf. juruensis (SUT088)*	X. cf. juruensis (SUT140)*
Stromata			
Shape	Short cylindrical to irregular with acute sterile apices (1 mm long), with short hair	Cylindrical to irregular with acute sterile apices, with thin stalk and no hair	Short cylindrical to irregular with acute sterile apices, short stalk with short hair
Color	Externally blackish with brownish gray peeling layer, internally white	Externally blackish with brownish gray peeling layer, internally white	Externally blackish with brownish gray peeling layer, internally white
Texture	Fairly hard	Fairly hard	Fairly hard
Surface	Roughened with perithecial contours	Roughened with perithecial contours	Roughened with perithecial contours
Perithecia			
Shape	Subglobose	Subglobose	Subglobose
Size	0.2-0.6 mm diameter	0.2-0.4 mm diameter	0.2-0.5 mm diameter
Ostiole	Umbilicate to slightly raise	Umbilicate to slightly raise	Umbilicate to slightly raise
Apical apparatus	Rectangular, 5-7 μm high x 2-4 μm broad	Rectangular, 4.5-7 μm high x 2-3.8 μm broad	Rectangular, 5-7 µm high x 2-3.8 µm broad
Ascospores			
Color	Brown	Brown	Brown
Shape	Ellipsoid-inequilateral, with rounded ends	Ellipsoid-inequilateral, with rounded ends	Ellipsoid-inequilateral, with rounded ends
Size	(10-)11.3-13.8 x 3.8-5 μm	12.5-15 x 3.8-5 μm	12.5-14(-15) x 3.8-5 μm
Germ slit	Straight slightly spore length	Straight slightly spore length	Straight slightly spore length
Habitat	On wood	On wood	On wood
Location	Ratchaburi	Songkhla	Nakhon Ratchasima
Specimen examined	SUT035	SUT088	SUT140

 Table 17. (Continued).

Character	X. cf. juruensis (170)*	X. juruensis var. microspora *	X. maitlandii (Dennis) D. Hawksw. *
Stromata			
Shape	Long cylindrical to irregular with acute sterile apices, with thin stalk and no hair	Cylindrical to irregular, terete to compressed, with hair-like apiculi on stipe	Cylindrical to gregarious, with acute sterile apices (1 mm diam), smooth stipe
Color	Externally blackish with brownish gray peeling layer, internally white	Externally blackish with brown peeling outer layer, internally white	Externally blackish with dark brown peeling outer layer, internally white
Texture	Fairly hard	Fairly hard	Fairly hard
Surface	Roughened with perithecial contours	Roughened by peeling layer	Smooth except for peeling layer
Perithecia			
Shape	Subglobose	Subglobose	Subglobose
Size	0.3-0.5 mm diameter	0.4-0.6 mm diameter	0.3-0.5 mm diameter
Ostiole	Umbilicate to slightly raise	Slightly raised	Slightly raised
Apical apparatus	Rectangular, 5-7 μm high x 2-4 μm broad	Rectangular, 1.4-1.8 µm high x 1.4-1.5 µm broad	Quadrate, 2 $\mu$ m high x 2 $\mu$ m broad
Ascospores			
Color	Brown	Brown	Brown to dark brown
Shape	Ellipsoid-inequilateral, with rounded	Ellipsoid-inequilateral with rounded to acute	Ellipsoid-inequilateral with broadly rounded
	ends	ends	ends
Size	11.3-12.5 x 3.8-5 μm	8.8-10 x 3.8-5 μm	11.3-12.5 x 3.8-5 μm
Germ slit	Straight slightly spore length	Straight less than spore length	Straight less than spore length
Habitat	On wood	On wood	On wood
Location	Nakhon Ratchasima	Nakhon Ratchasima	Nakhon Ratchasima
Specimen examined	SUT170	SUT129, SUT138, and SUT139	SUT177

 Table 17. (Continued).

Character	X. mellisii (Berk.) Cooke *	X. multiplex (Kunze) Fr. *	X. muscula Lloyd. *
Stromata			
Shape	Fertile part cylindrical to cylindric-	Cylindrical, with acute sterile apices, smooth	Cylindrical with sterile apices, on short
	conical, with acute sterile apices, on narrow hirsute stipes	stipe, arising from enlarged tomentose base	stipes
Color	Externally blackish with grey to brown	Externally blackish with light brown outer	Externally white with black ostioles,
	outer peeling layer, internally white to creamy white	peeling layer, which splited longitudinally into narrow strips, internally white	internally creamy white
Texture	Fairly hard	Fairly soft	Woody
Surface	Smooth except for peeling layer and ostiolar discs	Nodulose due to slightly protruding perithecial contours, and smooth	Slightly roughened by ostioles
Perithecia		•	
Shape	Immersed, subglobose	Partially immersed, subglobose	Subglobose
Size	0.3-0.4 mm diameter	0.5 mm diameter	0.2-0.4 mm diameter
Ostiole	Inconspicuous to finely papillate	Papillate	Umbilicate to slightly raised
Apical apparatus	Rectangular, 3-3.5 µm high x 2-2.5 µm broad	Rectangular, 1.5-2 $\mu$ m high x 1.5 $\mu$ m broad	Quadrate, 1-1.5 µm high x 1-1.5µm broad
Ascospores			
Color	Brown to dark brown	Brown	Light brown
Shape	Ellipsoid-inequilateral with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-inequilateral with broadly rounde ends
Size	12.5-15 x 3.8-5 μm	11.3-13.8(-15) x 3.8-5 μm	6-9(-10) x 3-3.5(-4) μm
Germ slit	Straight less than spore length	Straight full length	Straight full length
Habitat	On wood	On wood	On wood
Location	Ratchaburi, Trad	Ratchaburi	Nakhon Ratchasima
Specimen examined	SUT074 and SUT192	SUT028	SUT029

Table 17. (Cont	tinuea).
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Character	X. psidii J.D. Rogers & Hemmes*	X. schweinitzii Berk. & M.A. Curtis*	X. scruposa (Fr.) Fr.*
Stromata			-
Shape	Upright, cylindrical to more or less conical with acute sterile apices, bearing embedded to prominent perithecia	Cylindrical to clavate to irregular, with rounded fertile apices, with long or short stipes or sessile	Cylindrical to clavate to highly irregular, subglobose, on short or long stipes, with rounded or flattened fertile apices, on short to long narrowed smooth stipes
Color	Externally blackish, internally white	Externally brownish black to dull black, internally white	Externally yellowish brown to dark brown, internally white to creamy white
Texture	Fairly soft	Fairly hard	Woody to fairly hard
Surface	Roughened with perithecia	Cracked into minute scales, and rugulose	Rugose and usually roughened by warts
Perithecia			
Shape	Subglobose	Partially immersed, subglobose	Immersed, subglobose
Size	0.2-0.3 mm diameter	0.4-0.6 mm diameter	0.3-0.5 mm diameter
Ostiole	Umbilicate	Inconspicuous, umbilicate, appearing as small hemispherical black disks in between the dark brown scales	Inconspicuous, umbilicate, appearing as hemispherical black discs in between the brown scales
Apical apparatus	Cubic, 2 µm high x 2 µm broad	Rectangular, 4.5-5 $\mu$ m high x 3.8-5 $\mu$ m broad	Rectangular, constricted subapically, 4-5 µm high x 3-3.5 µm broad
Ascospores			
Color	Brown	Brown to dark brown	Light brown
Shape	Ellipsoid-inequilateral to somewhat fusoid, with rounded to acute ends	Ellipsoid-inequilateral with narrowly rounded ends	Ellipsoid-inequilateral with narrowly rounded to pinched ends
Size	(7.5-)8.8-10(-12.5) x 3.8-4 μm	18.8-21.3 x 6.3-7.5 μm	17.5-21.3(-22.5) x (5-)6.3-7.5 μm
Germ slit	Straight full length	Straight to slightly spiralling, obliquely oriented to the long axis of the spore, less than spore length	Straight to slightly sigmoid, slightly obliquely oriented to the long axis of the spore, less than spore length
Habitat	On Pod	On wood	On wood
Location	Songkhla	Trad	Petchaboon
Specimen examined	SUT124, SUT125, and SUT126	SUT201	SUT117

 Table 17. (Continued).

Character	<i>Xylaria</i> species 2 *	X. telfairii (Berk.) Fr.*	<i>Xylaria</i> sp. nov.*
Stromata			
Shape	Cylindrical, with rounded fertile	Cylindrical to fusiform, not branched or	Cylindrical, gregarious, with narrowly rounded
	apices, smooth stipe	occasionally branched near the base,	fertile apices, smooth stipe, which was
		with rounded fertile apices, smooth stipes, concolorous to the fertile part	longitudinally furrowed or wrinkled
Color	Externally blackish with dark brown sloughing scales, internally yellow	Externally pale yellow, clay-colored to orange brown, internally white	Externally dark brown to black, internally white
Texture	Woody	Hard to very hard	Woody
Surface	Rough, cracked into rounded or	Cracked into minute scales	Rough, finely reticulately cracked into small
	angular dark brownish scales		angular closely spaced scales so as to outline the individual perithecia
Perithecia			individual pertinecta
Shape	Immersed, subglobose	Partially immersed, subglobose	Immersed, sometimes vaguely evident in outline,
<b>T</b> .		, ,	subglobose
Size	0.3- 0.5 mm diameter	0.5-0.7 mm diameter	0.3- 0.5 mm diameter
Ostiole	Slightly raised	Inconspicuous, minute, black, and	Slightly papillate
		punctiform	
Apical apparatus	Rectangular, 1.5-2 µm high x 1.5 µm broad	Rectangular, constricted subapically, 4.5- 5 $\mu$ m high x 3.8-5 $\mu$ m broad	Quadrate to rectangular, 1.5 µm high x 1.5-2 µm broad
Ascospores	bload	5 µiii iligii x 5.8-5 µiii bload	bload
Color	Brown	Dark brown	Light brown to brown to dark brown
Shape	Ellipsoid-equilateral with narrowly	Ellipsoid-inequilateral with narrowly	Ellipsoid-equilateral with narrowly rounded ends
	rounded ends	rounded to pinched ends	r i i
Size	(7.5-)10-12.5 x 3.75-5 μm	17.5-20 x 5-6.25 μm	(7.5-)8.8-10 x (2.5-)3.8-5 μm
Germ slit	Slightly sigmoid full spore length	Straight to slightly sigmoid, obliquely	Straight full length
		oriented to the long axis of the spore, less	
		than spore length	
Habitat	On wood	On wood	On wood
Location	Kanchanaburi, Nakhon Ratchasima, Trad	Trad	Chiang Rai, Kanchanaburi, Nakhon Ratchasima, Trad
Specimen examined	SUT127, SUT128, SUT130, SUT132,	SUT206	SUT006, SUT012, SUT027, SUT031, SUT033,
	SUT134, SUT171, SUT195, SUT271,		SUT034, SUT087, SUT093, SUT131, SUT133,
	and SUT274		SUT136, SUT141, SUT143, SUT155, SUT172,
			SUT197, SUT198, SUT272, SUT273, and SUT275

 Table 17. (Continued).

Character	Xylaria taxonomic species 1 *	Xylaria taxonomic species 2 *	Xylaria taxonomic species 3 *
Stromata			
Shape	Cylindrical, with rounded fertile apices, short hair on stromata and stalk	Cylindrical, bearing completely immersed perithecia, with attenuated or acute sterile apices	Prostrate or upright, each stroma consisting of a rachis bearing scattered to crowded, naked perithecia, extended upward into sho acute apices
Color	Externally blackish with dark brown outer peeling layer, internally creamy white	Externally blackish brown to black with dull black peeling of outer later, internally white	Externally black, internally white
Texture	Fairly soft	Woody	Fairly soft
Surface	Smooth except for peeling layer	Smooth except for peeling outer layer	Rugose and usually roughened by warts
Perithecia			
Shape	Subglobose	Immersed, subglobose	Subglobose
Size	0.3- 0.5 mm diameter	0.4-0.8 mm diameter	0.4-0.8 mm diameter
Ostiole	Slightly papillate	Umbilicate to slightly raised	Papillate
Apical apparatus	Rectangular, 1-1.5 µm high x 1-1.5µm broad	Not observed	Rectangular, 1-1.5 µm high x 1-1.5µm broa
Ascospores			
Color	Brown	Brown to dark brown	Brown
Shape	Ellipsoid-inequilateral with broadly rounded ends	Ellipsoid-inequilateral with narrowly rounded to pinched ends	Ellipsoid-inequilateral with broadly rounded to pinched ends
Size	12.5-15(-16) x 5-6.3 μm	(7.5-)8.8-10 x 3.8 μm	6.25-7.5 x 2.5-3.8 μm
Germ slit	Straight full length	Straight full length	Straight less than spore length
Habitat	On wood	On wood	On wood
Location	Ratchaburi	Trad	Trad
Specimen examined	SUT075	SUT203	SUT204

<b>Table 17.</b> (Co	ntinued).
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Character	Xylaria taxonomic species4 *	Biscogniauxia capnodes (Berk.) YM. Ju & J.D. Rogers*	Biscogniauxia sp. nov. (SUT290)*
Stromata			
Shape	Clavate to clavate-cylindrical, with rounded fertile apices, on short stipes	Applanate	Applanate
Color	Externally copper- to bronze-colored to brown with black of ostioles, internally creamy white	Black	Externally black and internally yellow
Texture	Very hard	Very hard	Very hard
Surface	Smooth except for ostiolar slightly raised	Smooth	Smooth
Perithecia			
Shape	Completely immersed, subglobose	Obovoid to tubular	Obovoid to tubular
Size	0.5-0.7 mm diameter	0.2-0.3 mm diameter x 0.3-0.5 mm high	0.3-0.4 mm diameter x 0.4-0.5 mm high
Ostiole	Umbilicate to slightly raised	Slightly higher than stromatal surface	Slightly papillate
Apical apparatus Ascospores	Not observed	Not observed	Discoid, 1.5 $\mu$ m high x 2.5-3 $\mu$ m
Color	Dark brown	Dark brown	Dark brown
Shape	Ellipsoid-inequilateral with narrowly rounded	Ellipsoid, nearly equilateral with narrowly round ends	Ellipsoid, nearly equilateral with narrowly round ends
Size	21.3-25 x 8.8-10 μm	10-12.5 (-13.8) x 6.3-7.5 μm	9.2-11.9 x 5.4-6.7 μm
Germ slit	Straight to curving, oriented obliquely to the long axis of the spore, less than spore lengthStraight germ slit spore-lengthStraight germ slit spore-length		
Habitat	On wood	On wood	On wood
Location	Trad	Trad	Songkhla
Specimen examined	SUT207	SUT212	SUT290

<b>Table 17.</b> (C	continued).
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Character	Kretzschmaria species *	Nemania species *
Stromata		
Shape	Clustered subglobose fertile head with short stalk, not branched	Erumpent to superficial
Color	Externally blackish, internally white	Black
Texture	Woody	Very hard
Surface	Cracked into minute angular scales	Smooth with slightly papillate
Perithecia	C C	
Shape	Completely immersed, subglobose	Completely immersed, subglobose
Size	0.2-0.3 mm diameter	0.5-0.6 mm diameter
Ostiole	Inconspicuous papillate	
Apical apparatus	Not observed	Rectangular, 2-3 µm high x 1-1.5µm broad
Ascospores		
Color	Brown to dark brown	Brown to dark brown
Shape	Ellipsoid-inequilateral with rounded ends	Ellipsoid-inequilateral with rounded ends
Size	8.8-10 x 3.8-5 μm	8.9-11.7 x 4.7-6 μm
Germ slit	Straight spore length	Straight spore length
Habitat	On wood	On wood
Location	Songkhla	Trad
Specimen examined	SUT101	SUT258


**Figure 48.** *Xylaria anisopleura* (Mont.) Fr. (SUT205); (a) and (b) stromatal form (Bars = 1 cm and 1 mm respectively), (c) perithecia (Bar = 2 mm), (d) ascospores with germ slits (arrowed) (Bar = 10  $\mu$ m), and (e) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 49.** *Xylaria badia* Pat. (SUT076); (a) stromatal form (Bar = 1 cm), (b) perithecia (Bar = 1 mm), (c) stromatal form (Bar = 0.2 mm), (d) ascospores (Bar = 10  $\mu$ m), (e) apical apparatus (arrowed) (Bar = 1  $\mu$ m), (f) straight germ slit less than spore length (Bar = 1  $\mu$ m), and (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).

*Xylaria beccari* SUT092 (Figure 50) examined closely fitted the species *Xylaria beccari* Lloyd. as described by Lloyd (1924).

*Xylaria brachiata* SUT078 and SUT175 (Figure 51) examined were similar to *X. brachiata* Sacc. described by Lloyd (1919) except for ascospore sizes, which were (8-)10-11.3(-12.5) x  $3.8-5 \mu m$  and (11-14(-16) x  $5-6 \mu m$ ) respectively.

*Xylaria cubensis* SUT089, SUT090, SUT193, SUT194, and SUT199 (Figure 52) examined were similar to *Xylaria cubensis* (Mont.) Fr. described by Rogers and Samuels (1986), Rogers *et al.* (1988), González and Rogers (1989), and Thienhirun (1997). These collections had slightly smaller ascospores ((6.3-)7.5-8.8 x 3.8-5  $\mu$ m) compared to those given by Rogers and Samuels (1986), Rogers *et al.* (1988), and González and Rogers (1989) (8-10.5 x 4-5  $\mu$ m). But these *Xylaria* species were similar to specimens found in Thailand by Thienhirun (1997). *Xylaria cubensis* has been found in various tropical, subtropical, and temperate localities of the world.

*Xylaria inthino-velutina* SUT091 and SUT123 (Figure 53) examined were very similar to *Xylaria inthino-velutina* (Mont.) Fr. (Dennis, 1957; González and Rogers, 1989; and Thienhirun, 1997), but they were slightly different in ascospore size range, which were (7.5-)8.8-10(-12.5) x 3.8-4  $\mu$ m and 10-11.3 x 5-5.6  $\mu$ m respectively. This taxon is also closely related to *X. culleniae* except for ascospore size of *X. culleniae*, which is smaller (7.5-8.8 x 3-3.8  $\mu$ m). *Xylaria inthino-velutina* usually occurs on legume fruits.



**Figure 50.** *Xylaria beccari* Lloyd. (SUT092); (a) and (b) stromatal forms (Bars = 1 and 2 mm respectively), (c) perithecia (Bar = 1 mm), (d) ascospores (Bar = 5  $\mu$ m), (e) apical apparatus (arrowed) (Bar = 1  $\mu$ m), (f) straight germ slit spore length (Bar = 1  $\mu$ m), and (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 51.** *Xylaria brachiata* Sacc. (SUT078); (a) and (b) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (c) perithecia (Bar = 0.5 mm), (d) ascospores (Bar = 10  $\mu$ m), (e) straight germ slit spore length (arrowed) (Bar = 5  $\mu$ m), (f) apical apparatus (arrowed) (Bar = 5  $\mu$ m), and (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 52.** *Xylaria cubensis* (Mont.) Fr. (SUT089); (a) and (b) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (c) perithecia (Bar = 0.5 mm), (d) ascospores (Bar  $= 5 \mu$ m), (e) apical apparatus (arrowed) (Bar  $= 2 \mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 53.** *Xylaria inthino-velutina* (Mont.) Fr. (SUT123); (a) and (b) stromatal forms (Bars = 1 cm and 0.2 mm respectively), (c) perithecia (Bar = 0.2 mm), (d) ascospores (Bar =  $10 \ \mu m$ ), (e) apical apparatus (arrowed) (Bar =  $1 \ \mu m$ ), (f) straight germ slit spore length (Bar =  $2 \ \mu m$ ), (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar =  $1 \ cm$ ), and (h) exudates from anamorph (Bar =  $0.1 \ mm$ ).

*Xylaria* cf. *juruensis* SUT035 (Figure 54), *Xylaria* cf. *juruensis* SUT088 (Figure 55), *Xylaria* cf. *juruensis* SUT140 (Figure 56), and *Xylaria* cf. *juruensis* SUT170 (Figure 57) examined were similar to both *X. juruensis* P. Henn. and *X. multiplex* (Kunze.) Fr. (Dennis, 1957 and 1961; González and Rogers, 1989; and Thienhirun, 1997). All of them differed slightly in stromatal form, apical apparatus, and ascospore shape and size. Their ascospores were in the same size range and they were between *X. juruensis* (9-11(-12) x (3.5-)4-4.5  $\mu$ m), and *X. multiplex* (14.5-17(-18) x 5-5.5(-6.5)  $\mu$ m).

*Xylaria juruensis* var. *microspora* SUT129, SUT138, and SUT139 (Figure 58) closely matched the species as described by Thienhirun (1997) except that the stipes of the taxon examined were shorter and broader, and the ascospores were smaller than the previous reported species.

*Xylaria maitlandii* SUT177 (Figure 59) was similar to *X. maitlandii* (Dennis) D. Hawksw. as described by González and Rogers (1989). This taxon was different from material described by Dennis (as *Xylosphaera*) from Africa (1958) which the fertile part had hair on.

*Xylaria mellisii* SUT074 and SUT192 (Figure 60) were similar to *Xylaria mellisii* (Berk.) Cooke. as described by Van der Gucht (1995) from Papua New Guinea.

*Xylaria multiplex* SUT028 (Figure 61) was similar to *X. multiplex* (Kunze) Fr. as described by Dennis (1957; 1961), González and Rogers (1989), and Thienhirun (1997) except for the ascospore size range of  $11.3-13.8(-15) \ge 3.8-5 \ \mu\text{m}$  cf. 9-10.5(-11) x 3.5-4  $\mu\text{m}$  (Dennis, 1957; 1961; Thienhirun, 1997; González and Rogers, 1989).



**Figure 54.** *Xylaria* cf. *juruensis* (SUT035); (a) and (b) stromatal forms (Bars = 0.5 cm and 0.5 mm respectively), (c) perithecia (Bar = 0.5 mm), (d) ascospores (Bar = 5  $\mu$ m), (e) straight germ slit spore length (Bar = 2  $\mu$ m), and (f) apical apparatus (arrowed) (Bar = 2  $\mu$ m).



**Figure 55.** *Xylaria* cf. *juruensis* (SUT088); (a) and (b) stromatal forms (Bars = 5 and 1 mm respectively), (c) acute apex (Bar = 1 mm), (d) perithecia (Bar = 0.5 mm), (e) ascospores (Bar = 5  $\mu$ m), (f) straight germ slit spore length (Bar = 5  $\mu$ m), (g) apical apparatus (arrowed) (Bar = 1  $\mu$ m), and (h) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 56.** *Xylaria* cf. *juruensis* (SUT140); (a) and (b) stromatal forms (Bars = 1 and 0.1 cm respectively), (c) stalk (Bar = 1 mm), (d) perithecia (Bar = 0.5 mm), (e) ascospores (Bar = 5  $\mu$ m), (f) apical apparatus (arrowed) (Bar = 1  $\mu$ m), and (g) straight germ slit spore length (Bar = 5  $\mu$ m).



Figure 57. *Xylaria* cf. *juruensis* (SUT170); (a) and (b) stromatal forms (Bars = 1 cm and 1 mm respectively), (c) stalk (Bar = 1 mm), (d) perithecia (Bar = 0.5 mm), (e) ascospores (Bar = 5  $\mu$ m), and (f) apical apparatus (arrowed) (Bar = 1  $\mu$ m).



**Figure 58.** *Xylaria juruensis* var. *microspora* (Thienhirun, 1997); (a), (b), (c), and (e) stromatal forms of SUT129, SUT138, SUT139, and SUT129 respectively (Bars = 1, 1, 1, and 0.01 cm), d) perithecia (Bar = 0.5 mm), (f) straight germ slit spore length (Bar = 2  $\mu$ m), and (g) ascospores with apical apparatus (arrowed) (Bar = 10  $\mu$ m).



Figure 59. *Xylaria maitlandii* (Dennis) D. Hawksw (SUT177); (a), (b) and (c) stromatal forms (Bars = 0.5 cm, 2 mm, and 0.2 mm respectively), (d) perithecia (Bar = 0.5 mm), (e) straight germ slit spore length (Bar = 2  $\mu$ m), and (f) ascospores (Bar = 10  $\mu$ m).



**Figure 60.** *Xylaria mellisii* (Berk.) Cooke. (SUT192); (a) and (c) stromatal forms (Bars = 0.2 cm and 0.4 mm respectively), (b) perithecia (Bar = 0.5 mm), (d) ascospores (Bar = 15  $\mu$ m), (e) straight germ slit spore length (Bar = 5  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 61.** *Xylaria* cf. *multiplex* (SUT028); (a) and (c) stromatal forms (Bars = 0.5 cm and 0.5 mm respectively), (b) perithecia (Bar = 0.5 mm), (d) ascospores (Bar = 10  $\mu$ m), (e) apical apparatus (arrowed) (Bar = 1  $\mu$ m), (f) straight germ slit spore length (arrowed) (Bar = 2  $\mu$ m), (g) cultural characteristics on PDA at 25°C after 4 weeks (Bar = 1 cm), and (g) exudates from anamorph (arrowed) (Bar = 0.1 mm).

*Xylaria muscula* SUT029 (Figure 62) appeared identical to the species *Xylaria muscula* Lloyd. described by Dennis (1957).

*Xylaria psidii* SUT124, SUT125, and SUT126 (Figure 63) were very close to the species *Xylaria psidii* J.D. Rogers & Hemmes. described by Rogers, Ju and Hemmes (1992), and Thienhirun (1997).

*Xylaria schweinitzii* SUT201 (Figure 64) fitted very well with the species *Xylaria schweinitzii* Berk. & M.A. Curtis as described by Rogers *et al.* (1988), González and Rogers (1989), and Thienhirun (1997), except that the ascospore size differed from specimens reported by Thienhirun (1997) which were longer, 21-26.3 x 6.5-8  $\mu$ m cf. 18.8-21.3 x 6.3-7.5  $\mu$ m, than the present study.

*Xylaria scruposa* SUT117 (Figure 65) matched very closely the species *Xylaria scruposa* (Fr.) Fr. as reported by Van der Gucht (1995) from Papua New Guinea.

*Xylaria* species 2 (SUT127, SUT128, SUT130, SUT132, SUT134, SUT155, SUT171, SUT195, SUT271 and SUT274) (Figure 66) examined was similar to *Xylaria* species 2 described by Thienhirun (1997). The taxon *Xylaria* species 2 was distinctive with its yellow-colored internal stromatal tissue and spiraling germ slit. Both taxa were widely distributed in Thailand.

*Xylaria* sp. nov. (Figure 67) was a new species. Characteristics of this taxon are as follows: stromata cylindrical, gregarious, with narrowly rounded fertile apices, smooth stipe, which was longitudinally furrowed or wrinkled; external colour dark brown to black, internal colour white; texture woody; surface rough, finely reticulately cracked into small angular closely spaced scales so as to outline the individual perithecia; perithecia immersed, sometimes vaguely evident in outline,

subglobose, 0.3- 0.5 mm diameter; ostiole slightly papillate; apical apparatus quadrate to rectangular, 1.5  $\mu$ m high x 1.5-2  $\mu$ m broad; ascospore light brown to brown to dark brown, ellipsoid-equilateral with narrowly rounded ends, (7.5-)8.8-10 x (2.5-)3.8-5  $\mu$ m; germ slit straight full length.

Specimens examined: Thailand, Suranaree University of Technology, Nakhon Ratchasima, 20 September 2003, Suwannasai, N. (Holotype SUT195), SUT006, SUT012, SUT131, SUT133, SUT136, SUT141, SUT143 SUT172; Ratchaburi Province, SUT027, SUT031, SUT033, SUT034; Songkhla Province, SUT087, SUT093; Trad Province, SUT197, SUT198; Kanchanaburi Province, SUT272, SUT273, and SUT275.

Colonies on PDA covering 9 cm Petri dish in two weeks at room temperature, 23-28°C, at first white, velvety, zonate, becoming overlaid with a grayish brown layer of felty mycelium darkening to brownish black and patchily covered in areas with a thin white mycelial layer; stromata cylindrical, unbranched, developing at periphery of zones, grayish black with whitish interiors.

*Xylaria telfairii* SUT206 (Figure 68) appeared identical the species *Xylaria telfairii* (Berk.) Fr. as described by Dennis (1961), Rogers *et al.* (1987 and 1988), Callan and Rogers (1990), and Thienhirun (1997).



**Figure 62.** *Xylaria muscula* Lloyd. (SUT029); (a) and (b) stromatal forms (Bars = 0.5 cm and 0.2 mm respectively), and (c) perithecia (Bar = 0.2 mm).



**Figure 63.** *Xylaria psidii* J.D. Rogers & Hemmes. (SUT125); (a), (b) and (c) stromatal forms (Bars = 0.5, 0.1, and 0.03 cm respectively), (d) ascospores (Bar = 5  $\mu$ m), (e) straight germ slit spore length (arrowed) (Bar = 2  $\mu$ m), (f) apical apparatus (arrowed) (Bar = 2 $\mu$ m), (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm), and (h) exudates from anamorph (arrowed) (Bar = 0.5 mm).



Figure 64. Xylaria schweinitzii Berk. & M.A. Curtis. (SUT201); (a) and (b) stromatal forms (Bars = 1 cm and 3 mm respectively), (c) ascospores (Bar = 10  $\mu$ m), and (d) straight to slightly spiraling germ slit (arrowed) (Bar = 2  $\mu$ m).



**Figure 65.** *Xylaria scruposa* (Fr.) Fr. (SUT117); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (b) perithecia (Bar = 0.5  $\mu$ m), (d) ascospores (Bar = 5  $\mu$ m), (e) apical apparatus (arrowed) (Bar = 2 $\mu$ m), (f) straight to slightly sigmoid germ slit slightly obliquely oriented to the long axis of the spore (arrowed) (Bar = 1  $\mu$ m), and (g) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



**Figure 66.** *Xylaria* species 2 (SUT155); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (b) perithecia (Bar =  $0.5 \mu$ m), (d) ascospores (Bar =  $10 \mu$ m), (e) slightly sigmoid germ slit (arrowed) (Bar =  $1 \mu$ m), and (f) cultural characteristics on PDA cultured at 25°C for 2 weeks (Bar =  $1 \mu$ m).



Figure 67. *Xylaria* sp. nov.(SUT195); (a) and (c) stromatal forms (Bars = 0.5 cm and 0.3 mm respectively), (b) perithecia (Bar =  $0.5 \mu$ m), (d) ascospores (Bar =  $10 \mu$ m), (e) apical apparatus (Bar =  $1 \mu$ m), (f) straight germ slit spore length (arrowed) (Bar =  $2 \mu$ m), (g) cultural characteristics on PDA cultured at 25°C for 2 weeks (Bar = 1 cm), and (h) exudates from anamorph (Bar = 0.2 mm).



**Figure 68.** *Xylaria telfairii* (Berk.) Fr. (SUT206); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (b) perithecia (Bar = 0.5  $\mu$ m), (d) ascospores (Bar = 10  $\mu$ m), (e) straight germ slit (arrowed) (Bar = 1  $\mu$ m), and (f) cultural characteristics on PDA cultured at 25°C after 2 weeks (Bar = 1 cm).

Eight collections examined were found to be unidentified species. They were placed into four groups according to their morphological characteristics. Firstly, Xylaria taxonomic species 1 (SUT075) (Figure 69) which had ascospore sizes and germ slit form similar to X. mellisii (Berk.) Cooke as recorded by Van der Gucht (1995) but the stromatal form was different. This taxon was no peeling layer on the external surface, and no apex. Secondly, Xylaria taxonomic species 2 (SUT203) (Figure 70) had peeling layer on the external surface liked X. juruensis var. microspora (Thienhirun, 1997) and X. multiplex (Kunze) Fr. (Dennis, 1961; González and Rogers, 1989; Thienhirun, 1997). Stipes were longer than those species and ascospore size was different. Thirdly, Xylaria taxonomic species 3 (SUT204) (Figure 71) which was close to X. gracillima (Fr.) Fr. in stromatal structure as described by Van der Gucht (1995). It was different in ascospore size and germ slit form, which were 10-12(-13) x 4-5  $\mu$ m and straight germ slit less than spore length for X. gracillima (Fr.) Fr. respectively. The specimen was unculturated. Lastly, Xylaria taxonomic species 4 (Figure 72) had a stroma similar to X. allantoidea (Berk.) Fr. but the ascospore size range was closer X. schweinitzii Berk. & M.A. Curtis as described by Van der Gucht (1995).

*Kretzschmaria* species SUT101 and *Nemania* species SUT258 were shown in Figures 73 and 74 respectively. *Biscogniauxia capnodes* SUT212 (Figure 75) fitted *Biscogniauxia capnodes* (Berk.) Y.-M. Ju & J.D. Rogers as reported by Ju and Rogers (1998) whilst *Biscogniauxia* species (SUT290) sp. nov. was another new species showed in Figure 76.



**Figure 69.** *Xylaria* taxonomic species 1 (SUT075); (a) and (c) stromatal forms (Bars = 0.5 cm and 0.5 mm respectively), (b) perithecia (Bar  $= 0.3 \mu m$ ), (d) ascospores (Bar  $= 12 \mu m$ ), (e) straight to slightly sigmoid germ slit less than spore length (arrowed) (Bar  $= 2 \mu m$ ), and (f) apical apparatus (Bar  $= 2 \mu m$ ).



Figure 70. *Xylaria* taxonomic species 2 (SUT203); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (b) perithecia (Bar  $= 0.5 \mu$ m), (d) ascospores (Bar  $= 10 \mu$ m), and (e) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



Figure 71. *Xylaria* taxonomic species 3 (SUT204); (a) and (b) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (c) straight germ slit spore length (arrowed) (Bar = 2  $\mu$ m), and (d) ascospores (Bar = 8  $\mu$ m).



Figure 72. *Xylaria* taxonomic species 4 (SUT207); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (b) perithecia (Bar = 0.5  $\mu$ m), (d) ascospores (Bar = 10  $\mu$ m), and (e) sigmoid germ slit (arrowed) (Bar = 5  $\mu$ m).



Figure 73. *Kretzschmaria* species (SUT101); (a) and (c) stromatal forms (Bars = 1 cm and 0.2 mm respectively), (b) perithecia (Bar = 0.3  $\mu$ m), and (d) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).



Figure 74. *Nemania* species (SUT258); (a) and (b) stromatal forms (Bars = 1 cm and 0.5 cm respectively), (c) apical apparatus (Bar = 2  $\mu$ m), and (d) ascospores (Bar = 12  $\mu$ m).



Figure 75. Biscogniauxia capnodes (Berk.) Y.-M. Ju & J.D. Rogers (SUT212); (a) stromatal forms (Bar = 1 cm), (b) ascospores (Bar = 0.5 μm), (c) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm), and (d) straight germ slit spore length (arrowed) (Bar = 2 μm).



**Figure 76.** *Biscogniauxia* sp. nov. (SUT290); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm, respectively), (d) ascospores (Bar =  $0.5 \mu$ m), (e) straight germ slit spore length (arrowed) (Bar =  $2 \mu$ m), apical apparatus (arrowed) (Bar =  $2 \mu$ m), and (g) cultural characteristics on PDA cultured at  $25^{\circ}$ C after 4 weeks (Bar = 1 cm).

## 4.3 Chemotaxonomic study of the selected xylariaceous fungi

Since species of *Xylaria* have been reported as endophytes in several plants, the pattern of their secondary metabolites is another possible way to identify species. Therefore, *Xylaria* species which have been recorded as endophyte were selected to study.

## **4.3.1** TLC analysis of secondary metabolites from agar plugs

Four isolates of *X. anisopleura* (ST2329), *Xylaria* species (ST2372), *X. cubensis* (ST2326), and *X. grammica* (ST2348) were selected to study on their secondary metabolite profiles. After growing culture on YES agar, the secondary metabolites were extracted and analyzed by TLC method. There were no spots observed under both visible light and UV light. This might be the result of low concentration of secondary metabolites. This method might not be suitable for those xylariaceous collections, although this technique was frequently used in several fungi such as *Penicillium*, *Chaetomium*, *Fusarium*, *Verticillium*, and *Metarhizium* (Filtenborg and Frisvad, 1980; Filtenborg, Frisvad, and Svendsen, 1983; Lund and Frisvad, 1994).

## **4.3.2** TLC analysis of secondary metabolites from cultural broth

## 4.3.2.1 Secondary metabolite extraction from 100 mL of cultural broth

Four isolates of *Xylaria* were examined for their secondary metabolite profiles by extracting the metabolites from their cultural broths. Two isolates of *Xylaria* endophytes obtained by Dr. Nuttaporn Ruchichakhon (Ruchikachorn, 2005) were included. The TLC pattern of secondary metabolites of all isolates was not clear and they were similar (Figure 77), even though they were different in their species identification by their morphological characteristics.



Figure 77. Secondary metabolite profiles of *Xylaria* isolates compared to those of *Xylaria* endophytes extracted from 100-mL cultural broth and analyzed by TLC method under visible light. UX5 = *Xylaria hypoxylon* endophyte, UX3 = *Xylaria apiculata* endophyte, X1 = *X. cubensis* ST2326, X2 = *X. arbuscula* var. *microspora* ST2372, X3 = *X. anisopleura* ST2329, and X4 = *X. grammica* ST2348.
The patterns of secondary metabolites observed in this analysis might be commonly found in *Xylaria* isolates. However, other different secondary metabolites might be not observed because of their low concentrations.

#### 4.3.2.2 Secondary metabolite extraction from 1 L of cultural broth

An isolate of *Xylaria*, *X. cubensis* ST2326, was selected to culture in 1-L MA broth, then extracted for secondary metabolites and analyzed by TLC. The secondary metabolite profile of *X. cubensis* ST2326 was compared to other *Xylaria* endophytes. The profile exhibited clearly the different patterns of each isolate (Figures 78 and 79). These profiles could be used to identify species especially endophytes. The compound, which had  $R_f$  values around 0.44-0.46, was commonly found in all isolates. *Xylaria cubensis* ST2326 consisted of two different bands which were 0.44 and 0.64  $R_f$  value. The profile of *X. cubensis* ST2326 was not similar to any endophyte isolates.

Although this chemical technique could be used to classify the xylariaceous endophytes, it was time-consuming. Also, it required several steps to analyze and used a large volume of fungal culture for extraction.



Figure 78. The pattern of secondary metabolites extracted from *X. cubensis* and other fungal endophytes by TLC method. Lanes: PD3, *Nodulisporium* sp. endophyte; UX3, *X. apiculata* endophyte; PX1, *X. longipes* endophyte; UX5, *X. hypoxylon* endophyte; N24, *Rosellinia arcuata* endophyte; UD2, *Daldinia concentrica* endophyte; U1, *Hypoxylon rickii* endophyte; 19, *X. apiculata* endophyte; 21, *X. mali* endophyte; and X1, *X. cubensis* ST2326. The number indicated the R<sub>f</sub> value of each compound.



Figure 79. Schematic of secondary metabolites extracted from *Xylaria cubensis* and other endophytes by TLC method. Lanes: PD3, *Nodulisporium* sp. endophyte; UX3, *X. apiculata* endophyte; PX1, *X. longipes* endophyte; UX5, *X. hypoxylon* endophyte; N24, *Rosellinia arcuata* endophyte; UD2, *Daldinia concentrica* endophyte; U1, *Hypoxylon rickii* endophyte; 19, *X. apiculata* endophyte; 21, *X. mali* endophyte; and X1, *X. cubensis* ST2326. The number indicated the R<sub>f</sub> value of each compound.

# 4.4 Nucleic acid studies of the selected xylariaceous fungi

Form the morphological and chemical taxonomic results of xylariaceous specimens showed that approximately 30% of them could not be identified. Therefore, the nucleic acid method of DNA sequencing was then applied to resolve this problem. The results of DNA sequences exhibited clearly relationships between xylariaceous species and could also be used to confirm the results indicating new species.

## 4.4.1 Group I: Astrocystis and Rosellinia

The genera *Astrocystis* and *Rosellinia* are very similar in their morphological characters. There are some disagreements over the status of both genera. Ju and Rogers (1990 and 1995) and San Martín and Rogers (1994) mentioned that *Astrocystis* was accommodated as *Rosellinia*-like fungi whereas Petrini and Whalley (1996) suggested that both genera were different and should be separated from each other. They were different in the stromata character of *Astrocystis*, which spitted from the host surface, and anamorph form, which was *Acanthodochium*. The nucleotide sequences of 18S ribosomal DNA and ITS1-5.8S-ITS2 regions were analyzed to clarify the differentiation of both genera.

## 4.4.1.1 18S rDNA sequence analysis

Genomic DNA of *A. mirabilis* samples (SUT051 and SUT056) were extracted from mycelia and *R. procera* samples (SUT102, SUT109, SUT113, and SUT114) were extracted from ascospores because they could not be cultured. An isolate of *Rosellinia* sp. ST2310 obtained by Dr. Surang Thienhirun was included. The quality and quantity of the DNA solution were measured and adjusted to a suitable concentration for PCR amplification. It was found that no PCR product

was obtained from NS1/NS8 primers, although several modified conditions were performed. Therefore, two new primers, NS4 and SR8R (Table 18), were added to resolve this problem. The amplification of 18S rDNA sequence was divided into two steps by using two sets of primers. The NS1/NS4 primers were used to amplify the fragment at position 20 to 1,131 of 18S rDNA. The SR8R/NS8 primers were used to amplify the fragment at position 732 to 1,769 of 18S rDNA (Figure 8). The program of amplification consisted of 1 cycle of 95°C for 5 min; 35 cycles of 95°C for 1 min, 53°C for 1.30 min, 72°C for 2 min; and the final cycle of 72°C for 10 min. The PCR reactions were carried out in the automated thermal cycle (i-cycle, BioRad, U.S.A.). The amplified 18S rDNA fragments are shown in Figure 80.

 Table 18.
 Nucleotide sequences of NS4 and SR8R primers.

Name	Sequence (5'- 3')	Target region <sup>a</sup>	Reference
NS4	CTTCCGTCAATTCCTTTAAG	SSU 1150-1131	White <i>et al.</i> 1990
SR8R	GAACCAGGACTTTTACCTT	SSU 732-749	Vilgalys, www, 1999

<sup>a</sup> Saccharomyces cerevisiae numbering



Figure 80. Gel electrophoresis of partial 18S rDNA fragments. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, 3, and 5, Astrocystis mirabilis (SUT051), A. mirabilis (SUT056) and Rosellinia sp. ST2310 using NS1 and NS4 primers respectively; 2, 4, and 6, Astrocystis mirabilis (SUT051), A. mirabilis (SUT056) and Rosellinia sp. ST2310 using SR8R and NS8 primers respectively.

For 18S rDNA amplification, only genomic DNA extraction from mycelium could be achieved by both set of DNA primers. Although several modified conditions were attempted, no PCR product was obtained from the genomic DNA extraction from ascospores. This might be because of the low concentration of genomic DNA extracted from ascospores, or the degradation of genomic DNA. The size of amplified 18S rDNA fragments of *A. mirabilis* (SUT051 and SUT056) and *Rosellinia* sp. (ST2310) from two primer sets were similar. They were approximately 1,000 bp obtained from the amplification by using NS1/NS4 primers and SR8R/NS8 primers. The both amplified fragments were then combined to obtain the whole 18S rDNA sequence. The length of the DNA sequences is summarized in Table 19.

Table 19.	The length	of 18S rDNA	sequences of $A$ .	mirabilis	SUT051,	SUT056, and
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Species	Location /source	1 <sup>st</sup> fragment (NS1/NS4 primers) (bp)	GenBank accession number	2 <sup>nd</sup> fragment (NS4/NS8 primers) (bp)	GenBank accession number	Total (bp)
A. mirabilis	Ratchaburi	1012	DQ322075	1072	DQ322076	2056
(SUT056)	Province					
A. mirabilis	Ratchaburi	1012	DQ322074	NO	ND	ND
(SUT051)	Province					
Rosellinia sp.	RFD*	1020	DQ322072	1202	DQ322073	2210
(ST2310)						

Rosellinia sp. ST2310 obtained from DNA sequence analysis.

\* The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand. NO = Not observed, ND = Not determined

Then, the whole 18S rDNA sequences of *A. mirabilis* (SUT056) and *Rosellinia* sp. (ST2310) including *R. necatrix* (AB014044) from GenBank database were aligned (Figure 81). The result indicated that there was dissimilarity between the genera *Astrocystis* and *Rosellinia*. The percent similarity of *A. mirabilis* (SUT056) to *Rosellinia* sp. (ST2310) and *R. necatrix* (AB014044) was 70.3% and 68.7% respectively (Table 2, Appendix C). Nevertheless, both genera were closely related and showed highly conserved regions in the beginning of 1,000 bp of 18S rDNA sequences, whilst the middle region exhibited the highest variation (Figure 81). Therefore, the partial 18S rDNA sequences of *A. mirabilis* (SUT056) and *Rosellinia* sp. (ST2310) including *R. necatrix* (AY083805) and *A. coccoes* (AY083804) available from GenBank database, which were amplified by using NS1/NS4 primers, were aligned (Figure 2, Appendix C). The result showed high similarity between the genera *Astrocystis* and *Rosellinia* ranging from 97% to 98%

similarity (Table1, Appendix C) and it confirmed the highly conserved region of both genera at the 5' end of 18S rDNA sequence (position of 20 bp to 1,131 bp; Figure 8).

AB014044 A.mirabilis R.sp.ST2301	1 1 1	102030405060CCGCGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTATTTGATAGTACCTTACTAC60CGGCGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTATTTGATAGTACCTTACTAC60CGGCGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTATTTGATTGTACCTTACTAC60
AB014044 A.mirabilis R.sp.ST2301	61 61 61	708090100110120TTGGATACCT GTGGTAATTC TAGAGCNNNT ACATGCTGAA ANATCCCGAC TCACGGAGGG120ATGGATAACC GTGGTAATTC TAGAGCTAAT ACATGCT-AA AAATCCCGAC TCACGGAGGG119TTGGATAACC GTGGTAATTC TAGAGCTAAT ACATGCT-AA AAATCCCGAC TCACGGAGGG119
AB014044 A.mirabilis R.sp.ST2301	121 120 120	130140150160170180ATGTATTTAT TAGATTAAAA ACCAATGCCCCTCGGGGCTT TCTGGTGATT CATAATAACT180ATGTATTTAT TAGATTAAAA ACCAATGCCCCTCGGGGCTT TCTGGTGATT CATAATAACT179ATGTATTTAT TAGATTAAAA ACCAATGCCCCTCGGGGCTT TCTGGTGATT CATAATAACT179
AB014044 A.mirabilis R.sp.ST2301	181 180 180	190200210220230240TCACGAATCGCACGGCCTTGCGCCGGCGATGGTTCATTCAAATTTCTGCCCTATCAACTT240TCTCGAATCGCATGGCCTTGCGCCGGCGATGGTTCATTCAAATTTCTGCCCTATCAACTT239TCTCGAATCGCATGGCCTTGCGCCGGCGATGGTTCATTCAAATTTCTGCCCTATCAACTT239
AB014044 A.mirabilis R.sp.ST2301	241 240 240	250260270280290300TCGATGGCAGGGTCTTGGCCTGCCATGGTTTCAACGGGTAACGGAGGGTTAGGCCTCGAC300TCGATGGCAGGGTCTTGGCCTGCCATGGTTACAACGGGTAACGGAGGGTTAGGCCTCGAC299TCGATGGCAGGGTCTTGGCCTGCCATGGTTACAACGGGTAACGGAGGGTTAGGCCTCGAC299
AB014044 A.mirabilis R.sp.ST2301	301 300 300	310       320       330       340       350       360         CCCGGAGAAG       GAGCCTGAGA       AACGGCTACT       ACATCCAAGG       AAGGCAGCAG       GCCGCGAAAT       360         CCCGGAGAAG       GAGCCTGAGA       AACGGCTACT       ACATCCAAGG       AAGGCAGCAG       GCGCGCAAAT       359         CCCGGAGAAG       GAGCCTGAGA       AACGGCTACT       ACATCCAAGG       AAGGCAGCAG       GCGCGCAAAT       359         CCCGGAGAAG       GAGCCTGAGA       AACGGCTACT       ACATCCAAGG       AAGGCAGCAG       GCGCGCAAAT       359
AB014044 A.mirabilis R.sp.ST2301	361 360 360	370       380       390       400       410       420         TACCCAATCC       CGACACGGGG       AGGTAGTGAC       AATAAATACT       GATACAGGGC       TCTTTTGGGT       420         TACCCAATCC       CGACACGGGG       AGGTAGTGAC       AATAAATACT       GATACAGGGC       TCTTTTGGGT       420         TACCCAATCC       CGACACGGGG       AGGTAGTGAC       AATAAATACT       GATACAGGGC       TCTTTTGGGT       419         TACCCAATCC       CGACACGGGG       AGGTAGTGAC       AATAAATACT       GATACAGGGC       TCTTTTGGGT       419
AB014044 A.mirabilis R.sp.ST2301	421 420 420	430440450460470480CTTGTAATTGGAATGAGTACAATTTAAATCCCTTAACGAGGAACAATTGGAGGGCAAGTC480CTTGTAATTGGAATGAGTACAATTTAAAT-CCTTAACGAGGAACAATTGGAGGGCAAGTC478CTTGTAATTGGAATGAGT-CAATTTAAATCCCTTAACGAGGAACAATTGGAGGGCAAGTC478
AB014044 A.mirabilis R.sp.ST2301	481 479 479	490500510520530540TGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTGCAGTA540TGGTGCCAGCAGCCGCGGTAA-TTCAGCTTCAATAGCGTATATTAAAGTTGGTGCAGTA537TGGTGCCAGCAGCCGCGGTAATTCAGCTCCAATAGCGTATATTAAAGTTGGTGCAGGTA538
AB014044 A.mirabilis R.sp.ST2301	541 538 539	550560570580590600AAAAGCTCGT AGTTGAACCT TGGGCCTGGC TGGCCGGTCC GCCTCACCGC GTGCACTGGT600AAA-GCTCGT AGTTGAACCT TGGGCCTGGC TGGCCGGTC-GCCTCAACGC GTGCACTGGT595AAA-GCTCGT AGTTGAACCT TGGGCCT-GC TGGCCGGTCC GC-TCAACGC GTGCACTGGT595
AB014044 A.mirabilis R.sp.ST2301	601 596 596	TCGGCCGGGC CTTTCCCTTT GGGGAGCCCT ATGCCCTTCA CTGGGTGTAG TGGGGAACCA 655

Figure 81. Sequence alignment of 18S rDNA Rosellinia sp. (ST2310), Rosellinia necatrix (AB014044), and A. mirabilis (SUT056).

670 680 690 700 710 720 661 GGACTTTTAC TGTGAAAAAA TTAGAGTGTT CAAAGCAGGC ATTTGCTCGA ATACATCAGC 720 AB014044 720 A.mirabilis GGACTTTTAC TGTGAAAAAA TTAGAGTGTT CAAAGCAGGC CTATGCTCGA ATACATCAGC 656 715 GGACTTTTAC TGTGAAAAAA TTAGAGTGTT CAAAGCAGGC CTATGCTCGA ATACATCAGC R.sp.ST2301 654 713 770 
 730
 740
 750
 760
 770
 780

 ATGGAATAAT AGAATAGGAC
 GTGTGGTTCT
 ATTTTGTTGG
 TTTCTAGGAC
 CGCCGTAATG
 780 780 AB014044 721 ATGGAATAAT AGAATAGGAC GTGTGGTTCT ATTTTGTTGG TTTCTAGGAC CGCCGTAATG A.mirabilis 716 775 ATGGAATAAT AGAATAGGAC GTGTGGTTCT ATTTTGTTGG TTTCTAGGAC CGCCGTAATG R. sp. ST2301 714 773 
 790
 800
 810
 820
 830
 840

 ATTAATAGGG ACAGTCGGGG GCATCAGTAT TCAATTGTCA GAGGTGAAAT TCTTGGATTT
 AB014044 781 840 A.mirabilis 776 ATTAATAGGG ACAGTCGGGG GCATCAGTAT TCAATTGTCA GAGGTGAAAT TCTTGGATTT 835 R. sp. ST2301 774 ATTAATAGGG ACAGTCGGGG GCATCAGTAT TCAATTGTCA GAGGTGAAAT TCTTGGATTT 833 
 850
 860
 870
 880
 890
 900

 ATTGAAGACT
 AACTACTGCG
 AAAGCATTTG
 CCAAGGATGT
 TTTCATTAAT
 CAGGAACGAA
 900 AB014044 900 841 ATTGAAGACT AACTACTGCG AAAGCATTTG CCAAGGATGT TTTCATTAAT CAGGAACGAA A.mirabilis 895 836 ATTGAAGACT AACTACTGCG AAAGCATTTG CCAAGGATGT TTTCATTAAT CAGGAACGAA R.sp.ST2301 834 893 960 AB014044 901 960 A.mirabilis 896 955 **R.sp.ST2301** 894 AGTTAGGGGA TCGAAGACGA TCAGATACCG TCGTAGTCTT AACCATAAAC TATGCCGACT 953 AB014044 961 1020 A.mirabilis AGGGATCGGA CGATGTTATT TTTTGACTCG TTCGGCACCT TACGAGAAAT CAAAGTC---956 1012 R.sp.ST2301 954 AGGGATCGGA CGATGTTATT TTTTGACTCG TTCGGCACCT TACGAGAAAT CAAAGTCTT-1012 1080 AB014044 1080 A.mirabilis 1025 R. sp. ST2301 1012 --- TCCT--- ----- TT GATCCT---- --- GCGGACG GG-GAGAAGG 1040 1090 1100 1110 1120 1130 1140 AB014044 1139 A.mirabilis 1025 -CCTGGA-TG CTGCGCGCTA --GCCCTATT ATCA--CCTA G---CC--GT -CAAACAAGG R.sp.ST2301 1041 TCCAAGA--- -ACAGGAT-- --TCAACAAT AGCAGGAAGA GGAGGCCTAG ACCCGGAGTT 1073 1092 
 1150
 1160
 1170
 1180
 1190
 120

 ....|....|
 ....|....|
 ....|....|
 ....|....|
 1140
 GGTTGGTGGT
 AGGCCCTCGA
 TATATGCTAG
 TCAGGTGGTA
 ATAATCTGCT
 GTATTAAATA
 1200 AB014044 1199 

 A. mirabilis
 1074
 GCTCAGT-- ---CCTC- -TAG
 TAAGG--- ------A

 R.sp.ST2301
 1093
 GANTAAGGC -ANGACACGA
 TATATAACA CAAGGCG-- ------A

 1093 1126 
 1210
 1220
 1230
 1240
 1250
 1260

 AB014044
 1200
 GTGAGGTTAT
 TCCTGGCGAC
 ATCCTCAAAAT
 TGCGGGGGAAG
 CCCTACAACA
 AAAGCAATGA

 A.mirabilis
 1094
 G----- -----GC-- G-C-T---- GCGGG TC-----G
 AAAGCA--- 1259 1112 R. sp. ST2301 1126 ------ CTTAGCCAT GTC----T GACAGAGAGAG ------G GGAGCAGAG-1159 
 1270
 1280
 1290
 1300
 1310
 1320

 1260
 CTACTAAGCG
 CGCCTTGAAA
 AAGAGCGCGT
 GGCGGAGCGT
 AACGGCTCCG
 GTACAGTAAG
 AB014044 1319 A.mirabilis 1113 CCACTA-C- --TCAT--- --AGCACTT CGC---CGC A--G----AGAGTG 1144 R.sp.ST2301 1160 CTACAC--CG GACCAT--- --GAGCAACT TGCGGAAGGT GA----- --ACCATACG 1201

Figure 81. (Continued).

1330 1340 1350 1360 1370 138 ....|....| ....| ....| ....| ....| ....| ....| ....| ....| ....| ....| ....| ....| ....| ....| ....| ....| ....| 1320 AACGTGATTG CCTGGGGTCA TCCGCAGCCA AGCTCCTTAT AGGGATATAT -GAGAGAAGG 1380 AB014044 A.mirabilis 1145 CA-G--ACTC AACG---T-A ---GCTA-TA CGC-AC---- ---ACGTAT -GCC-----R.sp.ST2301 1202 GAAG--GACA CTAAAGGTAA TAAGTCA-CT GGCTCA---- -AGGGTAGGT CGAG------1247 
 1390
 1400
 1410
 1420
 1430
 1440

 1379
 TTCAGAGACT
 TGACGGGGAT
 GGGTGAACTC
 GCAACCAGGT
 TCGCTTAAGA
 TAAAGTCCAT
 1438
 AB014044 A.mirabilis 1177 -TTGGCGA-- -G-CGAGA-- G------ ---ACCAT-- -CGC-AT-GA CA--G-GGA-1209 R. Sp. ST2301 1247 --CGAAGA-- GGATGAGGA- -----ACCG GCCGAGTGGT AACCATAAAC TAT-GAATGC 1295 
 1450
 1460
 1470
 1480
 1490
 150

 1439
 TAAAGGCACG
 AAAGTGTCCT
 TTTAACAACC
 CCCTATAACA
 GGGAGCCTGC
 GGCTTAATTT
 AB014044 1498 A.mirabilis 1209 -- ACAGTACG ----TGCGAA GTT---AAT- T----GAG-C TGAAGACTTC GGTAAGAT--1252 R. SP. ST2301 1296 TAGAGATGCG AAGATCGTTA GTTCTTGACT C-----GTG AGGAACGTAC GAGAAAAT--1347 
 1510
 1520
 1530
 1540
 1550
 156

 1499
 GACTCAACAC
 GGGGAAACTC
 ACCAGGTTAA
 CTGAACAGTT
 ACTGTCTGGG
 CCTGGAATAG
 1560 AB014044 1558 1252 -----GCAT AGAATAGGT- AC----- ---GGCAGCA ACCG-----A.mirabilis 1278 R. sp. ST2301 1347 ---- AAAGT CTTTGGGCTC TT----- --GGGCGAGT A-TGTCTG-- -----1379 
 1570
 1580
 1590
 1600
 1610
 1620

 AB014044
 1559
 TGATTTGTTT
 CGCTAGTGCT
 AGACACTTGT
 CTACGTGGGA
 AAGCTCCCCGA
 TTCGGACGTA
 1618

 A.mirabilis
 1278
 ------ ----TA-CACT
 CGACAGATG ------ ----GAACG-A
 1299

 R.sp. ST2301
 1379
 ----- ----AAGGCT
 -GAAACATAA
 TGAAGCGACA
 TAAGGCACGA
 CCAGAAAGAG
 1424

 1630
 1640
 1650
 1660
 1670
 168

 1619
 GAGCGGTGGC
 CTCGCTACCG
 TTGTCTAGTG
 CACACCAGCT
 GGTACAGGGA
 ACGCTAACCC
 1680 AB014044 1678 1341 R. sp. ST2301 1425 GAGCCATGCT TA----ATTG GAATAGAGGG TGAACTAGCA GGTTCCGGAA GAATGAGGGT 1480 1690 • • • | • • • • ' 
 1690
 1710
 1720
 1730
 174

 1679
 TACATTCGTA GGTATGCCAA TCCTGTGGCG AGCTCAGGTT
 GCGCTGAGCC
 GTTGCAACGC
 1740 AB014044 1738 A. mirabilis 1342 AGGA--CGT- -GAA-----A CCCT-TCGAC AGTGCA---- ----CGAATC GCTC--ATGA 1381 R. Sp. ST2301 1481 AGACAATGTA -GAA----CG CCTTTTAG-- AATTGA---- ----GGCGG GGTCCAAGGC 1524 AB014044 1798 A.mirabilis 1382 GCAGCAATGC TAGG-GC--- -----AGA CGTG-CT--A --TCAG-GCT AAA-----GG R.sp.ST2301 1525 GGGCTAAAGT GGGGAGAAA- -----AAG GTTGGCTTAA ----TGGACC CTT----AA 1419 1567 
 1810
 1820
 1830
 1840
 1850
 186

 1799
 GARATCGTAG
 CCTTCTTGAC
 TAGGTCCGAA
 TGTCCTAATC
 AAGGAGGGCA
 GGGCNCGGCT
 1860 AB014044 1858 1445 R. sp. ST2301 1568 AAAATT---- -----TCCCGA CGCCCC---- ----------CCC----1588 1920 AB014044 1918 1445 -----CACCA GGAG-CGGAG CCTGCG-GTC ------A AATTTGACTC AACACGGGGT A.mirabilis 1489 R. Sp. ST2301 1588 ----CCGCCG TTGCCCCCTC CCGCCTTATT ------ --TTGACCCC AC-CCGGGAA 1631 
 1930
 1940
 1950
 1960
 1970
 1980

 1919
 CGCCCCTCTG
 GAGAATGCAG
 ACACAATGAG
 GA-TTGACAG
 ATTGAGAGACT
 -CTTTCTTGA
 1976
 AB014044 A.mirabilis 1490 AACTCACCAG G----TCCAG ACACAATGAG GA-TTGACAG ATGGGGAGCC -CTTTTTTGA 1543 R. Sp. ST2301 1632 C-CTCCCCAG G----TCCCA GCCCATTAG GATTTCCCAG ATTGACAGCC TCTTTCCTCA 1686

Figure 81. (Continued).

202

AB014044 2029 A.mirabilis 1543 --TTTGGAGG GC-GGAG-GC CATGGCCGAC TTA--GTGGG TGGAGAAATT TGT-CCTGCC 1596 R. sp. ST2301 1687 T-TTTTGTCC GCTGGTGGTG CCTCGCCCGT CTTACGTTGC TCCATTGATT TGT-CTG--C 1742 
 2050
 2060
 2070
 2080
 2090
 2100

 AB014044
 2030
 TTAATTGCGA TAACGAACG- AGACATTTAC
 CTGCTAAATA GCCCGTATTG CTTTGGCAGT

 A.mirabilis
 1597
 TTAATTGCGA TAACCAACG- AGAAATTAC
 CGCCTAAAAA G-CCGTATGC TTTGGGCAGT
 2088 1654 R. Sp. ST2301 1743 TTAATTCCGA TGCCGACCGA AGACATTTAC CTGCTAAATA GCCCGTATTG CTTT-GCCA-1800 211021202130214021502160AB0140442089AC-GCTGGC--TNCTTAG--AGGGACTATCCGCTTAAGC-GGGTGGAAGTTGGA--TGCA.mirabilis1655TCCGCTCGCC-TTCTTTATTAGGCCCTCTCCGCTTACGCCGGGTGCAAGTTGGCATTGCR.sp. ST23011800-CGCTCGCTGCTTCTTAG--AGGGCCTCTCCGCTTCACCCGTTGCACCGT-TCGAT-C 2160 2139 1712 1854 
 2170
 2180
 2190
 2200
 2210
 2222

 2140
 AATAAC--AG
 GTCT-GTGAT
 -GCCCTTAGA
 TGTTCTGGGC
 CGCACGCGCC GTTACA-CTG
 2220 AB014044 2193 A.mirabilis 1713 CACTACCAAG GTCTTGTGTT CGCCCTTAGA TGTTCTGGCC TGCACGCGCC GTTCCT-CTG 1771 R. sp. ST2301 1855 CACTACC-AG GTC-TGTGAT -GCCCTTAGA TGTTCTGGCC CGCACGCG-C GTTCCTACTG 1910 
 2230
 2240
 2250
 2260
 2270
 2280

 AB014044
 2194
 ACAGAGG-AC
 AGCGC-AGTA
 C-TTCCTTAG
 TAGAGATACT
 T---GGGTAA
 TCTTGTTAAA
 2245

 A.mirabilis
 1772
 CCA-AGCCGC
 AGCGGCAGTT
 C-TTCCTTAG
 TAGAGATACT
 T---GGGTAA
 TCTTGTTACA
 1826
 R. Sp. ST2301 1911 ACAGAACGAC AGCGCAGGTC CTTTCCTTAG TAGAGATCCT TCGCGCGGTAA TCTTGTTAC-1969 

 2290
 2300
 2310
 2320
 2330
 2340

 2246
 CTCTGT---C
 GGCTGGGGG ATA-GAGCAT
 GCCATTATT
 GCTCTTCAA CG-AG-GAAT

 1827
 CCTCTGTCC
 GGCCTGGGGGC
 ATA-GAGCAT
 TGCAATTATT
 GCTCTTCAC CG-AG-GAAT

 AB014044 2297 A.mirabilis 1883 R. Sp. ST2301 1970 CCTC-TGT-C GTGCT-GGGC ATAC-AGCAT TGCAATTATT GCTCTTCACA CGTAGCGCCT 2025 2400 AB014044 2345 1884 TCCT-ATGTC -AGCGTAATG TCA-TCCACT -TGCG--TTG AT-TACTGTC CCTGCCC--T A.mirabilis 1934 R. sp. ST2301 2026 TCCTCATGTC TACCGTATAG TCATTCCACT CTGCGCTCTG ATCTCCTGTC CCTGCCCTAT 
 2410
 2420
 2430
 2440
 2450
 2460

 AB014044
 2345
 -TTGT--ACA
 -CCACCCCCC
 GTCGT-ACT
 ACCGA-TTGA
 -ATGGCTCAG
 TGA--G-GC 

 A.mirabilis
 1934
 -TTGT--TCA
 -CA-CCGCCC
 GTCGT-ACT
 CCCCCCTTGA
 TATGGCTCAG
 TCG-AG-GC 2393 1985 R. sp. ST2301 2086 GTCGTGATCA TCACCCCCCC GTCGCTCACT CCCTCCCTGA ATGAGCTCAG TCGTAGCGCT 2145 
 2470
 2480
 2490
 2500
 2510
 252

 2394
 TTTCGGACT ---GGCCCAG
 A-GGAGTC-G
 GCANCGACAC
 TTC--AGGGC
 CGGA-AAGTC
 AB014044 2444 A.mirabilis 1986 TTTCTGGTTC TAGGTCCCAG A-GGTGTCAG GCACCGACAC CTCCTAGGGC CGGCCAAGGT R.sp.ST2301 2146 CTTCTGAGTC TCACTCCAGG ACGGAGTCAC GCATACTGTC TACCTACAG- TGGCC--GTG 2044 2202 2530 ....|....| .... 2445 **ATCCAAACTC GGT** 2457 AB014044 A.mirabilis 2045 CTTGGAACTT T-T 2056 R. sp. ST2301 2203 AC---AACG- -CT 2210

Figure 81. (Continued).

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## 4.4.1.2 ITS sequence analysis

Two representatives of *A. mirabilis* (SUT051 and SUT056) isolates and one *Rosellinia* sp. (ST2310) isolate were investigated. The sequences of ITS1-5.8S-ITS2 were analyzed. The amplified ITS1-5.8S-ITS2 fragments of all isolates were similar being approximately 500 bp as shown in Figure 82.



Figure 82. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of Astrocystis and Rosellinia using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, Rosellinia sp. (ST2310); 2, A. mirabilis (SUT051); 3, A. mirabilis (SUT056).

After sequencing the ITS1-5.8S-ITS2 fragments, the boundaries of the ITS1 and ITS2 regions were determined by comparison to published sequences of the ITS region. The lengths of ITS1-5.8S-ITS2 sequences of *A. mirabilis* and *Rosellinia* sp. ST2310 are summarized in Table 20. The alignment of ITS1-5.8S-ITS2 sequences of *A. mirabilis* (SUT051 and SUT056) and *Rosellinia* sp. ST2310 was

performed including five available ITS1-5.8S-ITS2 sequences from the GenBank database; *R. arcuata* (AB017660), *R. pepo* (AB017659), *R. quercina* (AB017661), and *R. necatrix* (AB017657 and AB017658). The ITS1 region exhibited the highest variation whereas the 5.8S region was highly conserved and constant at 155 bp (Figure 3C in AppendixC). The ITS2 region showed only minor variation. The phylogenetic trees were then constructed using two methods of neighbour-joining method by the PHYLIP program (Felsenstein, 1995), and the maximum parsimony method by PAUP program (Swofford, 2000) as shown in Figure 83 and Appendix 1D respectively.

**Table 20.** The length of ITS1-5.8S-ITS2 sequences of A. mirabilis and Rosellinia sp.ST2310 obtained from DNA sequence analysis.

Species	Location/Source	ITS1	<b>5.8</b> S	ITS2	Total	GenBank
		(bp)	(bp)	(bp)	(bp)	accession
						number
A. mirabilis (SUT051)	Ratchaburi Province	172	155	156	483	DQ322078
A. mirabilis (SUT056)	Ratchaburi Province	172	155	156	483	DQ322076
<i>Rosellinia</i> sp.	RFD*	178	155	160	493	DQ322077
(ST2310)						

\* The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand.



**Figure 83.** Phylogenetic tree of *Astrocystis* and *Rosellinia* based on ITS1-5.8S-ITS2 sequences constructed by using the neighbour-joining method. *Daldinia eschscholzii* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replication.

The phylogenetic trees obtained from two methods revealed the same pattern. *Rosellinia* sp. ST2310 was grouped together with other species of *Rosellinia* from GenBank database, *R. arcuata* (AB017660), *R. pepo* (AB017659), *R. quercina* (AB017661), and *R. necatrix* (AB017657 and AB017658), while two isolates of *A. mirabilis* (SUT051 and SUT056) were separated with high 96% bootstrap support. This result indicated the dissimilarity between both genera from a genetic point of view.

In these molecular analyses, both 18S rDNA sequences and ITS1-5.8S-ITS2 sequences demonstrated the dissimilarlity between *Astrocystis* and *Rosellinia*, which was in agreement with the concepts of Petrini (1993, 2003), Læssøe

and Spooner (1994), and Whalley (1996) to separate Astrocystis from Rosellinia.

## 4.4.2 Group II: Camillea

*Camillea tinctor* is the common species of this genus found in Thailand, and is also wide spread in the tropics and subtropics (San Martín, González, and Rogers, 1993; Whalley, 1996). The variation of ITS1-5.8S-ITS2 sequences within these species, *C. tinctor*, *C. selangorensis*, and *C. leprieurii* provided by Dr. Margaret A. Whalley, was investigated.

## 4.4.2.1 Genomic DNA extraction and ITS amplification

Genomic DNA was extracted from mycelia of two collections of *C. tinctor* (SUT161 and SUT260), and also an isolate of reference strain *C. tinctor* (ST2321) obtained by Dr. Surang Thienhirun was included. Two more collections of *C. tinctor* (SUT099 and SUT211) were too old and no ascospore was available for genomic DNA extraction. One specimen of *C. selangorensis* (KS15) obtained from Dr. Margaret A. Whalley was used for DNA extraction from ascospores which had been separated under a stereomicroscope and confirmed before the extraction. A specimen of *C. leprieurii* could not be used for the extraction of genomic DNA because the specimen was covered with other fungal mycelia making it difficult to isolate the pure ascospores without the risk of subsequent DNA contamination. Therefore, only *C. tinctor* and *C. selangorensis* were amplified for their ITS1-5.8S-ITS2 regions by using ITS5 and ITS4 as forward and reverse primers respectively. The results of amplified fragments are shown in Figure 84.



Figure 84. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of *Camillea* using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, *Camillea tinctor* (ST2321); 2, *C. tinctor* (SUT161); 3, *C. tinctor* (SUT260); and 4, *C. selangorensis* (KS15).

The length of amplified ITS1-5.8S-ITS2 fragments of *C. tinctor* (SUT161, SUT260, and ST2321) and *C. selangorensis* (KS15) were similar in size being approximately 500 bp. Since genomic DNA of *C. selangorensis* (KS15) was extracted from ascospores, the amplified fragment was in low concentration. It was because the only small amount of ascospores was used to extract after observation and isolation under stereomicroscope to make sure they were no contamination from other fungus spores.

#### 4.4.2.2 ITS1-5.8S-ITS2 sequence analysis

The amplified ITS1-5.8S-ITS2 fragments of three *C. tinctor* isolates and one *C. selangorensis* (KS15) specimens were performed. The length of ITS1-5.8S-ITS2 sequences is reported in Table 21.

 Table 21. The length of ITS1-5.8S-ITS2 sequences of Camillea tinctor and C.

 selangorensis obtained from DNA sequence analysis.

Species	Location/Source	ITS1	<b>5.8</b> S	ITS2	Total	GenBank
		(bp)	(bp)	(bp)	(bp)	accession
						number
C. tinctor (ST2321)	$RFD^{a}$	204	155	155	514	DQ322080
C. tinctor (SUT161)	Yasothorn Province	190	155	155	500	DQ322081
C. tinctor (SUT260)	Trad Province	186	155	155	496	DQ322082
C. selangorensis	Liverpool John	175	155	156	486	DQ322083
(KS15)	Moores University <sup>b</sup>					

<sup>a</sup> The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand.

<sup>b</sup> The specimen was provided by Dr. M.A. Whalley, Liverpool John Moores University, U.K., collected from Malaysia.

After sequencing the ITS1-5.8S-ITS2 fragments, the boundaries of the ITS1 and ITS2 regions were determined by comparison to published sequences of the ITS regions. The ITS1-5.8S-ITS2 sequences of both species ranged from 496 to 514 bp in length. The ITS1 region was high variable in length ranging from 186 to 204 bp whilst the 5.8S region was quite constant at 155 bp. The length of ITS2 region ranged from 155 to 159 bp. The ITS1-5.8S-ITS2 sequences of *C. tinctor* (SUT161, SUT260, and ST2321) and *C. selangorensis* (KS15), including DNA sequences of *C. tinctor* (AJ390421) available from the GenBank database, were aligned. The result revealed the high variation in ITS1 region as shown in Figure 85.

The complete ITS1-5.8S-ITS2 sequence alignment is given in Appendix 4C. The identity matrix is shown in the Table 22.



Figure 85. ITS1 sequence alignment of Camillea tinctor (AJ390421) from GenBank, C. tinctor (ST2321), C. tinctor (SUT161), C. tinctor (SUT260), and C. selangorensis (KS15) by using ClustalX and BioEdit programs. Blocks indicate the short tandem repeat (STR) sequences.

Species	<i>C. tinctor</i> (AJ390421)	<i>C. tinctor</i> (ST2321)	C. tinctor (SUT161)	C. tinctor (SUT260)	C. selangorensis (KS15)
C. tinctor (AJ390421)	1.000	0.909	0.890	0.881	0.700
C. tinctor (ST2321)		1.000	0.935	0.924	0.711
C. tinctor (SUT161)			1.000	0.986	0.745
C. tinctor (SUT260)				1.000	0.744
C. selangorensis (KS15)					1.000

Table 22. The identity matrix of ITS1-5.8S-ITS2 sequence comparison of Camillea

tinctor and C. selangorensis.

Note: 1.000 means 100% identity.

The ITS1-5.8S-ITS2 sequence comparison of the C. tinctor

(SUT161, SUT260, and ST2321) isolates varied from 88.1% to 98.6% identity which was a result of the insertion and/or deletion of DNA fragments in the ITS1 region. Andersen and Torsten (1997) reported the presence of a DNA motif repeated in tandem or short tandem repeats (STR) of ITS1 sequences, which caused an increased rate of mutation in the ITS1 sequence of these fungi. When using STAR software (Delgrange and Rivals, 2004), ITS1 sequences of different *C. tinctor* isolates examined exhibited eleven nucleotides repeated in tandem from three to five times (Figure 85). There were eight variation patterns of the STR motif found in the isolates of *C. tinctor* and *C. selangorensis* as shown in Table 23. All of these modifications were followed the basic motif 5' CTACCCTGTAG 3' as reported by Platas *et al.* (2001).

**Table 23.** Short tandem repeat motifs found in the isolates of *Camillea tinctor* and *C.selangorensis* (KS15). The gray characters are mutation point changedfrom the basic motif.

No.	STR motif (5' to 3')
1	CTACCCTGTAG
2	CTACCCTGGAG
3	CTACCCGGTAG
4	CTACCCTACAG
5	TTACCCTGGAG
6	TTACCTGTAAG
7	GTACCCCGGAG
8	GCACCCTACAG

The mechanisms of evolution of repetitive sequences are assumed to be shaped by both intra- or inter-strand recombinational effects such as unequal crossing over, or other mechanisms involving failures in the replication of the DNA such as slipped-strand mispairing (SSM) (Levinson and Gutman, 1987) or replication slippage (Pinder *et al.*, 1998). The SSM is a process in which misalignment intermediates are formed during DNA synthesis or recombination, as a result of the slippage of DNA strands in regions containing repeated nucleotides, or repeated sequences. This phenomenon causes short deletions or insertions and duplications (Levinson and Gutman, 1987). These might be the reason for genetic variation among species of *C. tinctor* (SUT161, SUT260, and ST2321) which presented the different numbers and patterns of STR motifs within the ITS1 sequences. Although ITS sequence analysis of *C. tinctor* (SUT161, SUT260, and ST2321) varied from 88.1% to 98.6%, they were placed in the same species. In addition, their morphological characteristics absolutely matched *C. tinctor* (Berk.) Læssøe, J.D. Rogers & Whalley described by Læssøe *et al.* (1989). *Camillea* 

*selangorensis* (KS15) also contained three repeated motifs in subsequently different patterns. The phylogenetic trees of *C. tinctor* and *C. selangorensis* were constructed based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method by the PHYLIP program (Felsenstein, 1995), and the maximum parsimony method by PAUP program (Swofford, 2000) as shown in Figure 86 and Appendix 2D respectively. The trees exhibited the separation of the two species, *C. selangorensis* and *C. tinctor*, from each other with high bootstrap support. The three isolates of *C. tinctor* (SUT161, SUT260, and ST2321) examined were grouped together with the two *C. tinctor* sequences (AJ390421 and AJ390422) from the GenBank database although they exhibited genetic variation within their ITS1 regions.



**Figure 86.** Phylogenetic tree of *Camillea* based on ITS1-5.8S-ITS2 sequences constructed by using the neighbour-joining method. *Daldinia eschscholzii* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replications.

#### 4.4.3 Group III: Daldinia

*Daldinia eschscholzii* and *D. concentrica* have been classified into five new species by Stadler *et al.* (2004) using anamorph characteristics and perispore ornamentation by SEM but there were still some serious limitations for uncultured specimens or failure of anamorph production in culture. The ITS1-5.8S-ITS2 sequence of *Daldinia eschscholzii* and *D. concentrica* were then investigated.

## 4.4.3.1 Genomic DNA extraction and ITS1-5.8S-ITS2 amplification

Genomic DNA of seven representatives of *D. eschscholzii* isolates were extracted from their mycelia. Two cultural isolates of *D. concentrica* (L1 and L2) obtained from Prof. Anthony J.S. Whalley were also included. Initially, the ITS1-5.8S-ITS2 amplification products most *Daldinia* isolates could not be achieved. It might be because of some inhibitors in DNA extracts which had brown to dark brown in colour corresponding to the fungus mycelia. Then, the DNA solutions were diluted into 1:50, 1:100, 1:200, 1:500, and 1:1,000 (v/v) before amplification. The fragments obtained, approximately 500 bp, were successfully amplified from ITS1-5.8S-ITS2 regions as shown in Figure 87.

## 4.4.3.2 ITS1-5.8S-ITS2 sequence analysis

The amplified ITS1-5.8S-ITS2 fragments of *D. eschscholzii* and *D. concentrica* were sequenced. The length of ITS1-5.8S-ITS2 sequences obtained is concluded in Table 24. The length of ITS1-5.8S-ITS2 sequences of all *D. eschscholzii* isolates was similar, ranging from 479 to 482 bp in size, whilst two isolates of *D. concentrica* (L1 and L2) were 499 bp. The comparison of ITS1-5.8S-ITS2 sequences revealed 95.4% to 99.7% identity within isolates of *D. eschscholzii*  examined but there was 100% identity within the *D. concentrica* specimens (Table 4, Appendix C).



Figure 87. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of *Daldinia* using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, *Daldinia eschscholzii* (SUT038); 2, *D. eschscholzii* (SUT039); 3, *D. eschscholzii* (SUT168); 4, *D. eschscholzii* (SUT169); 5, *D. eschscholzii* (SUT178); 6, *D. eschscholzii* (SUT209); 7, *D. eschscholzii* (SUT278); 8, *D. concentrica* (L1); and 9, *D. concentrica* (L2).

Species	Location/Source	ITS1	<b>5.8</b> S	ITS2	Total	GenBank
		(bp)	(bp)	(bp)	(bp)	accession
						number
D. eschschozii (SUT039)	Ratchaburi Province	164	155	162	481	DQ322084
D. eschschozii (SUT085)	Yasothorn Province	164	155	162	481	DQ322085
D. eschschozii (SUT168)	Yasothorn Province	164	155	163	482	DQ322086
D. eschschozii (SUT178)	Nakhon Ratchasima	164	155	163	482	DQ322087
	Province					
D. eschschozii (SUT209)	Trad Province	163	155	163	481	DQ322088
D. eschschozii (SUT278)	Kanchanaburi	163	155	159	477	DQ322089
	Province					
D. eschschozii (SUT322)	Chiang Rai Province	164	155	164	483	DQ322090
D. concentrica (L1)	Liverpool John	173	155	171	499	DQ322091
	Moores University*					
D. concentica (L2)	Liverpool John	173	155	171	499	DQ322092
	Moores University*					

**Table 24.** The length of ITS1-5.8S-ITS2 sequences of Daldinia eschscholzii and D.

concentrica obtained from DNA sequence analysis.

\* The specimen was provided by Prof. Anthony J.S. Whalley, Liverpool John Moores University, U.K.

The phylogenetic trees of *D. eschscholzii* and *D. concentrica* were constructed based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method by PHYLIP program (Felsenstein, 1995) and the maximum parsimony method by PAUP program (Swofford, 2000) as shown in Figure 88 and Appendix 3D, respectively. The ITS1-5.8S-ITS2 sequences of *Daldinia* species from GenBank database were also included in the phylogenetic tree construction. The trees contained three major clades.



**Figure 88.** Phylogenetic tree of *Daldinia* species based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replication.

Clade I consisted of D. grandis (AF176982), Daldinia sp.

(AY315403), D. loculata (AF176969, AF176968, AF176967), D. petrinia (AF176975, AF176974, AF176973), D. vericosa (AF163022), D. fissa (AF176981), Daldinia sp. (AF163023), and D. concentrica (AF163021). Clade II consisted of nine D. concentrica (AF176958, AY616681, AY616682, AY616684, AGTS305, AGTS306, AGTS396, L1, and L2). Clade III contained all the D. eschscholzii examined except for only one sequence of D. concentrica (AY616682), which was included. The sequences of D. eschscholzii examined were all grouped together in clade III including D. eschscholzii (AGTS384 and AY616684) from the GenBank database. This result confirmed that D. eschscholzii is a common species found in Thailand. Although D. concentrica (AY616682) was placed in the same clade as D. eschscholzii, it might be caused by genetic variation within the species or belonging to different species because D. concentrica has been separated into different new species as previously described by Stadler et al. (2004). In addition, another sequence of D. concentrica (AF163021) was placed in clade I with high bootstrap support, and this might be the same reasons as mentioned above. Most D. concentrica sequences from database were placed in clade II and these included two specimens examined, D. concentrica (L1 and L2).

## 4.4.4 Group IV: Hypoxylon

Since *Hypoxylon* is a large and complex genus with the high variation in morphological characteristics, several species could not be identified. The nucleic acid methodology was then applied to clarify species problem.

#### 4.4.4.1 Hypoxylon section Annulata

Thirty five collections belonging to nine species of *Hypoxylon* sect. *Annulata* were investigated for their nucleic acid sequences. Twelve isolates provided by Dr. Surang Thienhirun, and two specimens provided by Dr. Ju-Ming Yu as reference strains were also included. Genomic DNA was extracted from either cultural mycelia or ascospores depending on the culturable specimens. In case of coloured DNA solution, it was diluted as previously described in section 4.4.3.1. The length of amplified ITS1-5.8S-ITS2 fragments ranged from 500 to 1,000 bp as shown in Figure 89. The amplified ITS1-5.8S-ITS2 fragments were sequenced. The length of ITS1-5.8S-ITS2 sequences is listed in Table 25.

The ITS1-5.8S-ITS2 sequences of *Hypoxylon* sect. *Annulata* examined varied in length from 525 to 906 bp. This high variation resulted found in the ITS1 region, which ranged from 157 to 588 bp. The ITS2 sequences ranged from 154 to 170 bp whilst 5.8S sequences were highly constant at 155 bp. The extremely long ITS1 regions (716 to 906 bp) were found in four collections of *H. atroroseum* (SUT009 and SUT010), *Hypoxylon* sp. (ST2336), *Hypoxylon* taxonomic species 1 (SUT236, SUT242, SUT251, and SUT285), *H. stygium* (SUT058, SUT231, and SUT243) and *H. urceolatum* (SUT098). The extremely long ITS1 sequences of *H. atroroseum* (SUT009 and SUT010) and *H. stygium* (SUT058, SUT231, and SUT243) were similar to *H. atroroseum* (AJ390397) and *H. stygium* (AJ390409) respectively. Both *H. atroroseum* (AJ390397) and *H. stygium* (AJ390409) were available from the GenBank database as previously reported by Sánchez-Ballesteros *et al.* (2000). Moreover, *Hypoxylon* sp. (ST2336), *Hypoxylon* taxonomic species 1 (SUT236,

SUT242, SUT251, and SUT285), and *H. urceolatum* (SUT098), which had the long ITS1 sequences, were found in this study.



Figure 89. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of *Hypoxylon* sect. Annulata using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, *Hypoxylon nitens* (ST2313); 2, *H. stygium*; 3, *H. purpureonitens* (SUT001); 4, *H. atroroseum* (SUT009); 5, *H. purpureonitens* (SUT005); 6, *H. atroroseum* (SUT010); 7, *H. nitens* (SUT081); 8, *H. moriforme* (SUT220); 9, *H. nitens* (244); 10, *H. bovei* var. microspora (SUT025); 11, *H. cf. archeri* (SUT103); and 12, *H. purpureonitens* (SUT262).

# Table 25. The length of ITS1-5.8S-ITS2 sequences of Hypoxylon sect. Annulata

Species	Location/Source	ITS1	<b>5.8</b> S	ITS2	Total	GenBank
-		(bp)	(bp)	(bp)	(bp)	accession number
H. cf. archeri (SUT103)	Songkhla Province	209	155	161	525	DQ201121
H. cf. archeri (SUT105)	Songkhla Province	209	155	161	525	DQ201122
H. cf. archeri (ST2333)	RFD <sup>a</sup>	209	155	161	525	DQ201123
H. cf. archeri (ST2527)	RFD <sup>a</sup>	224	155	160	539	DQ201124
<i>H. atroroseum</i> (SUT009)	Nakhon Ratchasima Province	506	155	164	825	DQ223733
<i>H. atroroseum</i> (SUT010)	Nakhon Ratchasima Province	506	155	164	825	DQ223734
H. bovei var. microspora (SUT025)	Chaiyaphum Province	202	155	170	527	DQ322096
H. bovei var. microspora (Ju2)	The University of Taiwan <sup>b</sup>	226	155	167	548	DQ201127
H. bovei var. microspora (ST2579)	RFD <sup>a</sup>	226	155	167	548	DQ201129
H. bovei var. microspora (ST2406)	$RFD^{a}$	225	155	167	547	DQ201128
H. leptascum var. macrospora (ST2584)	RFD <sup>a</sup>	248	155	159	562	DQ322097
<i>H. moriforme</i> (SUT220)	Trad Province	230	155	165	550	DQ322129
H. nitens (Jul)	The University of Taiwan <sup>b</sup>	158	155	166	479	DQ223750
H. nitens (ST2313)	<b>RFD</b> <sup>a</sup>	158	155	166	479	DQ223751
H. nitens (ST2332)	RFD <sup>a</sup>	233	155	166	554	DQ322098
H. nitens (ST2436)	RFD <sup>a</sup>	158	155	166	479	DQ322099
H. nitens (ST2473)	$RFD^{a}$	158	155	166	479	DQ223752
<i>H. purpureonitens</i> (SUT001)	Nakhon Ratchasima Province	225	155	166	546	DQ322100
<i>H. purpureonitens</i> (SUT004)	Nakhon Ratchasima Province	225	155	169	549	DQ223753
<i>H. purpureonitens</i> (SUT005)	Nakhon Ratchasima Province	225	155	165	545	DQ322101
<i>H. purpureonitens</i> (SUT167)	Yasothorn Province	225	155	169	549	DQ223754
<i>H. purpureonitens</i> (SUT262)	Trad Province	225	155	169	549	DQ223755
H. purpureonitens (ST2448)	$RFD^{a}$	225	155	169	549	DQ223756
<i>H. purpureonitens</i> (ST2485)	$RFD^{a}$	225	155	169	549	DQ223757
H. stygium (SUT058)	Ratchaburi Province	477	155	164	796	DQ223760
H. stygium (SUT231)	Trad Province	588	155	163	906	DQ322102

obtained from DNA sequence analysis.

<sup>a</sup> The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand. <sup>b</sup> The specimen was provided by Dr. Ju-Ming Yu, The University of Taiwan, Taiwan.

# Table 25. (Continued).

Species	Location/Source	ITS1 (bp)	5.8S (bp)	ITS2 (bp)	Total (bp)	GenBank accession number
H. stygium (SUT243)	Trad Province	477	155	164	796	DQ223761
H. urceolatum (SUT098)	Songkhla Province	398	155	163	716	DQ322103
Hypoxylon sp. (ST2336)	RFD <sup>a</sup>	176	155	154	489	DQ322104
<i>Hypoxylon</i> taxonomic species 1 (SUT081)	Nakhon Ratchasima Province	560	155	155	870	DQ322105
<i>Hypoxylon</i> taxonomic species 1 (SUT236)	Trad Province	566	155	155	876	DQ322106
<i>Hypoxylon</i> taxonomic species 1 (SUT242)	Trad Province	566	155	155	876	DQ322107
<i>Hypoxylon</i> taxonomic species 1 (SUT244)	Trad Province	566	155	154	875	DQ322108
<i>Hypoxylon</i> taxonomic species 1 (SUT251)	Trad Province	566	155	155	876	DQ322109
Hypoxylon taxonomic species 1 (SUT285)	Kanchanaburi Province	566	155	154	875	DQ322110

<sup>a</sup> The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand.

<sup>b</sup> The specimen was provided by Dr. Ju-Ming Yu, The University of Taiwan, Taiwan.

#### A) ITS1-5.8S-ITS2 sequence analysis

Since there was a high variation in size of ITS1-5.8S-ITS2 region (476 bp to 906 bp), they could not be unambiguously aligned correctly. For sequence alignment, *Hypoxylon* sect. *Annulata* could be divided into 2 groups according to their ITS1-5.8S-ITS2 fragment sizes, which were 716 bp to 906 bp (group I) and 476 bp to 566 bp (group II) respectively. The group I composed of *H. atroroseum*, *H. stygium*, *H.* cf. *stygium* (SUT231), *Hypoxylon* taxonomic species 1, and *H. urceolatum* (SUT098). After their sequences were aligned with reference strains, *H. stygium* (AJ390409) and *H. atroroseum* (AJ390397), available from the GenBank database, the phylogenetic trees were constructed using both the neighbourjoining method (PHYLIP program; Felsenstein, 1995), and the maximum parsimony (PAUP program; Swofford, 2000). Similar patterns of phylogenetic trees were obtained from the two analysis methods (Figure 90 and Appendix 4D). The trees composed of two clades. Clade I consisted of *H. atroroseum* (SUT009, SUT010, and AJ390397), *H. stygium* (SUT058, SUT243, and AJ390409), *H. cf. stygium* (SUT231), and *H. urceolatum* (SUT098), whilst clade II consisted of only one species, *Hypoxylon* taxonomic species 1 (SUT081, SUT242, SUT244, SUT251, and SUT285).

and *H. atroroseum* (AJ390397) from the GenBank database were grouped together. But the two isolates of *H. stygium* (SUT058 and SUT243) examined were in another group with *H. stygium* (AJ390397) from the GenBank database. *Hypoxylon atroroseum* and *H. stygium* appeared to be closely related as shown by their 94 % identity.

Two isolates of *H. atroroseum* (SUT009 and SUT010)

The ITS1-5.8S-ITS2 sequence alignments of *H. atroroseum* (SUT009, SUT010, and AJ390397) and *H. stygium* (SUT058, SUT243, and AJ390397) revealed the insertion and/or deletion sequences of 28 bp (5' ATCTG CTCGAATAAAATTGCTTCAATAT 3') within the ITS1 region. This sequence fragment might be useful for the designer of a probes or markers for species specific detection. This molecular result was in agreement with their closely related morphological characteristics except that the stromata of *H. atroroseum* often have rosy surface tones and the conidiogenous structure of *H. atroroseum* is *Nodulisporium*-like, whereas that of *H. stygium* is *Periconiella*-like (Ju and Rogers, 1996). However, *H. atroroseum* and *H. stygium* were separated from each other with high 100% bootstrap support based on ITS1-5.8S-ITS2 sequence analysis.



Figure 90. Phylogenetic tree of *Hypoxylon* sect. *Annulata* containing extremely long ITS1 region (398 bp to 588 bp) based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replications.

In addition H. cf. stygium (SUT231) contained the largest

ITS1 region, 588 bp, and was branched from *H. stygium* but still showed close relationship to both taxa. *Hypoxylon* cf. *stygium* (SUT231) differed from *H. stygium* in stromatal form. *Hypoxylon* cf. *stygium* (SUT231) had conspicuous perithecial mounds and broad size of ostiolar disc (0.1-0.3 mm diameter). This might be genetic variation within the species or it might indicate a distinct taxon.

*Hypoxylon urceolatum* (SUT098) was separately branched from *H. atroroseum* (SUT009, SUT010, and AJ390397), *H. stygium* (SUT058, SUT243, and AJ390397), and *H.* cf. *stygium* (SUT231). In addition, *Hypoxylon urceolatum* has the distinctive characteristic of KOH-extractable pigment in purplish (Ju and Rogers, 1996). However, all taxa in clade I had a convex *truncatum*-type of ostiolar disc and small in the size of their ostiolar disc.

Clade II consisted of only one taxon, *Hypoxylon* taxonomic species 1 (SUT081, SUT242, SUT244, SUT251, and SUT285), which had a *bovei*-type of ostiolar disc. Although its morphological characteristics were close to both *H. nitens* and *H. bovei* var. *microspora*, the size of ITS1-5.8S-ITS2 region was absolutely different from those two taxa.

The group II (476 to 566 bp) composed of *H.* cf. *archeri* (SUT103, SUT105, ST2333, and ST2527), *H. bovei* var. *microspora* (SUT025, ST2406, ST2579, and Ju2), *H. leptascum* var. *macrosporum* (ST2584), *H. moriforme* (SUT220 and ST2332), *H. nitens* (ST2313, ST2436, ST2473, and Ju1), and *H. purpureonitens* (SUT001, SUT004, SUT005, SUT167, SUT262, ST2448, and ST2485). Sequence alignment of ITS1-5.8S-ITS2 was performed. The phylogenetic trees were constructed by using both the neighbour-joining method (PHYLIP)

program, Felsenstein, 1995), and the maximum parsimony method (PAUP program, Swofford, 2000). Both methods also exhibited the similar phylogenetic tree except for bootstrap valves. The trees were divided into three clades (Figure 91 and Appendix 5D).

Clade I consisted of *Hypoxylon* sp. (ST2336), which was

separated from other taxa. Clade II consisted of seven species, *H. annulatum* (AJ390395), *H. bovei* var. *microspora* (SUT025, ST2406, ST2579, and Ju2), *H. cohaerens* (AJ390406), *H. leptascum* var. *macrosporum* (ST2584), *H. moriforme* (SUT220 and ST2332), *H. nitens* (ST2313, ST2436, ST2473, and Ju1), and *H. purpureonitens* (SUT001, SUT004, SUT005, SUT167, SUT262, ST2448, and ST2485), which were clearly separated from each other. These isolates of *H. nitens* (ST2313, ST2436, and ST2473) were grouped together including a reference strain, *H. nitens* (Ju1). *Hypoxylon bovei* var. *microspora* (ST2406, ST2579, and SUT025) isolates were also clustered with a reference strain, *H. bovei* var. *microspora* (Ju2), although some variation within the species appeared to represent a distinctive taxon. *Hypoxylon bovei* var. *microspora* was placed close to *H. moriforme* (SUT220 and ST2332), and they were similar in their morphological characteristics except for the type and size of their ostiolar disc.

All of *H. purpureonitens* isolates (SUT001, SUT004, SUT005, SUT167, SUT262, ST2448, and ST2485) were grouped together and showed slightly variation among collections. Although the morphological characteristics of *H. purpureonitens* and *H. nitens* are very similar except for having purplish KOH-extractable pigments in *H. purpureonitens* (Ju and Rogers, 1996). They were completely different in molecular data.



Figure 91. Phylogenetic tree of *Hypoxylon* sect. *Annulata* based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Camillea tinctor* (AJ394021) is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replications.

Clade III contained only one species of *H*. cf. *archari* (SUT103, SUT105, ST2333, and ST2527). The morphological features of this taxon were similar to those of *H*. *archeri* Berk. and *H*. *michelianum* Ces. & De Not. Ascospore dimensions, stromatal form, and coloration were indicative of *H*. *archeri* but the distinctive white fringe surrounding the ostioles was reminiscent of *H*. *michelianum*. This taxon remains unknown.

#### **B) ITS2 sequence analysis**

Although the length of the ITS1 region of *Hypoxylon* sect. *Annulata* differed considerably (ranging from 157 to 588 bp), the length of ITS2 region was not so different (ranging from 147 to 170 bp). Therefore, ITS2 sequences of all *Hypoxylon* sect. *Annulata* taxa were aligned together, and the phylogenetic trees were constructed using both the neighbour-joining method (PHYLIP program, Felsenstein, 1995), and the maximum parsimony method (PAUP program, Swofford, 2000). Both methods exhibited the same pattern of trees except for their bootstrap valves. The trees contained three main clades (Figure 92 and Appendix 6D).

Clade I was a complex clade consisting of seven species, *H. nitens* (ST2313, ST2436, ST2473, and Ju1), *H. atroroseum* (SUT009, SUT010, AJ390397), *H. stygium* (SUT058, SUT243, and AJ390409), *H. cf. stygium* (SUT231), *H. leptascum* var. *microsporum* (ST2548), *H. urceolatum* (SUT098) and *Hypoxylon* taxonomic species 1 (SUT081, SUT242, SUT244, SUT251, and SUT285). Four specimens of *H. nitens* (ST2313, ST2436, ST2473, and Ju1) were grouped together in the same cluster, which was clearly separated from *Hypoxylon* taxonomic species 1 (SUT081, SUT242, SUT244, SUT251, and SUT285).


Figure 92. Phylogenetic tree of *Hypoxylon* sect. *Annulata* based on ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replications.

*Hypoxylon atroroseum* (SUT009, SUT010, and AJ390397), *H. stygium* (SUT058, SUT243, and AJ390409), and *H. cf. stygium* (SUT231) exhibited a similar pattern on ITS1-5.8S-ITS2 sequence analysis (Figure 90) and ITS2 sequence analysis (Figure 92). Thus, this result confirmed the close relationship among these taxa.

### Hypoxylon leptascum var. microsporum (ST2548) and H.

*urceolatum* (SUT098) were grouped together in the same cluster. Their morphological characters are also similar in convex *truncatum*-type of ostiolar disc, and overlap in ascospore size, but they differ in their KOH-extractable pigments (Ju and Rogers, 1996). *Hypoxylon leptascum* var. *microsporum* (ST2548) was greenish olivaceous whilst *H. urceolatum* (SUT098) was vinaceous purple or vinaceous grey.

Clade II consisted of six species including *H. cohaerens* (AJ390406), *H. bovei* var. *microspora* (SUT025, ST2406, ST2579, and Ju2), *H. purpureonitens* (SUT001, SUT004, SUT005, SUT167, SUT262, ST2448, and ST2485), *Hypoxylon* sp. (ST2336), *H. annulatum* (AJ390395), and *H. moriforme* (SUT220 and ST2332). The pattern of phylogenetic trees in clade II was also similar to the tree constructed from ITS1-5.8S-ITS2 sequence (Figure 91), which confirmed the close relationships within this clade except that *H. moriforme* (ST2336) and *H. annulatum* (AJ390395) placed as sister branch.

*Hypoxylon* sp. (ST2336) was placed in the same cluster as *H. annulatum* (AJ390395), which indicated a close relationship for both species. The morphological characters of *Hypoxylon* sp. (ST2336), which are usually has glomerate stromata, can look quite like *H. annulatum* when the stromata are hemispherical. The usually evident perithecial mounds and less massive stromata are

useful in identifying *H. moriforme*. Moreover, *H. annulatum* is a Northern temperate species, and is highly associated with *Quercus* (Ju and Rogers, 1996).

Clade III contained only *H.* cf. *archari* (SUT103, SUT105, ST2333, and ST2527) isolates (Figure 91).

From molecular analysis of ITS1-5.8S-ITS2 sequences and ITS2 sequence revealed the similarity of phylogenetic relationships based on both methods of the neighbour-joining and maximum parsimony. The trees exhibited a clear separation of the species complex within *Hypoxylon* sect. *Annulata*, and also indicated the range of genetic variation within each species (Figures 90, 91 and 92). Moreover, some taxa might prove to be the new taxa when further collections are made and examined.

### 4.4.4.2 Hypoxylon section Hypoxylon

Forty four representatives of *Hypoxylon* collections belonging to twenty one species were extracted for genomic DNA from either cultural mycelium or ascospores depending on the specimens. The amplified ITS1-5.8S-ITS2 fragments were approximately 500 to 600 bp (Figure 93). They were then sequenced. The sizes of ITS1-5.8S-ITS2 fragments are listed in Table 26.



Figure 93. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of *Hypoxylon* sect. *Hypoxylon* using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, *Hypoxylon investiens* (SUT041); 2, *H. investiens* (SUT063); 3, *H. lenormandii* (SUT046); 4, *H. lenormandii* (SUT180); 5, *H. hypomiltum* (SUT166); 6, *H. cf. perforatum* (SUT294); 7, *H. rubiginosum* (SUT146); 8, *H. kanchanapisekii* sp. nov. (SUT066); and 9, *H. sublenormandii* sp. nov. (SUT282).

Species	Location/Source	ITS1 (bp)	5.8S (bp)	ITS2 (bp)	Total (bp)	GenBank accession number
H. anthochroum (SUT233)	Trad Province	180	155	162	497	QD201125
H. anthochroum (SUT240)	Trad Province	180	155	162	497	QD201126
H. brevisporum (SUT256)	Trad Province	165	155	161	481	DQ322111
H. duranii (SUT223)	Trad Province	206	155	184	545	DQ322112
H. cf. ferruginuem (SUT070)	Ratchaburi Province	131	155	159	445	DQ322113
H. cf. fendleri (SUT061)	Ratchaburi Province	181	155	163	499	QD201130

 Table 26. The length of ITS1-5.8S-ITS2 sequences of Hypoxylon sect. Hypoxylon obtained from DNA sequence analysis.

\* The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand.

## Table 26. (Continued).

Species	Location/Source	ITS1 (bp)	5.8S (bp)	ITS2 (bp)	Total (bp)	GenBank accession
						number
H. cf. fendleri (SUT120)	Petchaboon Province	185	155	161	501	QD20113
H. cf. fendleri (SUT159)	Yasothorn Province	183	155	164	502	QD201132
H. cf. fendleri (SUT162)	Yasothorn Province	183	155	165	503	QD22373
H. cf. fendleri (SUT165)	Yasothorn Province	182	155	163	500	QD22373
H.cf. fendleri (SUT280)	Kanchanaburi Province	156	155	163	474	QD22373
H. haematostroma (SUT164)	Yasothorn Province	176	155	161	492	QD22373
H. haematostroma (SUT292)	Kanchanaburi Province	176	155	161	492	QD22373
H. haematostroma (SUT293)	Kanchanaburi Province	176	155	161	492	QD22374
H. hypomiltum (SUT166)	Yasothorn Province	213	155	160	528	QD32211
H. investiens (SUT041)	Ratchaburi Province	276	155	153	584	QD32211
H. investiens (SUT063)	Ratchaburi Province	230	155	155	540	QD32211
H. kanchanapisekii (SUT066) sp. nov.	Ratchaburi Province	209	155	162	526	QD22374
H. kanchanapisekii (SUT068) sp. nov.	Ratchaburi Province	209	155	162	526	QD22374
H. kanchanapisekii (SUT069) sp. nov.	Ratchaburi Province	209	155	162	526	QD22374
H. lenormandii (SUT016)	<b>Burirum Province</b>	188	155	160	503	QD22374
H. lenormandii (SUT046)	Ratchaburi Province	208	155	165	528	DQ32211
H. lenormandii (SUT180)	Nakhon Ratchasima Province	188	155	160	503	QD22374
H. lenormandii (ST2324)	RFD*	188	155	160	503	QD22374
H. monticulosum (SUT042)	Ratchaburi Province	171	155	165	491	QD22374
H. monticulosum (SUT080)	Nakhon Ratchasima Province	171	155	165	491	QD22374
H. monticulosum (SUT116)	Songkhla Province	171	155	165	491	QD22374
H. cf. perforatum (SUT020)	Burirum Province	301	155	156	612	QD32211
H. cf. perforatum (SUT218)	Trad Province	206	155	186	547	QD32211
H. cf. perforatum (SUT294)	Kanchanaburi Province	209	155	161	525	QD32212
H. rubiginosum (SUT215)	Trad Province	178	155	164	497	QD22375
H. rubiginosum (SUT221)	Trad Province	178	155	164	497	QD22375
H. subgilvum var. microsporum (SUT104)	Songkhla Province	148	155	164	467	QD32212

\* The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand.

## Table 26. (Continued).

Species	Location/Source	ITS1 (bp)	5.8S (bp)	ITS2 (bp)	Total (bp)	GenBank accession number
H. subgilvum var. microsporum (SUT108)	Songkhla Province	149	155	153	457	QD322122
<i>H. sublenormandii</i> sp. nov. (SUT250)	Trad Province	198	155	161	514	QD223762
<i>H. sublenormandii</i> sp. nov. (SUT282)	Kanchanaburi Province	198	155	161	514	QD223763
<i>H. suranareei</i> (SUT183) sp. nov.	Nakhon Ratchasima Province	199	155	162	516	QD223764
H. trugodes(SUT146)	Nakhon Ratchasima Province	182	155	168	505	QD322123
H. trugodes (SUT148)	Nakhon Ratchasima Province	181	155	152	488	QD322124
H. trogodes (SUT187)	Nakhon Ratchasima Province	181	155	158	494	QD322125
<i>Hypoxylon</i> taxonomic species 2 (SUT082)	Nakhon Ratchasima Province	130	155	166	451	QD322126
<i>Hypoxylon</i> taxonomic species 3 (SUT158)	Yasothorn Province	173	155	160	488	QD322127
<i>Hypoxylon</i> taxonomic species 4 (SUT237)	Trad Province	131	155	158	444	QD322128

\* The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand.

The length of ITS1-5.8S-ITS2 sequences of *Hypoxylon* sect. *Hypoxylon* ranged from 445 to 612 bp. The ITS1 sequences revealed the highest variation ranging from 131 to 209 bp. The ITS2 sequences ranged from 152 to 184 bp, whilst 5.8S sequences were highly constant at 155 bp. The sequences of two isolates, *H.* cf. *ferrugineum* (SUT017) and *H. macrocapum* (SUT045), could not be achieved. This might be because of either the variation of ITS1-5.8S-ITS2 sequences within the species or the direct sequencing of the amplified ITS1-5.8S-ITS2 fragments, which some fragments might contain a mutation and/or be GC rich. Therefore, their sequences could not be clearly performed.

The ITS1-5.8S-ITS2 sequences of *Hypoxylon* sect. *Hypoxylon* examined were aligned and their phylogenetic trees were constructed by using the neighbour-joining method by the PHYLIP program (Felsenstein, 1995), and the maximum parsimony method by the PAUP program (Swofford, 2000) (Figure 94 and Appendix 7D). The ITS1-5.8S-ITS2 sequences of related *Hypoxylon* species from the GenBank database, *H. fendleri* (AJ390400) and *H. perforatum* (AJ390407), were also included. The phylogenetic trees show four main clades.

Clade I contained five species which were *H. subgivum* var. *microsporum* (SUT108), *H. trugodes* (SUT148 and SUT187), *Hypoxylon* taxonomic species 2 (SUT082), *H.* cf. *ferruginium* (SUT070 and SUT237), *H. brevisporum* (SUT256). The stromatal surface colour of all species in this clade was mostly reddish brown to brownish vinaceous, and the KOH-extractable pigment was of the orange series. The phylogenetic tree showed that *H. subgivum* var. *microsporum* (SUT108) was distinctive and separated from other species in the same clade. Moreover, its ascospores were smaller than other species and it also had very orange pigment in KOH extraction.

Two isolates of *H*. cf. *ferrugineum* (SUT070 and SUT237) were grouped together and were placed as a sister branch of *Hypoxylon* taxonomic species 2 (SUT082). They had the same stromatal colour and KOH-extractable colour but they were different in ascospore size. The tree showed the closely relationship between both species.



**Figure 94.** Phylogenetic tree of *Hypoxylon* sect. *Hypoxylon* based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replications.

Clade II consisted of four species, *H. monticulosum* (SUT042, SUT080, and SUT116), *H. lenormandii* (SUT016, SUT180, and ST2324), *H. sublenormandii* sp. nov. (SUT046, SUT066, SUT068, and SUT069) and *H.* cf. *fendleri* (SUT061, SUT120, SUT159, SUT162, SUT165, and SUT280).

Hypoxylon monticulosum found in this study could be divided into two different types based on KOH-extractable pigments. Two representatives of *H. monticulosum* isolates, SUT042 and SUT080, were without apparent KOHextractable pigments as detailed by Ju & Rogers (1996) whereas *H. monticulosum* SUT116 had a purple colored extract. This does however agree with Ju & Rogers (1996) who state that "it is noteworthy that the purplish stromatal pigments dark livid to dark vinaceous of *H. monticulosum* and *H. submonticulosum* are easily detected in the young, rusty brown stromata but are hardly so in the mature, blackened stromata". The sequence alignment indicated 99% similarity and it was concluded that they represented the same taxon regardless of extractable pigment in KOH.

Three isolates of *H. lenormandii* (ST2324, SUT016, and SUT180) matched closely the description by Ju & Rogers (1996), and all collections were found on dicotyledonous wood from different forest areas. They were clearly separated from *H. sublenormandii* sp. nov. (SUT250 and SUT282), which occurred on bamboo, with high 100% bootstrap support. They also differed in morphological characters such as spore size, a more reddish brown stromatal surface color, and a straight germ slit (Table 16) and on the basis of this and the sequence data a new species was confirmed.

Initially, *H*. cf. *fendleri* (SUT061, SUT159, SUT162, SUT165, and SUT280) collections had been identified as *H. fendleri* since their

morphological characteristics matched to H. fendleri Berk. ex Cooke (Ju and Rogers 1996). However Ju and Rogers (1996) pointed out that *H. fendleri* and *H. retpela* Van der Gucht & Van der Veken are very similar stating "These two fungi differ mainly in the conspicuousness of their perispore ornamentation". The ornamentation in H. retpela is described as very conspicuous coil-like. However all the Thai collections had similar coiling, which was not noticeably conspicuous. Thus the description for H. fendleri (Ju & Rogers, 1996) was the nearest match. The phylogenetic result showed that all the Thai isolates (SUT061, SUT159, SUT162, SUT165, and SUT280) grouped together and were placed as a sister branch of *H. fendleri* (AJ390400) based on the GenBank database sequence with high bootstrap support. The percentage similarity of H. fendleri (AJ390400) to SUT061, SUT159, SUT162, SUT165, and SUT280 isolates was 85%, 85%, 85%, 85%, and 80% respectively. They are therefore quite different and as a result the Thai collections were recorded as H. cf. fendleri. This might be the result of a wide range of *H. fendleri* descriptions (morphological) or genetic variation within this taxon found in Thailand. More collections of specimens around the world are required for a better understanding of species delimitation and genetic variation within this taxon.

Clade III was a big clade and consisted of eleven species, *H. haematostroma* (SUT164, SUT292, and SUT293), *H. anthochroum* (SUT233 and SUT240), *H. kanchanapisekii* sp. nov. (SUT046, SUT066, SUT068, and SUT069), *H. rubiginosum* (SUT215 and SUT221), *H. investiens* (SUT041 and SUT063), *H. perforatum* (AJ390407), *H. cf. perforatum* (SUT020), *H. cf. perforatum* (SUT294), *H. suranareei* sp. nov. (SUT182), *H. hypomiltum* (SUT166), *Hypoxylon* taxonomic species 3 (SUT158). Three isolates of *H. haematostroma* (SUT164, SUT292, and

SUT293) were separated from other taxa in the same clade because their distinctive teleomorphic characteristics having red or orange red stromatal granules, constantly long tubular perithecia, and large ascospores (Ju and Rogers, 1996).

Hypoxylon anthochroum, H. kanchanapisekii sp. nov. (SUT046, SUT066, SUT068, and SUT069), H. rubiginosum (SUT215 and SUT221), and H. investiens were separated clearly from each other with high bootstrap support. Although H. anthochroum was considered to be a synonym of H. rubiginosum by Miller (1961), they are different in colour of KOH-extractable pigments. Two isolates of H. investiens (SUT041 and SUT063) exhibited genetic variation within the ITS1 region. These might be the appearance of insertion and/or deletion fragments of short repeated nucleotide sequences.

Hypoxylon cf. perforatum (SUT020), H. cf. perforatum (SUT294), and H. perforatum (AJ390407) from GenBank database grouped in the same branch and included H. suranareei sp. nov. (SUT182). Since H. perforatum described by Ju and Rogers (1996) had a wide range of stromatal surface colour, dark brick, grayish sepia, brown vinaceous, or umber, and also perispore ornamentation varied from smooth to inconspicuous coil-like ornamentation. Two isolates of H. cf. perforatum (SUT020) and H. cf. perforatum (SUT294) were different in stromatal surface colour but they were similar in having a smooth perispore. Importantly, both taxa are lacking white substance deposited around the ostioles which is usually found in this species (Ju and Rogers, 1996). The ITS1-5.8S-ITS2 sequence alignment of H. perforatum (SUT020) and H. cf. perforatum (SUT294) respectively, which might indicate different taxa. However, further investigations of more collections are required.

and SUT218). The ascospore size of *H. duranii* was similar to *H. anthochroum* but it differed in having conspicuous coil-like ornamentation of the perispore (Ju and Rogers, 1996), and the molecular data also revealed the different between both species very clearly.

The relationship between *Hypoxylon* sect. *Annulata* and *Hypoxylon* sect. *Hypoxylon* was then analyzed. Representatives of each species from both sections were aligned and the phylogenetic trees were constructed based on ITS2 sequences by using the neighbour-joining method by the PHYLIP program (Felsenstein, 1995), and the maximum parsimony method by the PAUP program (Swofford, 2000) as shown in Figure 95 and Appendix 8D, respectively. The species of *Hypoxylon* sect. *Annulata* appeared as a paraphylectic group with *Hypoxylon* sect. *Hypoxylon* sect. *Annulata* (H. cf. stygium (SUT231), H. atroroseum (SUT009), H. stygium (SUT058), Hypoxylon taxonomic species 1 (SUT081 and SUT285), *H. leptascum* var. macrosporum (ST2584) and *H. urceolatum* (SUT098)) were grouped together. The phylogenetic tree based on ITS2 sequence analysis did not support the concept of the division of *Hypoxylon* into two sections. The other nucleotide regions such as 28S rDNA or IGS may be more suitable candidates.



**Figure 95.** Phylogenetic tree of *Hypoxylon* based on ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replications.

## 4.4.5 Group V: Xylariaceous endophytes

Since members of *Xylaria* have been reported as common endophytes in many plants and there are serious limitations in species identification because of the lack of their teleomorph stage in culture, the *Xylaria* collections were selected to investigate for their ITS1-5.8S-ITS2 ribosomal nucleotide sequence analysis.

### 4.4.5.1 Genomic DNA extraction and ITS region amplification

Forty representatives of *Xylaria* specimens were extracted for genomic DNA including ten isolates of *Xylaria* obtained from Dr. Surang Thienhirun. Three more isolates, *Biscogniauxia* sp. nov. (SUT290), *Kretzschmaria* sp. (ST2325), and *Nemania* sp. (SUT258) were included. The amplified ITS1-5.8S-ITS2 fragments were approximately 500 to 600 bp (Figure 96).

### 4.4.5.2 ITS1-5.8S-ITS2 sequence analysis

The amplified ITS1-5.8S-ITS2 fragments were sequenced and the size of each specimen examined (Table 27).



Figure 96. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of *Xylaria* using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 base pair DNA ladder, Invitrogen); 1, *Xylaria* sp. nov. (SUT012); 2, *Xylaria* sp. nov. (SUT014); 3, *X. multiplex* (SUT028); 4, *X. badia* (SUT032); 5, *X. badia* (SUT076); 6, *X. mellisii* (SUT074); 7, *X. cf. juruensis* (SUT088); 8, *X. ianthino-velutina* (SUT091); 9, *X. ianthino-velutina* (SUT123); 10, *X. cubensis* (089); 11, *Xylaria* species 2 (SUT130); and 12, *Xylaria* species 2 (SUT195).

### ITS1 5.8S ITS2 Total GenBank **Species** Location/Source accession (bp) (bp) (bp) (bp) number X. anisopleura (SUT196) Trad Province 465 155 155 775 DQ322130 *X. anisopleura* (SUT205) Trad Province 141 155 DQ322131 155 451 RFD\* *X. anisopleura* (ST2329) 465 155 155 775 DQ322132 X. cf. apiculata (SUT203) 181 495 Trad Province 155 159 DQ322133 RFD\* X. arbuscula var. 182 155 162 499 DQ322134 microspora (ST2372) X. badia (SUT032) Ratchaburi Province 181 155 158 494 DQ322135 *X. badia* (SUT076) Ratchaburi Province 179 155 494 DQ322136 160 X. badia (SUT142) Nakhon Ratchasima 181 155 159 495 DQ322137 Province RFD\* 181 496 DQ322138 X. badia (ST2417) 155 160 X. beccari (SUT092) Songkhla Province 273 155 155 583 DQ322139 X. brachiata (SUT078) Ratchaburi Province 181 155 163 499 DQ322140 X. cubensis (SUT090) Songkhla Province 179 155 159 493 DQ322141 X. cubensis (ST2027) RFD\* 171 155 165 491 DO322142 X. cubensis (ST2326) RFD\* 188 155 163 506 DQ322143 209 155 *X. curta* (ST2382) RFD\* 162 526 DQ322144 494 X. grammica (ST2348) RFD\* 180 155 159 DQ322145 X. grammica (ST2363) RFD\* 155 490 DQ322146 180 155 X. ianthino-velutina Nakhon Ratchasima 177 155 156 488 DQ322147 Province (SUT123) X. cf. juruensis (SUT088) Songkhla Province 182 155 156 493 DQ322148 Nakhon Ratchasima 155 DQ322149 X. cf. juruensis (SUT140) 181 162 498 Province X. juruensis var. Nakhon Ratchasima 179 155 159 493 DQ322150 Province microspora (SUT129) X. juruensis var. Nakhon Ratchasima 181 155 159 495 DQ322151 Province microspora (SUT138) X. juruensis var. Nakhon Ratchasima 182 155 159 496 DQ322152 Province microspora (SUT139) X. maitlandii (SUT177) Nakhon Ratchasima 181 155 159 495 DQ322153 Province X. multiplex (SUT028) Ratchaburi Province 178 155 162 495 DQ322154 RFD\* X. multiplex (ST2298) 178 155 161 494 DQ322155 X. mellisii (SUT074) Ratchaburi Province 155 155 167 477 DQ322156 X. mellisii (SUT192) **Trad Province** 184 155 161 500 DQ322157 X. psidii (SUT124) Nakhon Ratchasima 181 155 159 495 DQ322158 Province

Table 27. The length of ITS1-5.8S-ITS2 sequences of different species of Xylaria,

\* The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand.

<b>Table 27.</b> (	Continued).
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Species	Location/Source	ITS1 (bp)	5.8S (bp)	ITS2 (bp)	Total (bp)	GenBank accession number
X. psidii (SUT125)	Nakhon Ratchasima Province	180	155	160	495	DQ322159
X. schweinitzii (SUT201)	Trad Province	151	155	156	462	DQ322160
X. schweinitzii (ST2349)	RFD*	151	155	156	462	DQ322161
<i>Xylaria</i> species 2 (SUT127)	Nakhon Ratchasima Province	174	155	158	487	DQ322162
<i>Xylaria</i> species 2 (SUT130)	Nakhon Ratchasima Province	174	155	157	486	DQ322163
<i>Xylaria</i> species 2 (SUT195)	Trad Province	174	155	157	486	DQ322164
Xylaria sp. nov. (SUT027)	Ratchaburi Province	178	155	149	482	DQ322165
Xylaria sp. nov. (SUT155)	Yasothorn Province	178	155	149	482	DQ322166
Xylaria sp. nov. (SUT198)	Trad Province	178	155	149	482	DQ322167
Xylaria sp. nov. (SUT200)	Trad Province	178	155	149	482	DQ322168
<i>Xylaria</i> taxonomic species 1 (SUT207)	Trad Province	176	155	155	486	DQ322169
Kretzschmaria sp. (ST2325)	RFD*	176	155	196	527	DQ322093
Nemania sp. (SUT258)	Trad Province	186	155	156	497	DQ322094
Biscogniauxia sp. nov. (SUT290)	Kanchanaburi Province	215	155	148	518	DQ322095

\* The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand.

When the amplified ITS1-5.8S-ITS2 fragments (451 to 775

bp) were sequenced, the highest variation was found in the ITS1 region ranging from 151 to 465 bp. But the 5.8S region was highly constant at 155 bp. The ITS2 region ranging from 148 to 165 bp, was slightly different. Two isolates of *X. anisopleura* (SUT196 and ST2329) exhibited extremely long ITS1 region, 465 bp, as found in some species of *Hypoxylon* sect. *Annulata*, which was described in section 4.4.4.1, whereas one isolate of *X. anisopleura* (SUT205) exhibited only 141 bp for its ITS1

region. Therefore, the ITS1-5.8S-ITS2 sequences of three *X. anisopleura* isolates were searched for repeated sequence motif by using STAR software (Delgrange and Rivals, 2004) and no repeated sequences were observed. However, their morphological characteristics were similar except for the stromatal form as shown in the Figure 47. More collections of specimens were required for a better understanding of genetic variation within this taxon.

The phylogenetic trees of *Xylaria* were constructed based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method by the PHYLIP program (Felsenstein, 1995), and the maximum parsimony method by the PAUP (Swofford, 2000). The ITS1-5.8S-ITS2 sequences of related *Xylaria* species from the GenBank database were also included. The tree contained four major clades (Figure 97 and Appendix 9D). Clade I consisted only one taxon *X. beccari* (SUT092), which was separated from other clades. Clade II contained twelve species *X. curta* (ST2382), *X. enteroteuca* (AF163033), *X. cornu-damae* (AF163031), *X. castorea* (AF163030), *X. acuta* (AF163026), *X. longipes* (AF163039), *X. floriana* (AF163034), *X. ianthino-velutina* (SUT123), *Xylaria* taxonomic species 1 (SUT027), *X. badia* (SUT032, SUT076, SUT142, and ST2417), *Xylaria* sp. nov. (SUT027, SUT155, SUT198, SUT200, and ST2298), and *Xylaria* species 2 (SUT127, SUT130, and SUT195). Each species in this clade was separated clearly from each other.



**Figure 97.** Phylogenetic tree of *Xylaria* based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replications.

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Clade III consisted of *X. polymorpha* (AF163042), *X. cubensis* (AY787733, SUT090, ST2027, and ST2326). All species in this clade had large stromata and hard tissue. Although three isolates of *X. cubensis* examined were placed as sister branches to *X. cubensis* (AY787733) from the GenBank database they showed high variation within the species. The ascospore size of *X. cubensis* (SUT090), (6.3-)7.5-8.8 x 3.8-5  $\mu$ m, was smaller than *X. cubensis* (Mont.) Fr., 8-10.5 x 4-5  $\mu$ m, described by Rogers and Samuels (1987). It could be a different taxon, *X. cf. cubensis*. This might also be the variation within species. In addition, *X. cubensis* is found in various tropical, subtropical and temperate localities of the world. Thus, it was possible that the *X. cubensis* isolates collected in Thailand might be different from the temperate zone in genetic data.

Clade IV consisted of *X. apiculata* (AF163027), *X.* cf. *apiculata* (SUT203), *X.* cf. *maitandii* (SUT177), *X. bambusicola* (SUT129, SUT138, and SUT139), *X.* cf. *juruensis* (SUT088 and SUT140), *X. arbuscula* var. *microspora* (SUT192 and ST2372), *X. mali* (AF163040), *X. hypoxylon* (AF163037), *X. grammica* (ST2348 and ST2363), *X. arbuscula* (ST and ST), *X. brachiata* (SUT078), *X.* cf. *multiplex* (SUT028), *X. mellisii* (SUT074), and *X. psidii* (SUT124 and SUT125). Three isolates of *X. bambusicola* examined were identical and very close to *X.* cf. *maitlandii* (SUT177) and *X.* cf. *apiculata* (SUT203).

Moreover, the host preference of *Xylaria* seems to be off limited taxonomic value in this analysis.

# 4.4.6 Phylogenetic analysis of xylariaceous fungi based on ITS1-5.8S-ITS2 sequences

The ITS1-5.8S-ITS2 sequence of each species of the Xylariaceae examined was aligned and the phylogenetic trees were constructed by using the neighbour-joining method by the PHYLIP program (Felsenstein, 1995), and the maximum parsimony method by the PAUP (Swofford, 2000) (Figures 98 and Appendix 10D). The tree constructed by the neighbour-joining was divided into three clades.

The species representatives of genera *Nemania*, *Astrocystis*, *Kretzschmaria*, *Rosellinia* were placed in Clade I, which demonstrated the close relationship between those genera.

Three different species of *Biscogniauxia* (SUT290, AF201706, and AJ390411) and *C. tinctor* isolates were grouped together (Figure 98 and Appendix 9D). The relationship of both genera reflected the nature of their bipartite stromata which differentiated them from species of *Hypoxylon* sensu Miller (Miller, 1960) and supported the current concept of *Hypoxylon* sensu Ju and Rogers (Ju and Rogers, 1996). Surprisingly, one species of *C. selangorensis* was placed to clade I. this might be the result of the presence of short repeated sequences in ITS1 region as described previously in section 4.4.2.2. However, more collections in *C. selangorensis* were required to obtain more molecular data, which could be used to explain the reliable relationship of this taxon.



**Figure 98.** Phylogenetic tree of xylariaceous fungi based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Diatrype disciformis* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replications.

Both species of *D. concentrica* and *D. eschscholzii* were in the same group, and placed as a sister branch of the *Hypoxylon* group in clade II. *Daldinia* and *Hypoxylon* were close as previously indicated by Bull (1791) although *Daldinia* had already been separated from *Hypoxylon* on the basis of alternating different stromatal anatomy of ring zones (Ju, Rogers, and San Martín, 1997; Stadler *et al.*, 2004).

Most species of *Hypoxylon* from both sections *Annulata* and *Hypoxylon* were placed in clade II except for *H. subgilvum* var. *microsporum* (SUT108) which was placed in clade III. This result exhibited the strong relationship within this genus and the similar finding for most species in *Xylaria*. They were mainly placed in clade I except for *X. cubensis* (SUT090) and *X. mellisii* (SUT074) which were placed in clade II and III respectively.

Therefore, all of the ITS1-5.8S-ITS2 sequences results and their relationships analyzed by using the phylogenetic trees proved to be valuable for taxonomic investigation from a molecular point of view as well as for developing a DNA sequence database. Additionally, this molecular data would be useful for the designation of specific primers and for the development of specific probes for the detection of species of certain *Xylaria* in environmental situations.

## **CHAPTER V**

## **CONCLUSION AND FUTURE PERSPECTIVE**

Species identification and classification of selected members of the xylariaceous fungi based on their molecular data were studied for resolving undescribed species relied on morphological and cultural characteristics. Three hundreds and thirty eight xylariaceous specimens were collected from natural habitats of 14 localities in different 11 provinces in Thailand. The specimens were identified and classified into species level. The high numbers of collected specimens belonged to genera *Hypoxylon* and *Xylaria* respectively. Both genera were also wide distribution, and found to reveal high variation in their morphological characteristics whereas the other xylariaceous genera were rarely represented especially *Astrocystis*.

Four xylariaceous isolates, *X. anisopleura* (ST2329), *Xylaria* sp. (ST2372), *X. cubensis* (ST2326), and *X. grammica* (ST2348), were selected for the study of secondary metabolite profiles using TLC comparing to xylariaceous endophytes. The profiles of secondary metabolites extracted from 100 mL cultural broth (2% malt extract broth containing 6% glucose) of the four isolates were similar, and did not exhibit any differences among species. Therefore, an isolate, *Xylaria cubensis* (ST2326), was cultured in 1-L cultural broth. Its secondary metabolites were extracted and analyzed by TLC method then compared to nine isolates of xylariaceous endophytes (Ruchikachorn, 2005). Each isolate had its different secondary metabolite profile analyzed by R<sub>f</sub> values. Although this technique is very useful to classify the

xylariaceous endophytes, it was time-consuming. Also, it needed high concentration of metabolites obtained from large volume of fungal culture for extraction.

One hundred and sixty nine representatives of xylariaceous fungi were investigated in their nucleotide sequences of 18S rDNA and/or the internal transcribed spacer (ITS) 1 and 2 regions including 5.8S rDNA. These nucleotide sequences were then compared to sequences from thirty eight reference specimens. It was found that 18S rDNA sequences of Astrocystis and Rosellinia which are very closely related genera according to their morphological characteristics were approximately 2,056 and 2,210 bp respectively. Nucleotide sequence of A. mirabilis (SUT056) exhibited 70.3% identity to Rosellinia sp. ST2310 and 68.7% identity to R. necatrix from GenBank database accession number AB014044. The results of ITS1-5.8S-ITS2 sequence analysis showed approximately 515 and 493 bp of A. mirabilis (SUT051 and SUT056) and Rosellinia sp. ST2310 respectively. ITS sequence comparison among both species ranged from 65.9% to 70.6% identity. However, molecular data of 18S rDNA and ITS1-5.8S-ITS2 sequences demonstrated the dissimilarity between Astrocystis and Rosellinia, which confirmed the opinion of Petrini (1993) and Whalley (1996) and disagreed with Ju and Rogers (1990) who combined Astrocystis with Rosellinia.

*Camillea tinctor*, which is the common species of *Camillea* found in Thailand were studied and compared to *C. selangorensis*. Three isolates of *C. tinctor* (SUT161, SUT260, and ST2321) and a reference specimen of *C. selangorensis* (KS15) were investigated on ITS1-5.8S-ITS2 sequences and their sizes ranged from 496 to 529 bp. ITS sequence comparison revealed the high variation within ITS1 region and it was found that the nucleotide repeated in tandem from three to five times, which might

caused by unequal crossing over or failures in the replication of the DNA. This result agreed with the previous report of the tandem repeated sequence found in Xylariales (Platas *et al.*, 2001). The phylogenetic tree of *Camillea* exhibited the separation of *C*. *selangorensis* and *C. tinctor* from each other but all *C. tinctor* examined were grouped together with *C. tinctor* sequences from GenBank database accession numbers AJ39041 and AJ39042 respectively.

*Daldinia eschscholzii* and *D. concentrica* were recently examined and five new species recognised by Stadler *et al.* (2004) based on anamorph characteristics and perispore ornamentation. In addition, some *Daldinia* collections could not be cultured and this caused problems in identification. Therefore, ITS1-5.8S-ITS2 sequences of seven *D. eschscholzii* representatives and two anamorphic isolates of *D. concentrica* (L1 and L2) were investigated. The sizes ranging from 479 to 499 bp were found. ITS sequence comparison and phylogenetic analysis of *Daldinia* examined including twenty sequences of *Daldinia* available from GenBank database indicated that all seven isolates of *D. eschscholzii* are the same species, which separated clearly from *D. concentrica* (L1 and L2).

Seventy nine isolates of *Hypoxylon* species from both sect. *Annulata* and sect. *Hypoxylon* were studied on ITS1-5.8S-ITS2 sequences. The sizes of ITS sequences varied from 445 to 906 bp. Most *Hypoxylon* sect. *Annulata*, *H. stygium*, *H. atroroseum*, *H.* cf. *stygium* (SUT231), *H. urceolatum*, and *Hypoxylon* taxonomic species 1, exhibited extremely long sequences in the ITS1 region. The whole ITS sequence alignment revealed the greatest variation in ITS1 region whereas 5.8S and ITS2 regions were more conserved. The phylogenetic tree showed clearly relationships of *Hypoxylon* species and could be used to solve the morphological

taxonomic problems.

Fifty nine isolates of *Xylaria* species and three isolates belonged to *Biscogniauxia* sp. (SUT290), *Kretzschmaria* sp. (ST2325), and *Nemania* sp. (SUT258), were investigated on ITS1-5.8S-ITS2 sequences. The sizes of ITS sequences ranged from 451 to 775 bp. Alignments of the *Xylaria* species sequences exhibited the greatest variation in the ITS1 regions whilst the 5.8S sequences gave approximately 99% similarity for all isolates tested. The phylogenetic tree showed clear separation of each species.

Therefore, these molecular data showed clearly relationships within xylariaceous species examined and also could be used to confirm results of the finding of new species. From this study, the xylariaceous fungi were identified as belonging to nine genera; *Astrocystis, Biscogniauxia, Camillea, Daldinia, Hypoxylon, Kretzschmaria, Nemania, Rosellinia* and *Xylaria*, and were represented by fifty nine species, including seven new species, *Hypoxylon kanchanapisekii* sp. nov., *Hypoxylon sublenormandii* sp. nov., *Hypoxylon suranareei* sp. nov., *Hypoxylon* taxonomic species 1 sp. nov., *Xylaria* species 2, *Xylaria* sp. nov., *Biscogniauxia* sp. nov.

The molecular data results from this study are valuable for the creation of DNA sequence database of the xylariaceous fungi found in Thailand. These nucleotide sequences can be used to design specific primers and DNA probes for certain species especially xylariaceous endophytes, which are difficult to identify. In addition, molecular data will be very useful for explaining the evolutionary and genetic variation of xylariaceous fungi found in Thailand comparing to other fungi form over the world.

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# **APPENDIX** A

# FUNGAL MEDIA AND REAGENTS

## 1. Reagents and medium used for morphological taxonomic study

#### 1.1 Potato Dextrose Agar (PDA)

Potato	300.00	g
Dextrose	40.00	g
Agar	15.00	g

Potato slices were boiled in 1000-mL distilled water for 30 min and filtrated. The potato solution was then mixed with dextrose and agar, and adjusted the volume to 1,000 mL with distilled water. The medium was sterilized by autoclaving for 10 minutes at 121°C, 15 lb/square inches after preparation.

#### 1.2 Melzer's reagent

Chloral hydrate	100.00	g
Potassium iodine	5.00	g
Iodine	5.00	g

The ingredients were dissolved and adjusted the volume to 100 mL with distilled water. The reagent was stored in dark bottle at room temperature.

### 1.3 10% KOH

Potassium hydroxide	10.00	g
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The ingredient was dissolved and adjusted the volume to 100 mL with distilled water.

## 2. Media used for chemical taxonomic study

The media were sterilized by autoclaving for 10 min at 121°C, 15 lb/square inches after preparation.

2.1	Yeast Extract Sucrose Agar (YES)		
	Yeast Extract	20.00	g
	MgSO <sub>4</sub> .5H <sub>2</sub> O	0.50	g
	Agar	15.00	g

The ingredients were dissolved and adjusted the volume to 1,000 mL with distilled water.

#### 2.2 2% Malt Extract Broth containing 6% glucose

Malt Extract Broth	20.00	g
Glucose	60.00	g

The ingredients were dissolved and adjusted the volume to 1,000 mL with distilled water.

## 3 Chemicals and reagents used for nucleic acid study

#### 3.1 Lysis buffer

Tris Base	6.06	g
EDTA $(C_{10}H_{14}N_2O_8Na_2.2H_2O)$	18.61	g
Sodium dodecylsulfate (SDS)	30.00	g
2-Mercaptoethanol	10.00	mL

The ingredients were dissolved and adjusted the volume to 1,000 mL with deionized water. Then, the solution was sterilized by autoclaving for 10 min at 121°C, 15 lb/square inches after preparation.

#### 3.2 Tris-EDTA (TE) Buffer

Tris Base	1.21	g
		$\mathcal{O}$

EDTA (
$$C_{10}H_{14}N_2O_8Na_2.2H_2O$$
) 0.37 g

The ingredients were dissolved and adjusted the volume to 1,000 mL with deionized water. Then, the solution was sterilized by autoclaving for 10 min at 121°C, 15 lb/square inches after preparation.

#### 3.3 Sodium acetate (3.0 M)

The chemical was dissolved in deionized water, adjusted to pH 5.2 with glacial acetic acid, and adjusted the volume to 100 mL with deionized water. Then, the solution was sterilized by autoclaving for 10 min at 121°C, 15 lb/square inches after preparation.

#### 3.4 RNAase (10 mg/mL)

RNAase 10.00 mg

The RNAase was dissolved in 10 mM Tris-HCl (pH 7.5), 15 mM NaCl and stored at -20°C.

3.5 Tris-borate (TBE) buffer (5X)		
Tris Base	54.00	g
Boric acid	27.50	g
EDTA (C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> O <sub>8</sub> Na <sub>2</sub> .2H <sub>2</sub> O)	0.37	g

The ingredients were dissolved and adjusted the volume to 1,000 mL with deionized water.

### 3.6 Gel loading buffer (6X)

Bromophenol blue	25.00	g
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The dye was dissolved and adjusted the volume to 10 mL with 40% sucrose in water.

#### 3.7 Ethidium bromide (10 mg/mL)

Ethidium bromide (Sigma)	1.00	g
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The chemical was dissolved and adjusted the volume to 10 mL with sterilized deionized water.

# **APPENDIX B**

# LOCATIONS AND DETAILS OF XYLARIACEOUS

# **COLLECTIONS**

Locations of xylariaceous collections and details of their morphological

characteristics were given in Tables 1B and 2B respectively.

Code	Location	Date
SUT001 - SUT007	Phu Luang, Nakhon Ratchasima Province	28 July 2003
SUT008 - SUT012	Nong Rawieng, Nakhon Ratchasima Province	9 August 2003
SUT013 - SUT021	Burirum Province	24 August 2003
SUT022 - SUT025	Chaiyaphum Province	22 August 2003
SUT026 - SUT076	Ratchaburi Province	28 August 2003
SUT077 - SUT082	Nong Rawieng, Nakhon Ratchasima Province	1 September 2003
SUT083 - SUT084	Bangkok	5 September 2003
SUT085 - SUT086	Yasothon Province	6 September 2003
SUT087 - SUT116	Songkhla Province	8 September 2003
SUT117 - SUT122	Petchaboon Province	10 September 2003
SUT123 - SUT152	Suranaree University of Technology, Nakhon	20-25 September 2003
	Ratchasima Province	
SUT153 - SUT169	Yasothon Province	15 November 2003
SUT170 - SUT191	Suranaree University of Technology, Nakhon	17 November 2003
	Ratchasima Province	
SUT192 - SUT268	Trad Province	19 November 2003
SUT269 - SUT271	Chiang Rai Province	10 December 2003
SUT272 - SUT323	Kanchanaburi Province	14 December 2003
SUT324 - SUT327	Chiang Rai Province	25 January 2003
SUT328 - SUT334	Chiang Mai Province	16 June 2002
SUT335 - SUT338	Nakhon Ratchasima Province	20 July 2002

**Table 1B.** Locations and collecting dates of xylariaceous collections.

Code	Species	Stromatal colour	KOH extracted pigments	Ascospore size (µn
SUT001	H. purpureonitens	Blackish with reddish brown	Purple	8.8-10x2.5-5
SUT002	H. purpureonitens	Blackish with reddish brown	Purple	8.8-11.5x2.5-5
SUT003	H. purpureonitens	Blackish with reddish brown	Purple	NF
SUT004	H. purpureonitens	Blackish with reddish brown	Purple	3.8-5x7.5-10
SUT005	H. purpureonitens	Blackish with reddish brown	Purple	3.8-5x7.5-10
SUT005	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	(7.5)8.8-10x3.8-5
SUT000	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	8.8-10x3.8-5
SUT007	Hypoxylon taxonomic	Black	Greenish olivaceous	0.0 10x5.0 5 NF
	species 1 sp. nov.			
SUT009	H. atroroseum	Brown vinaceous or chestnut	Greenish olivaceous	6.3-8.8x2.5-3.8
SUT010	H. atroroseum	Brown vinaceous or chestnut	Greenish olivaceous	5-6.3x2.5
SUT011	<i>Xylaria</i> sp.	Brownish black	Colorless	NF
SUT012	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	(7.5)8.8-10x3.8-5
SUT013	Daldinia eschscholzii	Brown vinaceous	Purple	11.3-13.8x5-6.3
SUT014	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	8.8-10x3.8-5
SUT015	Hypoxylon taxonomic species 1 sp. nov.	Black with shinny	Greenish olivaceous	NF
SUT016	H. lenormandii	Grayish sepia	Red	10-12.5x5
SUT017	H. cf. ferrugineum	Hazel	Orange	12.5-15(17.5)x5-7.
SUT018	H. lenormandii	Grayish sepia	Red	12.5-15x5-6.3
SUT019	Eutypa sp.	- ··· ··· ··· ··· ··· ···		-
SUT020	H. cf. perforatum	Gravish sepia	Yellowish brown	NF
SUT020	H. monticulosum	Brownish vinaceous to black	Colorless	(7.5)8.8-10x5-6.3
SUT021	H. lenormandii var.	Blackish brown	Red	5-7x2.5-3.8
CLIT022	microspora	Die shish harmanish ashite fain sa	01:	7510-295
SUT023	H. truncatum	Blackish brown with white fringe	Olivaceous	7.5-10x3.8-5
SUT024	H. stygium	Blackish with reddish brown	Greenish olivaceous	3.8-6.3x2.5-3.8
SUT025	H. bovei var. microspora	Black	Greenish olivaceous	7.5-10x3.8-5
SUT026	X. badia	Silvery brown	Colorless	10-12x3.8
SUT027	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	8.8-10x3.8-5
SUT028	X. cf. multiplex	Blackish with light brown with peeling layer	Colorless	11.3-13.8x3.8-5
SUT029	X. muscula	White with black ostioles	Colorless	NF
SUT030	<i>Xylaria</i> sp. nov.	Black	Colorless	NF
SUT031	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	8.8-10x3.8-5
SUT031	Xytaria sp. nov. X. badia	Silvery brown	Colorless	9.8-12x3.8-5
		•	Colorless	
SUT033	<i>Xylaria</i> sp. nov.	Dark brown to black		(7.5)8.8-10x3.8-5
SUT034	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	8.8-10x3.8-5
SUT035	X. cf. juruensis	Blackish with light brown with peeling layer	Colorless	(10)11.3-13.8x3.8-
SUT036	X. ianthino-velutina	Black	Colorless	(7.5)8.8-10x3.8
SUT037	D. eschscholzii	Brown vinaceous	Purple	10-12.5x5-6.3
SUT038	D. eschscholzii	Brown vinaceous	Purple	10-13.8x3.8-5
SUT039	D. eschscholzii	Brown vinaceous	Purple	11.3-12.5x5-6.3
SUT040	H. fendleri	Brown vinaceous	Orange	(5)7.5-10x3.75
SUT041	H. investiens	Brown vinaceous	Dull green	7.3-8.8x2.5-3.8
SUT042	H. monticulosum	Brownish vinaceous to black	Colorless	6.3-7.5x2.5-3
SUT043	H. monticulosum	Brownish black	Purple	6.3-7.5x2.5-3.8
SUT045	H. monticulosum	Brownish black	Purple	6.3-7.5x2.5-3.8
SUT044	H. anthochroum	Brown vinaceous or chestnut	Dull green	8.8-11.25x3.8-5
SUT045	H. sublenormandii sp.	Reddish brown	Reddish brown	(8.8)12.5-15x5-6.2
SUT047	nov. A. mirabilis	Black	Colorless	10-13x5
SUT047 SUT048	A. mirabilis A. mirabilis		Colorless	
		Black		10-12.5x3.8-5
SUT049	A. mirabilis	Black	Colorless	(8.8)9-12.5x3.8-5
SUT050	Not Xylariaceae	-	Colorless	-
SUT051	A. mirabilis	Black	Colorless	(8.8)9-13.5x3.8-5
SUT052	Not Xylariaceae	-	Colorless	-
SUT053	Not Xylariaceae	-	Colorless	-
SUT054	A. mirabilis	Black	Colorless	NF
SUT055	A. mirabilis	Black	Colorless	10-13.8x5
SUT056	A. mirabilis	Black	Colorless	11.2-13.8x5
SUT057	A. mirabilis	Black	Colorless	10-13.8x3.8-5

 Table 2B.
 More details of xylariaceous collections.

 $\overline{NF} = Not found.$ 

Code	Species	Stromatal colour	KOH extracted pigments	Ascospore size (µm)
SUT059	H. monticulosum	Brownish vinaceous to black	Purple	6.3-7.5x2.5
SUT060	H. monticulosum	Brownish vinaceous to black	Purple	7.5-8.8x2.5-3
SUT061	H. cf. fendleri	Brownish vinaceous	Orange	10-12.5x3.8-5
SUT062	H. haematostroma	Orange red or rust	Orange red	NF
SUT062	H. investiens	Brownish vinaceous	Dull green	(5)7.5-8.8x(2.5)3.8-5
		Orange red or rust	2	(5)7.5-8.8x(2.5)5.8-1 NF
SUT064	H. haematostroma	-	Orange red	
SUT065	H. lenormandii	Grayish sepia	Red	(8.8)10-12.5x3.8-5
SUT066	<i>H. kanchanabhisakii</i> sp. nov.	Dull reddish brown	Reddish brown	(8.8)10-11.3x3.8-5
SUT067	<i>H. kanchanabhisakii</i> sp. nov.	Dull reddish brown	Reddish brown	(7.5)10-11.3x3.8
SUT068	<i>H. kanchanabhisakii</i> sp. nov.	Dull reddish brown	Reddish brown	10-11.3(13)x(2.5) 3.8-5
SUT069	H. kanchanabhisakii sp. nov.	Dull reddish brown	Reddish brown	10-11.3x3.8-5
SUT070	H. cf. ferrugineum	Brown vinaceous	Orange	(5)16.5-17.5x6.6-7.5
SUT071	H. kanchanabhisakii sp. nov.	Dull reddish brown	Reddish brown	10-11.3(12.5)x3.8-5
SUT072	<i>H. kanchanabhisakii</i> sp. nov.	Dull reddish brown	Reddish brown	(12.5)11.3-10x3.8-5
SUT073	H. monticulosum	Brownish vinaceous to black	Colorless	7.5-8.8x3.8
SUT074	X. mellisii	Blackish with gray to brown outer peeling layer	Colorless	NF
SUT075	<i>Xylaria</i> taxonomic species 1	Dark brown to black	Colorless	12.5-15(16.5)x5-6.3
SUT076	X. badia	Silvery brown	Colorless	9.8-12x3.8-5
SUT077	X. psidii	Black	Colorless	8.8-3.8-5
SUT078	X. brachiata	Brown outer peeling layer	Colorless	(8.8)10-
				11.3(12.5)x3.8-5
SUT079	H. cf. archeri	Blackish brown	Hazel	8.8-10x3.8-5
SUT080	H. monticulosum	Brownish vinaceous to black	Colorless	7.5-8.8x3.8-5
SUT081	Hypoxylon taxonomic species 1 sp. nov.	Black with shinny	Greenish olivaceous	7.5-10x3.8-5
SUT082	Hypoxylon taxonomic species 2	Brown vinaceous	Yellowish brown	(8.8)11.3- 12.5(17.5)x5-7.5
SUT083	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	7.5-8.8x3.8
SUT084	D. eschscholzii	Brown vinaceous	Purple	10-13.8 x 5-7.5
SUT085	D. eschscholzii	Brown vinaceous	Purple	10-13.5 x 3.8-6.3
SUT086	D. eschscholzii	Brown vinaceous	Purple	9.5-14x3.8-6.3
SUT087	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	7.5-8.8(10)x3.8
SUT087	X. cf. juruensis	Blackish with light brown with peeling layer	Colorless	12.5-15x3.8-5
SUT089	X. cubensis	Bronze becoming dark with age	Colorless	7.5-8.8x3.8
	X. cubensis X. cubensis		Colorless	
SUT090		Bronze becoming dark with age		7.5-8.8x3.8
SUT091	X. ianthino-velutina	Black	Colorless	(7.5)8.8- 10(12.5)x3.8-5
SUT092	X. beccari	Brownish black	Colorless	(5)6.3-7.5x2.5
SUT093	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	(7.5)8.8-10x3.8-5
SUT094	H. monticulosum	Brownish vinaceous to black	Purple	7.5-8.8x3.8
SUT095	H. subgilvum var. microsporum	Dark brick	Orange	(5)6.5-8.8x2.5
SUT096	B. capnodes	Black	Colorless	NF
SUT097	B. capnodes	Black	Colorless	10-12.5(-13.8)x6.3- 7.5
SUT098	H. urceolatum	Black	Colorless	10-12.5x2.5-5
SUT099	C. tinctor	Black	Colorless	NF
SUT100	H. purpureonitens	Blackish with reddish brown	Purple	7.5-10x2.5-5
SUT101	Kretzschmaria sp.	Black	Colorless	8.8-10x3.8-5
SUT102	R. procera	Black	Colorless	(70)100- 135(162.5)x8.8-15
SUT103	H. cf. archeri	Blackish brown	Hazel	8.8-10x2.5-5
SUT103 SUT104	H. subgilvum var.	Dark brick	Orange	8.8-10x2.5-5 (3.8)5-7.5x2.5-3.8
01.001.05	microsporum	D1 1'11		0.0.10.00.5
SUT105	H. cf. archeri	Blackish brown	Hazel	8.8-10x3.8-5
SUT106	H. monticulosum	Brownish vinaceous to black	Purple	6.3-7.5x3.8

Code	Species	Stromatal colour	KOH extracted pigments	Ascospore size (µm)
SUT107	H. cf. fendleri	Brownish vinaceous	Orange	NF
SUT108	H. subgilvum var. microsporum	Dark brick	Orange	(2.5)6.3-8.8x2.5-3
SUT109	R. procera	Black	Colorless	(77.5)90-117.5x10- 12.5
SUT110	Nemania sp.	Black	Colorless	NF
SUT111	Nemania sp.	Black	Colorless	NF
SUT112	H. cf. archeri	Blackish brown	Hazel	8-10x3.8-5
SUT113	R. procera	Black	Colorless	(70)100-112.5x10- 12.5
SUT114	R. procera	Black	Colorless	(65)95-125x10-15
SUT115	H. monticulosum	Brownish vinaceous to black	Purple	6.3-7.5x2.5-3.8
SUT116	H. monticulosum	Brownish vinaceous to black	Purple	(6.3)7.5- 8.8(11.3)x3.8
SUT117	X. scrupora	Yellowish brown to dark brown	Colorless	17.5- 21.3(22.5)x(5)6.3-7.5
SUT118	Xylaria sp. nov.	Dark brown to black	Colorless	10-11.5x3.2-3.7
SUT119	X. anisopleura	Dark brown to dull black	Colorless	8.8-10x3.8-5
SUT120	H. cf. fendleri	Brownish vinaceous	Orange	(8.8)10-12.5x3.8-5
SUT121	H. anthochroum	Brown vinaceous or chestnut	Olivaceous	10.8-13(14)x4-6
SUT122	Biscogniauxia sp.	Black	Colorless	9.2-11.9 x 5.4-6.7
SUT123	X. ianthino-velutina	Black	Colorless	7.5-8.8(10)x3.8-5
SUT124	X. psidii	Black	Colorless	(7.5)8.8-10x3.8-5
SUT125	X. psidii	Black	Colorless	(7.5)8.8-10x3.8-5
SUT126	X. psidii	Black	Colorless	7.5-8.8(10)x3.8-5
SUT127	Xylaria species 2	Dark brown to black	Colorless	(8.8)10- 11.3(12.5)x2.5-3.8
SUT128	Xylaria species 2	Dark brown to black	Colorless	8.8-10x3.8-5
SUT129	X. juruensis var. microspora	Blackish with brown peeling outer layer	Colorless	(7.5)10-2.5x3.8
SUT130	Xylaria species 2	Dark brown to black	Colorless	8.8-10x3.8-5
SUT131	Xylaria sp. nov.	Dark brown to black	Colorless	10-11.3(13.8)x3.2- 3.7
SUT132	Xylaria species 2	Dark brown to black	Colorless	8.8-10x3.8-5
SUT133	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	8.8-10x3.8-5
SUT134	Xylaria species 2	Dark brown to black	Colorless	10-11.3x2.5-3.8
SUT135	Xylaria sp. (Immature)	Dark brown to black	Colorless	NF
SUT136	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	(7.5)8.8-10x3.8-5
SUT137	X. juruensis var. microspora	Blackish with brown peeling outer layer	Colorless	(7.5)10-11.3x3.8-5
SUT138	X. juruensis var. microspora	Blackish with brown peeling outer layer	Colorless	8.8-10x3.8-5
SUT139	X. juruensis var. microspora	Blackish with brown peeling outer layer	Colorless	8.8-10x3.8-5
SUT140	X. cf. juruensis	Blackish with brown peeling outer layer	Colorless	12.5-13.8(15)x3.8-5
SUT141	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	(7.5)8.8-10x2.5-3.8
SUT142	X. badia	Silvery brown	Colorless	(8.8)10-11.3x3.8
SUT143	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	8.8-10x3.8-5
SUT144	H. lenormandii	Grayish sepia	Red	10-11.3x3.8-5
SUT145	H. cf. fendleri	Brownish vinaceous	Orange	8.8-11.3x3.8-5
SUT146	H. rubiginosum	Brown vinaceous	Yellowish brown	10-11.2x5-6.3
SUT147	H. lenormandii	Grayish sepia	Red	10-12.5x3.8-5
SUT148	H. rubiginosum	Brown vinaceous	Yellowish brown	10-11.3x3.8-5
SUT149	H. rubiginosum	Brown vinaceous	Yellowish brown	11.3-12.5x5-6.3
SUT150	H. sublenormandii sp. nov.	Reddish brown	Orange	8.8-10x3.8
SUT151	H. lenormandii	Grayish sepia	Red	11.3-12.5x3.8-5
SUT151 SUT152	H. cf. fendleri	Brown	Orange	8.8-10x3.8-5
SUT152 SUT153	H. anthochroum	Brown vinaceous or chestnut	Dull green	NF
	H. anthochroum	Brown vinaceous or chestnut	Dull green	10-12.5x3.8-5
SUT154				

Code	Species	Stromatal colour	KOH extracted pigments	Ascospore size (µm)
SUT156	B. capnodes	Black	Colorless	12.5-15(18.8)x6.3-
	1			7.5
SUT157	H. rubiginosum	Brown vinaceous	Yellowish brown	10-11.3x3.8-5
SUT158	Hypoxylon taxonomic species 2	Dark brick to brown vinaceous	Yellowish brown	10-11.3x3.8-5
SUT159	H. cf. fendleri	Brownish vinaceous	Orange	(8.8)10-11.3(15)x3.8- 5
SUT160	H. purpureonitens	Blackish with reddish brown	Purple	10-11.3x3.8-5
SUT161	C. tinctor	Black	-	15-17.5x6.3-7.5
SUT162	H. cf. fendleri	Brownish vinaceous	Orange	8.8-10x3.8-5
SUT163	H. cf. fendleri	Brownish vinaceous	Orange	10-12.5x3.8-5
SUT164	H. haematostroma	Orange red or rust	Orange red	15-16.3x6.3-7.5
SUT165	H. cf. fendleri	Brownish vinaceous	Orange	10-12.5x3.8-5
SUT166	H. hypomiltum	Blackish with brown peeling outer layer	Yellowish brown	7.5-2.5x3.8
SUT167	H. purpureonitens	Blackish with reddish brown	Purple	(6.3)7.5-10x3.8
SUT168	D. eschscholzii	Brown vinaceous	Purple	10-12.5x5-6.3
SUT169	D. eschscholzii	Brown vinaceous	Purple	11.3-12.5x5-6.3
SUT170	X. cf. juruensis	Blackish with brown peeling outer	Colorless	11.3-12.5x3.8-5
	U	layer		
SUT171	Xylaria species 2	Dark brown to black	Colorless	10-11.3x2.5-3.8
SUT172	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	(6.3)7.5-8.8x3.8
SUT173	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	7.5-10x3.8-5
SUT174	X. cf. apiculata	Black	Colorless	7.5-8.8x3.8
SUT175	X. brachiata	Brown outer peeling layer	Colorless	10-12.5x3.8-5
SUT176	X. cf. apiculata	Black	Colorless	8.8-10x3.8-5
SUT177	X. maitlandii	Blackish with brown peeling outer layer	Colorless	8.8-10x3.8-5
SUT178	D. eschscholzii	Brown vinaceous	Purple	10-12.5(15)x5-6.3
SUT179	H. monticulosum	Brownish vinaceous to black	Colorless	7.5-8.8(10)x2.5
SUT180	H. lenormandii	Grayish sepia	Red	10-12.5x3.8-5
SUT181	H. lenormandii	Grayish sepia	Red	10-12.5x3.8-5
SUT182	H. suranarii sp. nov.	Dark brown to black	Yellowish orange	12.5-13.8x5-6.3
SUT183	H. suranarii sp. nov.	Dark brown to black	Yellowish orange	12.5-13.8x5-6.3
SUT184	H. suranarii sp. nov.	Dark brown to black	Yellowish orange	(10)12.5-13.8x5-6.3
SUT185	H. monticulosum	Brownish vinaceous to black	Purple	6.3-7.5(8.8)x2.5-3.8
SUT186	H. cf. fendleri	Brownish vinaceous	Orange	10-11.3x5-6.3
SUT187	H. trugodes	Sepia	Yellow	10-11.3x5-6.3
SUT188	Hypoxylon taxonomic species 1 sp. nov.	Black with shinny	Greenish olivaceous	7.5-8.8x2.5-3.8
SUT189	H. monticulosum	Brownish vinaceous to black	Purple	7.5-8.8x2.5-3.8
SUT190	H. cf. fendleri	Brownish vinaceous	Orange	6.3-7.5x2.5
SUT191	H. cf. fendleri	Brownish vinaceous	Orange	15-16.3x6.3-7.5
SUT192	X. mellisii	Black	Colorless	12.5-15x3.8-5
SUT193	X. cubensis	Bronze becoming dark with age	Colorless	7.5-8.8x3.8
SUT194	X. cubensis	Bronze becoming dark with age	Colorless	7.5-8.8x3.8
SUT195	Xylaria species 2	Dark brown to black	Colorless	10-11.3x2.5-3.8
SUT196	X. anisopleura	Dark brown to dull black	Colorless	(20)23.8- 25(27.5)x7.5-8.8
SUT197	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	6.3-7.5(8.8)x3.8
SUT198	Xylaria sp. nov.	Dark brown to black	Colorless	(6.5)7.5-8.8(10)x3.8
SUT198	X. cubensis	Bronze becoming dark with age	Colorless	(6.2)7.5-8.8x3.8
SUT200	Xylaria sp. nov.	Dark brown to black	Colorless	7.5-8.8x3.8
SUT200 SUT201	X. schweinitzii	Brownish black to dull black	Colorless	18.8-21.3x6.3-7.5
SUT201 SUT202	X. schweinign X. cubensis	Bronze becoming dark with age	Colorless	6.3-7.5x3.8
SUT202	Xylaria taxonomic	Black	Colorless	(7.5)8.8-10x3.8
SUT204	species 2 Xylaria taxonomic species 3	Black	Colorless	6.3-7.5x2.5-3.8
SUT205	X. anisopleura	Dark brown to dull black	Colorless	(17.5)18.8-2.3x6.3- 7.5
SUT206	X. telfairii	Pale yellow, clay-colored to orange brown	Colorless	17.5-20x5-6.3
SUT207	<i>Xylaria</i> taxonomic species 4	Copper- to bronze-colored to brown	Colorless	21.3-25x8.8-10

 $\overline{NF} = Not found.$ 

SUT208			pigments	Ascospore size (µm)		
01177000	X. anisopleura	Dark brown to dull black	Colorless	20-21.5x7.5-8		
SUT209	D. eschscholzii	Brown vinaceous	Purple	NF		
SUT210	B. capnodes	Black	Colorless	11.3-12.5x6.3-7.5		
SUT211	C. tinctor	Black	Colorless			
SUT212	B. capnodes	Black	Colorless	10-12.5(13.8)x6.3- 7.5		
SUT213	H. nitens	Black with shinny	Greenish olivaceous	7.5-9.5x3.8-5		
SUT214	H. atroroseum	Brown vinaceous or chestnut	Greenish olivaceous	3.8-5x1.5-2.5		
SUT215	H. rubiginosum	Reddish brown	Yellowish brown	8.8-10x3.8-5		
SUT216	H. moriforme	Black	Greenish olivaceous	7.5-8.8x3.8-5		
SUT217	H. rubiginosum	Reddish brown	Yellowish brown	(7.5)8.8-10x3.8-5		
SUT218	H. cf. perforatum	Brown vinaceous	Yellowish brown	8.8-10(11.3)x3.8-5		
SUT218 SUT219	H. atroroseum	Blackish brown	Greenish olivaceous	5-6.3x2.5		
SUT219 SUT220	H. moriforme	Black with shinny	Greenish olivaceous	7.5-8.8x3.8		
		5	Yellowish brown			
SUT221	H. rubiginosum	Reddish brown		7.5-8.8(10)x3.8		
SUT222	H. stygium	Blackish with reddish brown	Greenish olivaceous	5-6.3x2.5		
SUT223	H. duranii	Brown vinaceous or chestnut	Reddish brown	8.8-10x3.8-5		
SUT224	H. duranii	Brown vinaceous or chestnut	Reddish brown	8.8-10x3.8-5(6.3)		
SUT225	H. monticulosum	Brownish vinaceous to black	Purple	6.3-7.5x2.5(3.8)		
SUT226	H. stygium	Blackish with reddish brown	Greenish olivaceous	5-6.3x2.5		
SUT227	H. monticulosum	Brownish vinaceous to black	Colorless	6.3-7.5(8.8)x2.5-3.8		
SUT228	H. monticulosum	Brownish vinaceous to black	Colorless	NF		
SUT229	H. stygium	Blackish with reddish brown	Greenish olivaceous	5-6.3x2.5		
SUT230	H. stygium	Blackish with reddish brown	Greenish olivaceous	5-6.3x2.5		
SUT231	H. stygium	Blackish with reddish brown	Greenish olivaceous	5-6.3x2.5-3		
SUT232	H. monticulosum	Brownish vinaceous to black	Colorless	7.5-8.8x2.5-3.8		
SUT233	H. anthochroum	Brown vinaceous or chestnut	Dull green	10-11.3x3.8-5		
SUT234	H. subgilvum	Hazel to dark brick	Orange	8.8-10x3.8-5		
SUT235	H. monticulosum	Brownish vinaceous to black	Colorless	0.0 10x5.0 5 NF		
SUT235	<i>Hypoxylon</i> taxonomic species 1 sp. nov.	Black	Greenish olivaceous	7.2-9.8x3.1-4.4		
SUT237	H. cf. ferrugineum	Brown vinaceous to rusty brown	Orange	(12.5)13.4-17.8x5.3- 8.3		
SUT238	<i>Hypoxylon</i> taxonomic species 1 sp. nov.	Black with shinny	Greenish olivaceous	7.9-9.1x3.2-4.1		
SUT239	H. duranii	Brown vinaceous or chestnut	Reddish brown	8.5-10.8x4-5.6		
SUT240	H. anthochroum	Brown vinaceous or chestnut	Dull green	10.8-13(14)x4-6		
SUT241	<i>Hypoxylon</i> taxonomic species 1 sp. nov.	Black with shinny	Greenish olivaceous	7.5-8.9x2.8-4		
SUT242	H. bovei var. microspora	Black with shinny	Greenish olivaceous	7.3-9x3.1-4		
SUT243	H. stygium	Blackish with reddish brown	Greenish olivaceous	4.7-6.5x1.8-2.4		
SUT245	<i>Hypoxylon</i> taxonomic species 1 sp. nov.	Black with shinny	Greenish olivaceous	7.6-9.1x2.8-4.2		
SUT245	H. stygium	Blackish with reddish brown	Greenish olivaceous	5.5-6.4x1.7-2.4		
SUT246	<i>Hypoxylon</i> taxonomic species 1 sp. nov.	Black with shinny	Greenish olivaceous	7.3-8.7x3-4		
SUT247	H. stygium	Blackish with reddish brown	Greenish olivaceous	4.6-5.9x1.8-2.6		
SUT247 SUT248	H. duranii	Brown vinaceous or chestnut	Reddish brown	(6.7)8.1-9.5x4.3-5		
SUT248 SUT249	H. stygium	Blackish with reddish brown	Greenish olivaceous	(0.7)8.1-9.5x4.5-5 7.5-8.8x3.1-4.4		
SUT250	H. sublenormandii sp. nov.	Reddish brown	Reddish brown	7.5-8.8x5.1-4.4 8.9-11.3x3.4-4.7		
SUT251	<i>Hypoxylon</i> taxonomic species 1 sp. nov.	Black	Greenish olivaceous	7-8.2x3.4-4		
SUT252	H. duranii	Brown vinaceous or chestnut	Reddish brown	8.5-10.4x4.5-5.5		
SUT253	H. stygium	Blackish with reddish brown	Greenish olivaceous	4.8-5.9x1.8-2.2		
SUT254	H. duranii	Brown vinaceous or chestnut	Reddish brown	8.2-9x(3.9)4.4-5.4		
SUT255	<i>Hypoxylon</i> taxonomic species 1 sp. nov.	Black with shinny	Greenish olivaceous	6.6-8x3.4-4.6		
	H. brevisporum	Brown vinaceous or chestnut	Hazel	6.1-7.2x2.7-3.7		
SUT256		Blackish with reddish brown	Greenish olivaceous	5.6-6.3x2.1-2.8		
	H stvaium		Greenish Unvaceous	J.U-U.JAZ.1-Z.O		
SUT257	H. stygium Nemania sp		Colorless			
SUT257 SUT258	Nemania sp.	Black	Colorless Reddish brown	8.9-11.7x4.7-6		
SUT256 SUT257 SUT258 SUT259 SUT260			Colorless Reddish brown Colorless			

Code	Species	Stromatal colour	KOH extracted pigments	Ascospore size (µm)
SUT262	H. purpureonitens	Blackish with reddish brown	Purple	8.6-12.3x3.6-4.6
SUT263	H. anthochroum	Brown vinaceous or chestnut	Dull green	9.7-12.2x4-5.4
SUT264	H. monticulosum	Brownish vinaceous to black	Purple	5.8-7.2x2.4-4.1
UT265	H. monticulosum	Brownish vinaceous to black	Purple	6.8-9x2.8-3.9
UT266	H. monticulosum	Brownish vinaceous to black	Purple	6.9-8.5x3-3.6
UT267	H. monticulosum H. monticulosum	Brownish vinaceous to black	1	0.9-0.5X5-5.0 NF
			Purple	
UT268	D. eschscholzii	Brown vinaceous	Purple	10.8-13.2x5.8-6.6
UT269	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	8.9-10.3x3.9-5
UT270	X. cubensis	Bronze becoming dark with age	Colorless	7.5-8.8x3.8-5
UT271	X. cubensis	Bronze becoming dark with age	Colorless	(6.3)7.5-8.8x3.8-5
UT272	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	7.5-9x4.1-4.9
UT273	Xylaria sp. nov.	Dark brown to black	Colorless	7.1-9x3.6-4.8
UT274	<i>Xylaria</i> species 2	Dark brown to black	Colorless	9.5-12.1x3.4-4.5
UT275	<i>Xylaria</i> sp. nov.	Dark brown to black	Colorless	7.6-8.8x4-5
UT276	<i>Xylaria</i> taxonomic species 5	Dark brown to black	Colorless	12.5-14.7x4.5-6.2
UT277	X. cubensis	Bronze becoming dark with age	Colorless	7.5-9x3.8-5
UT278	D. eschscholzii	Brown vinaceous	Purple	10-12.3x4.7-6.1
UT279	H. cf. fendleri	Brownish vinaceous	Orange	NF
UT280	H. cf. fendleri	Brownish vinaceous	Orange	8.4-10.9x3.9-5
UT281	H. duranii	Brown vinaceous or chestnut	Reddish brown	9.7-11.7x4.5-5.5
UT282	H. sublenormandii sp. nov.	Reddish brown	Reddish brown	9-11.8x4.5-5.1
UT283	H. lenormandii	Gravish sepia	Red	9.5-11.9x4.6-5.9
			Reddish brown	
SUT284	H. duranii	Brown vinaceous or chestnut		9-10.9x4-5.3
UT285	Hypoxylon taxonomic species 1 sp. nov.	Black with shinny	Greenish olivaceous	6.1-9x2.3-4
SUT286	H. monticulosum	Brownish vinaceous to black	Purple	NF
UT287	H. monticulosum	Brownish vinaceous to black	Purple	6.8-8.4x3.3-4
UT288	H. nitens	Black with shinny	Greenish olivaceous	7.2-8.6x3.1-4.3
UT289	H. monticulosum	Brownish vinaceous to black	Colorless	NF
UT290	Biscogniauxia sp.	Black	Colorless	9.2-11.9x5.4-6.7
	ē 1			
UT291	H. cf. fendleri	Brownish vinaceous	Orange	8.8-10x3.8-5
UT292	H. haematostroma	Orange red or rust	Orange red	13-15.2x6.3-7.9
UT293	H. haematostroma	Orange red or rust	Orange red	15.6-17.9x7.1-8.6
UT294	H. rubiginosum	Brown vinaceous	Yellowish brown	8.8-11.3x5
SUT295	H. monticulosum	Brownish vinaceous to black	Purple	6.3-7.5x2.5-3.8
UT296	H. rubiginosum	Brown vinaceous	Yellowish brown	(8.8)10-11.3x5-6.3
UT297	H. rubiginosum	Brown vinaceous	Yellowish brown	8.8-10x3.8-5
UT298	H. nitens	Black with shinny	Greenish olivaceous	10-12.5x3.8-5
UT299	H. nitens	Black with shinny	Greenish olivaceous	10-11.3(12.5)x3.8-
u maga	TT 1 1			5(6.3)
SUT300	H. lenormandii	Grayish sepia	Red	8.8-10x2.5-3.8
SUT301	H. monticulosum	Brownish black to black	Colorless	6.3-7.5x3.8-5
UT302	H. monticulosum	Brownish black to black	Colorless	6.3-7.5x3.8
SUT303	<i>H. sublenormandii</i> sp. nov.	Brownish black to black	Reddish brown	8-10x3.8-5
SUT304	H. sublenormandii sp. nov.	Brownish black to black	Reddish brown	8-10x3.8-5
UT305	<i>H. sublenormandii</i> sp. nov.	Brownish black to black	Reddish brown	8-10x3.8-5
SUT306	H. duranii	Brown vinaceous or chestnut	Reddish brown	9-10.9x4-5.3
UT307	H. lenormandii	Grayish sepia	Red	8.8-10x2.5-3.8
UT308	H. cf. fendleri	Brownish vinaceous	Orange	8.8-10x2.3-5.8
			•	
UT309	X. badia	Silvery brown	Colorless	8.8-11.3x3.8-5
UT310	X. badia	Silvery brown	Colorless	8.8-11.3x3.8-5
UT311	H. sublenormandii sp. nov.	Reddish brown	Reddish brown	(10)11.3-13.8x5-7.5
UT312	H. lenormandii	Grayish sepia	Red	11.3-12.5x3.8-5
UT313	H. lenormandii	Grayish sepia	Red	8.8-11.3x3.8-5
UT314	H. fendleri	Brownish vinaceous	Orange	8.8-12.5x5-6.3
	U		•	
UT315	H. monticulosum	Brownish vinaceous to black	Purple	6.3-7.5x3.8-5
UT316	H. nitens	Black	Greenish olivaceous	10-12.5x3.8-5(6.3)
UT317	H. nitens	Black	Greenish olivaceous	10-12.5x3.8-5(6.3)
UT318	H. purpureonitens	Blackish with reddish brown	Purple	8.8-12.5x3.8-5

Code	Species	Stromatal colour	KOH extracted pigments	Ascospore size (µm)
SUT319	H. purpureonitens	Blackish with reddish brown	Purple	8.8-11.5x2.5-5
SUT320	H. rubiginosum	Brown vinaceous	Yellowish brown	8.8-10x3.8-5
SUT321	Xylaria sp. nov.	Dark brown to black	Colorless	8.9-10.5x3.8-5
SUT322	Xylaria sp. nov.	Dark brown to black	Colorless	8.9-10.5x3.8-5
SUT323	Xylaria species 2	Dark brown to black	Colorless	10-11.3x2.5-3.8
SUT324	X. cubensis	Bronze becoming dark with age	Colorless	7.5-8.8x3.8
SUT325	H. monticulosum	Brownish vinaceous to black	Purple	6.9-8.5x3-3.6
SUT326	D. eschscholzii	Brown vinaceous	Purple	10-13.5 x 3.8-6.3
SUT327	H. stygium	Blackish with reddish brown	Greenish olivaceous	7.5-8.8x3.1-4.4

# **APPENDIX C**

## NUCLEOTIDE SEQUENCE DATA

Nucleotide sequence results of 18S rDNA and ITS1-5.8S-ITS2 regions were presented in dendrogram for example in Figure 1C using ITS5 primer.

TG TCA A CA 1A C CA GA C G T TGC C TC G GCA G GC C GC G C GC C A C C TC TC TC A G G G G C G C G C G C G C G C C A C G C T C 70 C C GC C G GC 140 <sup>T</sup> T G A 200 210 TC TC TTGGTTC TGGCA TCGA 220 230 GAAGAAC GCAGC GAAA 250 260 270 280 290 300 A T G T GA A T T GCA GA A T TCA G T GA A T C GA A T C T T TGA AC GC A C A T T GC GC C C A T T an <mark>n</mark>a T 330 340 350 TC GA GC G TCA T T TCAAC C C T TAA GC C 310 TAGTGGGCA 360 370 380 T T A G T G T T G G G A G C C T A C G G A G A C G T A Gl TGCTGC 410 420 CGGA GTCG GtAC GCAC TC TA 390 CC 400 CAAA G TTA GT G 430 440 450 460 A C G TA G TAA T TA T C TA T C TC G C C T G T G A G C C G G A C C G G t C сста O TA G C T 460 470 G t C C C T G C G t A A A 500 510 520 GTA GAA TA C C GC T G A C T A g C A TATCAA T A G CG 480 CACA TCTAA 490 GAICCITC 0.0 GΤ GA TCA 520 FCAATA GCG

Figure 1C. Sequence electrophenogram of ITS1-5.8S-ITS2 region of Astrocystis mirabilis (SUT051) using ITS5 primer.

SUT051 SUT056 ST2301 90 90 90 90 AY083804 1 AY083805 1 TCACGGAGGG ATGTATTTAT TCACGGAGGG ATGTATTTAT TCACGGAGGG ATGTATTTAT TCACGGAGGG ATGTATTTAT TCACGGAGGG ATGTATTTAT ТАСАТТАААА АССАЛОССС СТСССССС CTCCCCCCTT CTCCCCCCCTT CTCCCCCCCTT SUT051 SUT056 ST2301 ACATGCI ACATGCI ACATGCI ACATGCI 91 91 91 91 91 -AA AAATCCCGAC -AA AAATCCCGAC -AA AAATCCCGAC -GA AAATCCCGAC AY083804 AY083805 179 TAT TAGAT 
 SUT051
 180

 SUT056
 180

 ST2301
 180

 AY083804
 180

 AY083805
 181
 280 290 300 310 320 33 ACAACGGTA ACCACGGTT ACCCCCACACA ACCCCTACT ACAACGGTA ACCACGGTT ACCCCTCAC COCCACACA ACCCCTACT ACAACGGTA ACCACGGTT ACCCCTCAC COCCACACA GACCCTACA AACCCCTACT ACAACCGGTA ACCACGGTT ACCCCTCAC COCCACACAG GACCCTCACA AACCCCTACT CAACCCGTA ACCACGGTT ACCCCTCACC COCCACACAG GACCCTCACA AACCCCTACT CAACCCGTA ACCGACGGTT ACCCCTCACC COCCACACAG GACCCTCACA AACCCCTACT 
 SUT051
 270

 SUT056
 270

 ST2301
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 AY083804
 270

 AY083805
 271
 370 380 390 400 410 420 430 440 45 TACCAATCC CGACACGGG AGGTAGTGAC AATAAATACT GATACAGGC TCTTTTGGGT CTTGTAATTG GAATGAGTAC AATTAAAT TACCAATCC CGACACGGG AGGTAGTGAC AATAAATACT GATACAGGGC TCTTTTGGGT CTTGTAATTG GAATGAGTAC AATTAAATACT TACCAATCC CGACACGGG AGGTAGTGAC AATAAATACT GATACAGGGC TCTTTTGGGT CTTGTAATTG GAATGAGTAC AATTAAATC TACCAATCC CGACACGGGG AGGTAGTGAC AATAAATACT GATACAGGGC TCTTTTGGGT CTTGTAATTG GAATGAGTAC AATTAAATC TACCAATCC CGACACGGGG AGGTAGTGAC AATAAATACT GATACAGGGC TCTTTTGGGT CTTGTAATTG GAATGAGTAC AATTTAAATC 360 360 360 360 361 507051 SUT056 ST2301 AY083804 AY083805 460 470 480 490 500 510 520 530 54 ccttaaccae gaacaatrice accordinate treetectae according a treatment cataloger a tattaacta generative according to the treatment of the treat SUT051 SUT056 ST2301 449 449 449 450 451 AY083804 AY083805 550 560 570 580 590 600 610 620 AMA-GCTOGT AGTTGAACCT TGGGCCTGGC TGGCCGGTC- GCCTCAACGC GTGCACTGGT TCGGCCGGGC CTTTCCCTTT AAA-GCTCGT AGTTGAACCT TGGGCCTGGC TGGCCGGTC- GCCTCAACGC GTGCACTGGT TCGGCCGGGC CTTTCCCTTT AAA-GCTCGT AGTTGAACCT TGGGCCTGGC TGGCCGGGC GCCCCACGC GTGCACTGGT TCGGCCGGGC CTTTCCCTCT AAAA-GCTCGT AGTTGAACCT TGGGCCTGGC TGGCCGGTCC GCCTCACCGC GTGCACTGGT TCGGCCGGGC CTTTCCCTCT AAAAGCTCGT AGTTGAACCT TGGGCCTGGC TGGCCGGTCC GCCTCACCGC GTGCACTGGT TCGGCCGGGC CTTTCCCTCT AAAAGCTCGT AGTTGAACCT TGGGCCTGGC TGGCCGGTCC GCCTCACCGC GTGCACTGGT TCGGCCGGGC CTTTCCCTCT SUT051 538 SUT056 538 ST2301 539 À¥083804 540 À¥083805 541 640 650 660 670 690 690 700 710 721 ATGCCTTCA CTGGCTGTAC TGGCAAACA GCACTTTTAC TGGCAAAAA TTAGAGTGTT CAAACCAGC CTATGCTCGA ATACATCAGC ATGCCTTCA CTGGCTGTAC TGGCAAACA GCACTTTTAC TGGCAAAAA TTAGAGTGTT CAAAGCAGC CTATGCTCGA ATACATCAGC ATGCCCTCA CTGGCTG-G TAGGGAACCA GGACTTTTAC TGTGAAAAAA TTAGAGTGTT CAAAGCAGGC CTATGCTCGA ATACATCAGC ATGCCCTCA CTGGCTGTAG CGGGGAACCA GGACTTTTAC TGTGAAAAAA TTAGAGTGTT CAAAGCAGGC CTATGCTCGA ATACATCAGC ATGCCCTCA CTGGCTGTAG CGGGGAACCA GGACTTTTAC TGTGAAAAAA TTAGAGTGTT CAAAGCAGGC CTATGCTCGA ATACATCAGC ATGCCCTCA CTGGCTGTAG CGGGGAACCA GGACTTTTAC TGTGAAAAAA TTAGAGTGTT CAAAGCAGGC CTATGCTCGA ATACATCAGC SUT051 SUT056 ST2301 626 626 626 630 631 730 740 750 760 770 780 780 810 ATGGATAAT AGATAGGAC GIGTOGTICT ATTITIGTIGG TITCTAGGAC GOCGETAATG ATTIATAGGA CAGTOGGG GCATAGTAT ATGGATAAT AGATAGGAC GIGTOGTICT ATTITIGTIGG TITCTAGGAC GOCGETAATG ATTIATAGGG ACAGTOGGG GCATAGTAT ATGGATAAT AGATAGGAC GIGTOGTICT ATTITIGTIGG TITCTAGGAC GOCGETAATG ATTIATAGGG ACAGTOGGG GCATAGTAT ATGGAATAAT AGATAGGAC GIGTOGTICT ATTITIGTIGG TITCTAGGAC GOCGETAATG ATTIATAGGG ACAGTOGGG GCATAGTAT ATGGAATAAT AGATAGGAC GIGTOGTICT ATTITIGTIGG TITCTAGGAC GOCGETAATG ATTIATAGGG ACAGTOGGG GCATAGTAT ATGGAATAAT AGATAGGAC GIGTOGTICT ATTITIGTIGG TITCTAGGAC GOCGETAATG ATTIATAGGG ACAGTOGGG GCATAGTAT 
 SUT051
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 AY083805
 721
 820 TCANTECTA GAGGTGAAAT TCTTCGATTT ATTGAAGACT AACTACTCCG AAAGCATTTG CCAAGGATGT TTTCATTAAT CAGGAACGAA TCANTECTA GAGGTGAAAT TCTTCGATTT ATTGAAGACT AACTACTCCG AAAGCATTTG CCAAGGATGT TTTCATTAAT CAGGAACGAA TCANTECTA GAGGTGAAAT TCTTCGATTT ATTGAAGACT AACTACTCGC AAAGCATTTG CCAAGGATGT TTTCATTAAT CAGGAACGAA TCANTECTA GAGGTGAAAT TCTTCGATTT ATTGAAGACT AACTACTCGC AAAGCATTTG CCAAGGATGT TTTCATTAAT CAGGAACGAA TCANTEGTCA GAGGTGAAAT TCTTCGATTT ATTGAAGACT AACTACTCGC AAAGCATTTG CCAAGGATGT TTTCATTAAT CAGGAACGAA TCAATTGTCA GAGGTGAAAT TCTTCGATTT ATTGAAGACT AACTACTCGC AAAGCATTTG CCAAGGATGT TTTCATTAAT CAGGAACGAA TCAATTGTCA GAGGTGAAAT TCTTCGATTT ATTGAAGACT AACTACTCGC AAAGCATTTG CCAAGGATGT TTTCATTAAT CAGGAACGAA 
 SUT051
 806

 SUT056
 806

 ST2301
 804

 AY083804
 810

 AY083805
 811
 TCG TCG TCG TCG TCG 896 896 894 900 901 985 985 ST2301 AY083804 AY083805 1000 TROGCACCT TACCACAAAT CAAAGTC 1012 TROGCACCT TACCACAAAT CAAAGTC 1012 TROGCACCT TACCACAAAT CAAAGTC 1010 TROGCACCT TACGACAAAT CAAAGTC 1010 TROGCACCT TACGACAAAT CAAAGTT 1017 
 SUT051
 986

 SUT056
 986

 ST2301
 984

 AY083804
 990

 AY083805
 991

Figure 2C. Multiple sequence alignment of partial 18S rDNA using NS1 and NS4 primers of Astrocystis mirabilis (SUT051, SUT056) and Rosellinia sp. (ST3201) examined compared to DNA sequences from GenBank database, R. necatrix (AY083805) and A. cocoes (AY083804), by using ClustalX and BioEdit program.

AB017660 AB017657 AB017658 AB017659 AB017661 ST2301 SUT051 SUT056	I       AAAGAGTTCT AT-AACTOCC AAAACCCATE TEAACATACC ACACETTECC TOGGCAGGTC GETOCTA-       CCCCGAAGTC 7         1       ACAGAGCCGA CA-AGCTCCC AAACCCATE TEAACATACC ACACETTECC TOGGCAGGTC GETOCTA-       CCCCGAAGTC 7         1       ACAGAGCTTA CTTAACTCCC AAACCCCATE TEAACATACC TACGTTECC TOGGCAGGTC GETOCTA-       CCCCGGAAGTC 6         1       ACAGAGTTTA CTTAACTCCC AAACCCCATE TEAACATACC TACGTTECC TOGGCAGGTC GETOCTA-       CCCCGGAAGGTC 7         1       ACAGAGTTTA TA-ACTCCC AAACCCCATE TEAACATACC TACGTTECC TOGGCAGGTC GACCTCA-       CCCCGGGAGGG 7         1       ATAGAGTTTA CA-ACTCCC -       AAACCCCATE TEAACATACC AACCTTECC TOGGCAGGC GETOCCAC CTCTCAGG 7         1       ATAGAGTTTA CA-ACTCCC -       AAACCCATE TEAACATACC AGACTTECC TOGGCAGGC GETOCCAC CTCTCAGG 7	77 77 79 78 75 76 76
▲B017660 ▲B017657 ▲B017658 ▲B017659 ▲B017661 ST2301 SUT051 SUT056	90       100       110       120       130       140       150       160         78       CCCTACCCTG       TTAGGGCCTA       CCCGGGGCCAA       -CCTGCCGC       GGC       CC       ACGAAAC       -CCTGT       1         78       CCCTACCCTG       TTAGGGCCTA       CCCGGTGGCC       GGCGGCCAA       -CCTGCCGC       GGC       ACGAAAC       -CCTGT       1         78       CCCTACCCTG       TTAGGGCCTA       CCCGGTGGCC       GGCGGCCAA       -CCTGCCGGC       GGC       ACGAAAC       -TCTGT       1         70       CCCGAGAA       -ACGGCC       -CCGGCCGGC       GGC       CC       ACGAAAC       -TCTGT       1         70       CCCGAGAA       -AGGGCC       -CCGGCCGGC       GGC       CC       ACGAAAC       -TCTGT       1         70       CCCGAGAA       -AGGGGCC       GGCGCGC       CC       ACGAAAC       -TCTGT       1         70       CCCTACCTG       TAGGGCCAGA       CCTGGCGGC       GGC       CC       CCGAAAC       -TCTGT       1         70       CCTACCTG       TAGGGCCTA       CCCGGGGCTAAC       -CCCGCCGGC       GCC       CC       CCAAAAC       -TCTGT       1         70       GCCGACGCC       GCAAGGCCTG </th <th>L42 L42 L42 L43 L43 L40 L56 L56</th>	L42 L42 L42 L43 L43 L40 L56 L56
AB017660 AB017657 AB017658 AB017659 AB017661 ST2301 SUT051 SUT056	143       TTAGC—ATT       GA-AT-TCTG       AACAC—       —ATAACTAA       ATAACTAA       ATAACTAA       ACTTCCAACA       ACGGATCTCT       TGGTTCTGGC       2         143       TTAGC—ATT       GA-AT-TCTG       AACAC       —ATAACTAA       ATAACTAA       ACTACTAACA       ACGGATCTCT       TGGTTCTGGC       2         119       CTAGCC-ATT       GATATCTCIG       AACTIG       —ATAACTAA       ATCAGTTAAA       ACTITCCAACA       ACGGATCTCT       TGGTTCTGGC       2         144       TITATCACATT       AGAAT-TCTG       AATCT       —ATAACTAA       ATAACTAA       ACTITCCAACA       ACGGATCTCT       TGGTTCTGGC       2         141       TTAATCACT       AGAATCTGTG       AATT       —ATAACTAA       ATAAGTAAA       ACTTTCCAACA       ACGGATCTCT       TGGTTCTGGC       2         157       TTAATTCTG       AATACTGTIG       AATTCTAACAA       ACTTACACA       ACGGATCTCT       TGGTTCTGGC       2	211 211 211 215 210 236 236
AB017660 AB017657 AB017658 AB017659 AB017661 ST2301 SUT051 SUT051 SUT056	212 ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACAT 2 212 ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA TCGAATCATT GAACGCACAT 2 192 ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA TCGATCATTT GAACGCACCAT 2 216 ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA TCGATCATTT GAACGCACCAT 2 211 ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCAT 2 213 ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCAT 2 214 ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCAT 2 215 ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACCAT 2	291 291 291 271 295 290 316 316
AB017660 AB017657 AB017658 AB017659 AB017661 ST2301 SUT051 SUT051 SUT056	292 TGCGCCCATT AGTATTCTAG TGGGCATGCC TGTTCGAGCG TCATTTCAAC CCTTAAGCCC CTGTTGCTTA GTGTTGGGGG 3 292 TGCGCCCCATT AGTATTCTAG TGGGCATGCC TGTTCGAGCG TCATTTCAAC CCTTAAGCCC CTGTTGCTTA GTGTTGGGGG 3 272 TGCGCCCCGT AGTATTCTAG CGGGCATGCC TGTTCGAGCG TCATTTCAAC CCTTAAGCCC CACCTGCTTA GTGTTGGGGG 3 296 TGCGCCCATT AGTATTCTAT TGGGCATGCC TGTTCGAGCG TCATTTCAAC CCTTAAGCCC CCGTTGCTTG GTGTGGGGG 3 317 TGCGCCCATT AGTATTCTAG TGGGCATGCC TGTTCGACGG TCATTTCAAC CCTTAAGCCC CTGTTGCTTA GCGTTGGGGG 3	371 371 351 375 370 393 393
▲B017660 ▲B017657 ▲B017658 ▲B017659 ▲B017661 ST2301 SUT051 SUT056	410 420 430 440 450 460 470 480 372 CCTGCAGGCC -CTGCT-GCA GCCCTCGAA GTCAGTGGCG GAGTCGGTCA CACACTCTAG ACGTAGTAGA TTTCTCATCT 372 CCTGCAGCGC -CTGCT-GCA GCCCCTCGAA GTCAGTGGCG GAGTCGGTCA CACACTCTAG ACGTAGTAGA TTTCTCATCT 372 CCTGCAGCGC -CTGCT-GCA GCCCCTCGAA GTCAGTGGCG GAGTCGGTCA CACACTCTAG ACGTAGTAGA TTTCTCATCT 372 CCTGCAGCGC -CTGCT-GCA GCCCCTCGAA GTCAGTGGCG GAGTCGGTCA CACACTCTAG ACGTAGTAGA TTTCTCATCT 374 CCTACGGCGA	449 449 431 448 444 459 459
AB017660 AB017657 AB017658 AB017659 AB017661 ST2301 SUT051 SUT056	490500510520530450GOCCTATGGTT-GTGCCGGTCOCCTGCCGTAAAACACCCC-CCTATACCAA499450GOCCTATGGTT-GTGCCGGTCOCCTGCCGTAAAACACCCC-CCTATACCAA499450GOCCTATGGTT-GTGCCGGTCOCCTGCCGTAAAACACCCC-CCTATACCAA499450GOCCTGCGGCG-GOGCCGGTCOCCTGCCGTAAAACCCCCC-CCTATACCAA499450GOCCTGTGGGTGCCTGCCGTCOCCTGCCGTAAAACCCCCCACTTATCCAAA482449GOCCTGTAGTGTGGACCGGTCOCCTGCCGTAAAACCCCCCAATTACAAA499445GCCCTGTAGTGTGGACCGGTCOCCTGCCGTAAAACCCCCCAATTACAAGA495460GCCTGGAAGTOGGACCGGTCOCTTGCGAAAACCCCCAAATTAAAGG506460GCCTGGAAGTCGGACCGGTCCCTGCCGAAAACCCCCAAATTAAAGG506	

Figure 3C. The ITS1-5.8S-ITS2 sequence alignment of Astrocystis mirabilis (SUT056, SUT051) and Rosellinia sp. (ST2301) examined compared to DNA sequences from GenBank database, R. arcuata (AB017660), R. pepo (AB017659), R. quercina (AB017661), and R. necatrix (AB017657 and AB017658), by using ClustalX and BioEdit programs for phylogenetic tree construction in Figure 83. Arrows indicate the start and the stop of 5.8S rDNA sequences.

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AJ390421 AJ390422 ST2321 AJ390423 SUT161 SUT260 KS15	10       20       30       40       50       60       70       80         1       GCGAGTTAAT       TACAAACTCC       AAACCCATGT       GAACTTACT       GCGTTGCCT       CGCGAGTTG       CCTGCCGAG       TG       CC       74         1       GCGAGTTAAT       TACAAACTCC       AAACCCACGT       GAACTTACT       GCTGTGCCT       CGCGCAGGTTG       CCTGCCGAG       TG       CC       74         1       GCGAGTTAAT       TACAAACTCC       AAACCCACGT       GAACTTACT       ACTGTTGCCT       CGGCAGGTTG       TGCTGCGCGG       TGAAGTCC       74         1       GCGAGTTAAT       TACAAACTCC       AAACCCCACGT       GAACTACCT       ATTGTTGCCT       CGGCAGGTTG       TGCTGCGCGG       TGAGAGTTCC       80         1       ACGAGTTAAT       TACAAACTCC       AAACCCACGT       GAACGTACCT       ATGTGTGCCT       CGGCAGGTGC       TACGTGCGCG       TGCTGCGCGG       TGCTGCGCGG       TGCTGCGCGG       74         1       ACGAGTTAAT       TACAAACTCC       AAACCCACGT       GAACGTACCT       ACTGTGCGCT       CGCCAGGTG       TGCTGCGGCG       70       74         1       ACGAGTTAAT       TACAAACTCC       AAACCCACGT       GAACGTACCT       ACTGTGCGCT       CGCGCAGGTG       70       70 <t< th=""></t<>
▲J390421 ▲J390422 ST2321 ▲J390423 SUT161 SUT260 KS15	90       100       110       120       130       140       150       160         75       TACCCTGGAG       TGGCTTACCC       TGGAGTAGCT       ACCCTGTAGT       GCCTACCCTG       GAGTAGGAC       CCCGCAGCCC       GCAACCAGAC       154         75       TACCCTGGAG       TACCTGGAG       TGGAGTAGCT       ACCCTGTAGT       GCCTACCCTG       GAGTAGGAC       CCCGCAGCCC       GCAACCAGAC       154         75       TACCTGGAG       TACCTGGAG       TACCTGGAG       TACCTGGAG       TACCTGGAG       CACCGCG       GCAACCAGAC       154         75       TACCTGGAG       TACCTGGAG       TACCTGGAG       TACCTGGAG       CCCGCGAGCC       CCACCGCG       CCAACCAGAC       154         75       TACCTGGAG       TACCTGGAG       TACCTGGAG       TACCTGGAG       TACCTGGAG       154         75       TACCTGGAG       TACCTGGAG       TGCAGTAGCT       ACCCTG       CAGCGC       CCAACCAGAC       125         71       TACCTGGAG       TACCTGGAG       TGCCTGAGCT       ACCCTG       CAGCGC       CCACCAGAC       121         60       TACCTGGAG       TGCCTTACCT       GCAAGCGGC       ACCCGG       TACCCTGGAG       110
AJ390421 AJ390422 ST2321 AJ390423 SUT161 SUT161 SUT260 KS15	170 180 190 200 210 220 230 240 155 CTGCCAGAGG ACCTCTGAAC TCTTTTTTAC ACTGGAACTC TGAAACTATT ATACAAACAA GTTAAAACTT TCAACAACGG 234 155 CTGCCAGAGG ACCTCTGAAC TCTTTTTTAC ACTGGAACTC TGAAACTATT ATACAAACAA GTTAAAACTT TCAACAACGG 234 140 CTGCCGAAGG ACCTCTGAAT TCTTTTTTAC ACTGGAACTC TGAAACTATT ATACAAATAA GTTAAAACTT TCAACAACGG 219 155 CTGTCGGAGG ACCTCTTAAAC TCTATTTTAT AACGTATCTC TGAAACTATT ATACAAATAA GTTAAAACTT TCAACAACGG 234 126 CTGCCGAAGG ACCTTTAAAC TCTTTTTTAC CCCGGAACTC TGAAACTATT ATACAAATAA GTTAAAACTT TCAACCAACGG 205 122 CTGCCGAAGG ACCTTAAAT TCTTTTTTAC CCCCGGAACTC TGAAACTATT ATATAAATAA GTTAAAACTT TCAACCAACGG 201 111 CCGCCGGCGC CCCATTAAAC TCTGTTTAAT ACTGGAATCT TGAACTATT AACTAAAACTT TCAACAACGG 188
AJ390421 AJ390422 ST2321 AJ390423 SUT161 SUT260 KS15	250 260 270 280 290 300 310 320 235 ATCTCTTGGT TCTGGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA 314 235 ATCTCTTGGT TCTGGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA 314 220 ATCTCTTGGT TCTGGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA 314 235 ATCTCTTGGT TCTGGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA 314 206 ATCTCTTGGT TCTGGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA 314 206 ATCTCTTGGT TCTGGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA 314 206 ATCTCTTGGT TCTGGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA 285 202 ATCCTTGGT TCTGGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA 281 189 ATCTCTTGGT TCTGGCATCG ATGAAGAACG CAGCGAAATG CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA 268
AJ390421 AJ390422 ST2321 AJ390423 SUT161 SUT260 KS15	330 340 350 360 370 381 390 400 315 ATCTTIGAAC GCACATIGCG CCTAACAGTA TICIGTIAGG CATGCCTGIT CGAGCGTCAT TICAACCCCC AAGCCCTAIT 304 315 ATCTTIGAAC GCACATIGCG CCTAACAGTA TICIGTIAGG CATGCCTGIT CGAGCGTCAT TICCAACCCCC AAGCCCTAIT 304 315 ATCTTIGAAC GCACATIGCG CCTAATAGTA TICIGTIAGG CATGCCTGIT CGAGCGTCAT TICCAACCCCC AAGCCCTAIT 315 ATCTTIGAAC GCACATIGCG CCTAATAGTA TICIGTIAGG CATGCCTGIT CGAGCGTCAT TICCAACCCCC AAGCCCTAIT 316 ATCTTIGAAC GCACATIGCG CCTAATAGTA TICIGTIAGG CATGCCTGIT CGAGCGTCAT TICCAACCCCC AAGCCCTAIT 317 316 ATCTTIGAAC GCACATIGCG CCTAATAGTA TICIGTIAGG CATGCCTGIT CGAGCGTCAT TICCAACCCCC AAGCCCTAIT 316 ATCTTIGAAC GCACATIGCG CCTAATAGTA TICIGTIAGG CATGCCTGIT CGAGCGTCAT TICCAACCCCC AAGCCCTAIT 317 316 ATCTTIGAAC GCACATIGCG CCTAATAGTA TICIGTIAGG CATGCCTGIT CGAGCGTCAT TICCAACCCCC AAGCCCTAIT 318 317 317 317 317 317 317 317 317 317 317
AJ390421 AJ390422 ST2321 AJ390423 SUT161 SUT260 KS15	410420430440450460470480395TCCTTGACGT TOGGAGTTTA COGAAACG-T AATTCCTCAA ATATACTOGC GGAGCTAGGT COTGCTCTAA GCGTAGTAAT473395TCCTTGACGT TOGGAGTTTA COGAAACG-T AATTCCTCAA ATATACTOGC GGAGCTAGGT COTGCTCTAA GCGTAGTAAT473380TGCTTGACGT TOGGAGTTTA COGAAACG-T AATTCCTCAA ATATACTOGC GGAGCTAGGT COTGCTCTAA GCGTAGTAAC458395TCCTTGACGT TOGGAGTTTA COGAAACG-T AATTCCTCAA ATATACTOGC GGAGCTAGGT COTGCTCTAA GCGTAGTAAC473380TGCTTGACGT TOGGAGTTTA COGAAACG-T AATTCCTCAA ATATACTOGC GGAGCTAGGT COTGCTCTAA GCGTAGTAAC473360TGCTTGACGT TOGGAGTTTA COGAAACG-T AATTCCTCAA ATATACTOGC GGAGCTAGGT COTGCTCTAA GCGTAGTAAC473361TGCTTGACGT TOGGAGTTTA COGAAACG-T AATTCCTCAA ATATACTOGC GGAGTTAGGT COTGCTCTAA GCGTAGTAAC444362TGCTTGACGT TOGGAGCTTA COGAAACG-T AATTCCTCAA ATATACTOGC GGAGTTAGGT COTGCTCTAA GCGTAGTAAC440349TGCTTGACGT TOGGAGCCTA CCGTAGCGGT AGCCCCTTAA AATTACTGGC GGAGTTGGC CGGCTCTAA GCGTAGTAAC428
AJ390421 AJ390422 ST2321 AJ390423 SUT161 SUT260 KS15	490500510520530474TATATTCTCGCHICLGCAGCCGGTCTAGGTCHIGCCGTAAAGCCCTATATTHTTCT474TATATTCTCGCHICLGCAGCCGGTCTAGGTCHIGCCGTAAAGCCCTATATTHTTCT529474TATATTATCGCHICLGCAGCCGGTTAGGTCCIGCCGTAAAGCCCTATATTHTTCT514474TTATTCTCGCHICLGCAGCCGGCTTAGGTCCIGCCGTAAAACCCTATATTHTTCT514474TTATTCTCGCHICLGCAGCCGGCTTAGGTCCIGCCGTAAAACCCTATATTHTTCT529445TATATTCTCGCHICLGCAGCCGGCTTAGGTCCIGCCGTAAAACCCTATATTHTTCT500441TATATTCTCGCHICLGCAGCCGGCTTAGGTCCIGCCGTAAAACCCTATATTHTCT496429TATTATCTCGCCTATTAGTTGGACCGGTCCCCIGCCGTAAAACCCTA-ATTHT-C-481

Figure 4C. The ITS1-5.8S-ITS2 sequence alignment of *Camillea tinctor* (AJ390421, AJ390422), *C. tinctor* (ST2321), *C. obularia* (AJ390423), *C. tinctor* (SUT161), *C. tinctor* (SUT260), and *C. selangorensis* (KS15) by using ClustalX and BioEdit programs for phylogenetic tree construction in Figure 86. Arrows indicate the start and the stop of 5.8S rDNA sequences.

		10					60	70		90	100						
	14																100
SUT209	1							GTTCGCCCTG									136
SUT178	1							GTTCGCCCTG									137
SUT278	1							GTTCGCCCTG									136
SUT039	1							GTTCGCCCTG									135
AY616684	1							GTTCGCCCTG									137
SUT168	1							GTTCGCCCTG									137
SUT322	1							GTTCGCCCTG				A CONTRACTOR OF					137
SUT085	1							GTTCGCCCTG									137
AY616682	1							G									129
AY616681	1							GCTTACCCTG							TCTGTTTTAA		146
AY616682	1	CCGAGTTATC	TAAACTCC-A	ACCCTTTGTG	AAACTTACCG	TCGTTGCCTC	GGCGGGCTGC	GCTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGCGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAC-	TCTGTTTTAA	TACCG-AATC	146
AY616683	1	CCGAGTTATC	TAAACTCC-A	ACCCTTTGTG	AAACTTACCG	TCGTTGCCTC	GGCGGGCTGC	GCTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGCGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAC-	TCTGTTTTAA	TACCG-AATC	146
AF176955	1	CCGAGTTATC	TAAACTCC-A	ACCCTTTGTG	AAACTTACCG	TCGTTGCCTC	GGCGGGCTGC	GCTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGCGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAC-	TCTGTTTTAA	TACCG-AATC	146
AF176958	1	CTGAGTTATC	TAAACTCC-A	ACCCTTTGTG	AAACTTACCG	TCGTTGCCTC	GGCGGGGCTGC	GCTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGCGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAC-	TCTGTTTTAA	TACCG-AATC	146
AF176954	1	CTGAGTTATC	TAAACTCC-A	ACCCTTTGTG	AAACTTACCG	TCGTTGCCTC	GGCGGGCTGC	GCTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGCGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAC-	TCTGTTTTAA	TACCG-AATC	146
L1	1	CTGAGTTATC	TAAACTCC-A	ACCCTTTGTG	AAACTTACCG	TCGTTGCCTC	GGCGGGCTGC	GCTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGCGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAC-	TCTGTTTTAA	TACCG-AATC	146
L2	1	CTGAGTTATC	TAAACTCC-A	ACCCTTTGTG	AAACTTACCG	TCGTTGCCTC	GGCGGGCTGC	GCTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGCGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAC-	TCTGTTTTAA	TACCG-AATC	146
AF176957	1	CTGAGTTATC	TAAACTCC-A	ACCCTTTGTG	AAACTTACCG	TCGTTGCCTC	GGCGGGCTGC	GCTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGCGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAC-	TCTGTTTTAA	TACCG-AATC	146
AF176969	1	CTGAGTTATC	TAAACTCCCA	ACCCTTTGTG	AACCTTACCG	TCGTTGCCTC	GGCGGGCTGT	ACTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGTGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAT-	TCTATTTTAC	TACTG-TATC	147
AF176968	1	CTGAGTTATC	TAAACTCCCA	ACCCTTTGTG	AACCTTACCG	TCGTTGCCTC	GGCGGGCTGT	ACTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGTGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAT-	TCTATTTTAC	TACTG-TATC	147
AF176967	1	CTGAGTTATC	TAAACTCCCA	ACCCTTTGTG	AACCTTACCG	TCGTTGCCTC	GGCGGGGCTGT	ACTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGTGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAT-	TCTATTTTAC	TACTG-TATC	147
AY315403	1	CTGAGTTATC	TAAACTCCCA	ACCCTTTGTG	AACCTTACCG	TCGTTGCCTC	GGCGGGGCTGT	ACTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGTGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAT-	TCTATTTTAC	TACTG-TATC	147
AF176982	1	CTGAGTTATC	TAAACTCCCA	ACCCTTTGTG	AACCTTACCG	TCGTTGCCTC	GGCGGGGCTGT	ACTTACCCTG	TAG-CTACCC	TGTAGCTACC	CGGTAGGTGC	GCTCCAAGCC	CGCCGGTGGA	CCACTAAAT-	TCTATTTTAC	TACTG-TATC	147
AF176975	1	CTGAGTTATC	TAAACTCCCA	ACCCTATGTG	AACCTTACCG	TCGTTGCCTC	GGCGGGGCCGC	GCTTACCCGG	TAG-CTACCC	TGTAGCTACC	CGGTAGGTGC	GCTATAGGCC	CGCCGGTGGA	CTACTCAACT	CTGTTTTTAA	TACTG-TATC	148
AF176974	1	CTGAGTTATC	TAAACTCCCA	ACCCTATGTG	AACCTTACCG	TCGTTGCCTC	GGCGGGGCCGC	GCTTACCCGG	TAG-CTACCC	TGTAGCTACC	CGGTAGGTGC	GCTATAGGCC	CGCCGGTGGA	CTACTCAACT	CTGTTTTTAA	TACTG-TATC	148
AF176973	1	CTGAGTTATC	TAAACTCCCA	ACCCTATGTG	AACCTTACCG	TCGTTGCCTC	GGCGGGGCCGC	GCTTACCCGG	TAG-CTACCC	TGTAGCTACC	CGGTAGGTGC	GCTATAGGCC	CGCCGGTGGA	CTACTCAACT	CTGTTTTTAA	TACTG-TATC	148
AF163022	1	CTGAGTTATC	TAAACTCCCA	ACCCTATGTG	AACCTTACCG	TCGTTGCCTC	GGCGGGCTGT	GCTTACCCGG	TAG-CTACCC	TGTAGCTACC	CGGTAGGTAC	GCTGCAAGCC	-GCCGGTGGA	CCACTAAAG-	GGTTTAAT	TACTG-TATC	144
AF163023	1		AACTCCCA	ACCCTATGTG	AACCTTACCG	TNGTTGC-TC	GENGGGCTGT	G-TTACCCGG	TAGTACCC	TG	-GCTAGGTAC	G-TGCAAGCC	-GCCGGTGGA	CCACTAAACC	TCTGTTTAAT	TACTGCTATC	123
AF163021	1		-AAACTCCCA	ACCCTATGTG	AACCTTACCG	TNGTTGC-TC	GENGEGCTGT	G-TTACCCGG	TAGTACCC	TG	-GCTAGGTAC	G-TGCAAGCC	-GCCGGTGGA	CCACTAAACC	TCTGTTTAAT	TACTGCTATC	124
AF176981	1	CTGAGTTATC	TAAACTCCCA	ACCCTATGTG	AACCTTACCG	TCGTTGCCTC	GGCGGGCTGT	GCTTACCCGG	TAG-CTACCC	TGTAGCTACC	CGGTAGGTAC	GCTGCAAGCC	CGCCGGTGGA	CCACTAAAC-	TCTGTTTAAT	TACTG-TATC	147
	- C			A STATE OF A	12000		COLUMN STORES		and the second se	Constant of the second second		STORE STREET, STORE STORE STREET, STORE ST	7.79.79.79.79	CARGE AND		STATISTICS AND	

Figure 5C. The ITS1-5.8S-ITS2 sequence alignment of *Daldinia eschscholzii* (SUT209, SUT178, SUT278, SUT039, SUT168, SUT322, SUT085, AY616684) and *D. concentrica* (L1 and L2) examined compared to DNA sequences from GenBank database, *D. concentrica* (AY616682, AY616681, AY616683, AY176955, AY176958, AY1769584), *D. grandis* (AF176982), *D. loculata* (AF176969, AF176968, AF176967), *D. petrinia* (AF176975, AF176974, AF176973), *D. vericosa* (AF163022), *D. fissa* (AF176981), *Daldinia* sp. (AF163023, AY315403), by using ClustalX and BioEdit programs for phylogenetic tree construction in Figure 88. Arrows indicate the start and the stop of 5.8S rDNA sequences.

		16			0 19												0
SUT209	137		TCAACTTAAT					CGATGAAGAA									286
SUT178			TCAACTTAAT														
SUT278			TCAACTTAAT					CGATGAAGAA									
SUT039			TCAACTTAAT														
AY616684	138	TCTGAATGCT	TCAACTTAAT	AAGTTAAAAC	TTTCAACAAC	GGATCTCTTG	GTTCTGGCAT	CGATGAAGAA	CGCAGCGAAA	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACATTG	CGCCCATTAG	287
SUT168	138	TCTGAATGCT	TCAACTTAAT	AAGTTAAAAC	TTTCAACAAC	GGATCTCTTG	GTTCTGGCAT	CGATGAAGAA	CGCAGCGAAA	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACATTG	CGCCCATTAG	287
SUT322			TCAACTTAAT														
SUT085			TCAACTTAAT														
AY616682			TCAACTTAAT														
AY616681			TCAACTTAAT														
AY616682 AY616683			TCAACTTAAT														
AF176955			TCAACTTAGT														
AF176958			TCAACTTAAT														
AF176954			TCAACTTAAT														
L1			TCAACTTAAT														
L2	147	TCTGAATGCT	TCAACTTAAT	AAGTTAAAAC	TTTCAACAAC	GGATCTCTTG	GTTCTGGCAT	CGATGAAGAA	CGCAGCGAAA	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACATTG	COCCCATTAG	296
AF176957	147	TCTGAATGCT	TCAACTTAAT	AAGTTAAAAC	TTTCAACAAC	GGATCTCTTG	GTTCTGGCAT	CGATGAAGAA	CGCAGCGAAA	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACATTG	CGCCCATTAG	296
AF176969	148	TCTGAATGCT	TCAACTTAAT	AAGTTAAAAC	TTTCAACAAC	GGATCTCTTG	GTTCTGGCAT	CGATGAAGAA	CGCAGCGAAA	TGCGATAAGT	AATGTGAATT	GCAGAATTCA	GTGAATCATC	GAATCTTTGA	ACGCACATTG	CGCCCATTAG	297
AF176968			TCAACTTAAT														
AF176967			TCAACTTAAT														
AY315403			TCAACTTAAT					CGATGAAGAA									
AF176982			TCAACTTAAT														
AF176975 AF176974			TCAACTTAAT														
AF176973			TCAACTTAAT														
AF163022			TCAACTTAAT														
					-												
		31	0 320			350	360	370				410	420	430	440	400	
SUT209	287	TATTCTAGTG	GGCATGCCTG	TTCGAGCGTC												ATACC-ATTC	429
SUT209 SUT178			GGCATGCCTG GGCATGCCTG		ATTTCAACCC	TTAAGCCCCT	GTTGCTTAGC	GTTGGGAATC	T-AGGTCTCC	AGGGC	CTAGTTCCCC	AAAGTCATCG	GCGGAGTCGG	AGCGTACTCT	CAGCGTAGTA		429 430
SUT178 SUT278	288 287	TATTCTAGTG TATTCTAGTG	GGCATGCCTG GGCATGCCTG	TTCGAGCGTC TTCGAGCGTC	ATTTCAACCC ATTTCAACCC ATTTCAACCC	TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT	GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC	GTTGGGAATC GTTGGGAATC GTTGGGAATC	T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC	AGGGC AGGGC AGGGC	CTAGTTCCCC . CTAGTTCCCC . CTAGTTCCC	AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG (	GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG	AGCGTACTCT AGCGTACTCT AGCGTACTCT	CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA	ATACC-ATTC ATACC-ATTC	430 428
SUT178 SUT278 SUT039	288 287 286	TATTCTAGTG TATTCTAGTG TATTCTAGTG	GGCATGCCTG GGCATGCCTG GGCATGCCTG	TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC	ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC	TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT	GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC	GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC	T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC	AGGGC AGGGC AGGGC AGGGC	CTAGTTCCCC . CTAGTTCCCC . CTAGTTCCC CTAGTTCCCC .	AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG (	GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG	AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT	CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA	ATACC-ATTC ATACC-ATTC ATACC-ATTC	430 428 428
SUT178 SUT278 SUT039 AY616684	288 287 286 288	TATTCTAGTG TATTCTAGTG TATTCTAGTG TATTCTAGTG	GGCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTG	TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC	ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC	TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT	GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC	GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC	T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC	AGGGC AGGGC AGGGC AGGC	CTAGTTCCCC . CTAGTTCCCC . CTAGTTCCCC . CTAGTTCCCC . CTAGTTCCCC .	AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG (	GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG	AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT	CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA	ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC	430 428 428 429
SUT178 SUT278 SUT039 AY616684 SUT168	288 287 286 288 288	TATTCTAGTG TATTCTAGTG TATTCTAGTG TATTCTAGTG TATTCTAGTG	GGCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTG	TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC	ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC	TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT	GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC	GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC	T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC	AGGGC AGGGC AGGGC AGGC AGGC AGGGC	CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC :	AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG (	GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG	AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT	CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA	ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC	430 428 428 429 430
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SUT178 SUT278 SUT039 AY616684 SUT168 SUT322 SUT085	288 287 286 288 288 288 288 288 288 280 297	TATTCTAGTG TATTCTAGTG TATTCTAGTG TATTCTAGTG AATTCTAGTG TATTCTAGTG TATTCTAGTG TATTCTAGTG	GGCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTG GACATGCCTG	TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC TCCGAGCGTC TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC	ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC	TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCTA TTAAGCCCTA	GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTCGCTTAGC GTTGCTTAGC	GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTGGGGACC	T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC TGAGCCCTTC TGGCCCTTC	AGGGC AGGGC AGGGC AGGC AGGC AGGC AGGC AGGC AGGC AGGC	CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : GCAGTTCCTC :	AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( -AAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG (	GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG	AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCG-ACTCT AGCG-ACTCT GGCATACTCT	CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA GAGCGTAGTA AAGCGTAGTA	ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATATT-CTC ATATTTCTTC	430 428 428 429 430 430 420
SUT178 SUT278 SUT039 AY616684 SUT168 SUT322 SUT085 AY616682 AY616681	288 287 286 288 288 288 288 288 288 280 297 297	TATTCTAGTG TATTCTAGTG TATTCTAGTG TATTCTAGTG AATTCTAGTG TATTCTAGTG TATTCTAGTG TATTCTAGTG TATTCTAGTG	GGCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTA	TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC TTCGAGCGTC	ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC ATTTCAACCC	TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCTA TTAAGCCCTA TTAAGCCCTA	GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTCGCTTAGC GTTGCTTAGC GTTGCTTAGC	GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGACTC GTTGGGACTC GTTGGGACTC	T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC TGAGCCTTC TGAGCCTTC TGCGCTGTAC TGCGCTGTAC	AGGGC AGGGC AGGGC AGGC AGGC AGGC AGGC AGGGGC TTGTTACGGC	CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : CTAGTTCCCC : GCAGTTCCTC : GCAGTTCCTC :	AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTCATCG ( AAAGTGATTG (	GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTTAG	AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT GGCATACTCT	CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CAGCGTAGTA CA-CG-AATA GAGCGTAGTA AAGCGTAGTA	ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATATTC-CTC ATATTTCTTC ATATTTCTTC	430 428 428 429 430 430 420 420 424 446
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SUT178 SUT278 SUT278 SUT239 Ax616684 SUT168 SUT322 SUT085 Ax616682 Ax616682 Ax616682 Ax616682 Ax616682 Ax616682 Ax616682 Ax7176558 AF176958 AF176957 AF176957 AF176967 Ax7176974 AF176974 AF176974 AF176974 AF176972 AF176972 AF163022	288 287 286 288 288 288 280 297 297 297 297 297 297 297 297 297 297	TATTCTAGTG TATTCTAGTG	GCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTG GGCATGCCTA GGCATGCCTA GGCATGCCTA GGCATGCCTA GGCATGCCTA GGCATGCCTA GGCATGCCTA GGCATGCCTA GGCATGCCTA GGCATGCCTA GGCATGCCTA GGCATGCCTA GGCATGCCTA	TTCBAGGTC TTCBAGGTC	ATTICAACCC ATTICAACCC	TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCTT TTAAGCCCTA TTAAGCCTTA TTAAGCCTTA TTAAGCCTTA TTAAGCCTTA TTAAGCCTTA TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A	GTTGCTTAGC GCTGCTTAGC GCTGCTTAGT	GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAGTC GTTGGGAGTC GTTGGGAGTC GTTGGGAGTC GTTGGGAGTC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAACC GTTGGGAACC GTTGGGAATC	T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC TGGCCTGTAC TGGCCTGTAC TGGCCTGTAC TGGCCTGTAC TGGCCTGTAC TGCCCTGTAT TGCCCCGTAT TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG	AGGGC AGGC AGGC AGGC AGGC AGGC AGGC TTGTTACGC TTGTTACGC TTGTTACGC TTGTTACGC TTGTTACGC TTGTTACGC TTGTTACGC TTGTTACGC TTGTTACGC TTATAGG TTATAGG TTATAGG TTATAGG CTACGGC CTACGGC CTACGGC CTACAGC	CTAGTTCCCC CTAGTTCCCC CTAGTTCCCC CTAGTTCCCC CTAGTTCCCC CTAGTTCCCC CTAGTTCCCC CTAGTTCCCC GCAGTTCCTC	AAAGTCATCG ( AAAGTCATCG (	GCGGAGTCGG GCGAAGTCGG GCGAAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTCGG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG GCGGAGTTAG	AGGETACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGGGTACTCT AGGGTACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT	САВССЯТАРТА САВССЯТАРТА САВССЯТАРТА САВССЯТАРТА САВССЯТАРТА САВССЯТАРТА САВССЯТАРТА САВССЯТАРТА АЛВССЯТАРТА	ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATATTC-TTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATT-CTTC ATATT-CTTC CTATT-CTTC CTATT-CTTC CTATT-CTTC CTATT-CTTC ATATTCTTC ATATTCTTC ATATTCTTC	430 428 429 430 420 430 420 440 446 446 446 446 446 446 446 446 44
SUT178 SUT278 SUT278 SUT278 SUT278 SUT39 Ax516681 SUT322 SUT085 Ax516682 Ax516682 Ax516682 Ax516682 Ax516682 Ax516682 Ax176958 Ax176958 Ax176958 Ax176959 Ax176959 Ax176969 Ax176969 Ax176975 Ax176975 Ax176973 Ax176973 Ax176973 Ax176973	288 287 288 288 288 288 288 297 297 297 297 297 297 297 297 297 297	TATTCTAGTG TATTCTAGTG	GCCATGCCTG GCCATGCCTG GCCATGCCTG GCCATGCCTG GCCATGCCTG GCCATGCCTG GCCATGCCTA GCCATGCCTA GCCATGCCTA GCCATGCCTA GCCATGCCTA GCCATGCCTA GCCATGCCTA GCCATGCCTA GCCATGCCTA GCCATGCCTA GCCATGCCTA	TTCBAGGTC TTCBAGGTC	ATTICAACCC ATTICAACCC	TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCCT TTAAGCCCTA TTAAGCCCTA TTAAGCCTTA TTAAGCCTTA TTAAGCCTTA TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A TTAAGCCT-A	GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGC GTTGCTTAGT GTTGCTTAGT GTTGCTTAGT GTTGCTTAGT GTTGCTTAGT GTTGCTTAGT GTTGCTTAGT GTTGCTTAGT GTTGCTTAGT GTTGCTTAGT GCTGCTTAGT GCTGCTTAGT	GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAGTC GTTGGGAGTC GTTGGGAGTC GTTGGGAGTC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC GTTGGGAATC	T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC T-AGGTCTCC TGGGCTGTAC TGCGCTGTAC TGCGCTGTAC TGCGCTGTAC TGCGCTGTAC TGCGCTGTAC TGCGCTGTAT TGCCCTGTAT TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG TGCCCCGTAG	AGGGC AGGGC AGGGC AGGGC AGGGC AGGGC AGGGC TTGTTACGGC TTGTTACGGC TTGTTACGGC TTGTTACGGC TTGTTACGGC TTGTTACGGC TTGTTACGGC TTGTTACGGC TTGTTACGGC TTGTTACGGC TTGTTACGGC TTGTTACGGC TTTAGG TTATAGG TTACGGC CTACGGC CTACGGC CTACAGC	CTASTICCCC CTASTICCCC CTASTICCCC CTASTICCCC CTASTICCCC CTASTICCCC CTASTICCCC CTASTICCCC CTASTICCCC CASTICCTC GCASTICCTC GCASTICCTC GCASTICCTC GCASTICCTC GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT GCASTICCTT	AAAGTCATCG ( AAAGTCATCG (		AGGETACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGCGTACTCT AGGCATACTCT AGGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT GGCATACTCT	САВССТАРТА САВССТАРТА САВССТАРТА САВССТАРТА САВССТАРТА САВССТАРТА САВССТАРТА САВССТАРТА САВССТАРТА САВССТАРТА САВССТАРТА САВССТАРТА СОСТАРТА С	ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATACC-ATTC ATATTC-CTC ATATTTCTTC ATATTTCTTC ATATTTCTTC ATATTTCTTC ATATTTCTTC ATATTTCTTC ATATTTCTTC ATATTCTTC ATATT-CTTC ATATT-CTTC ATATT-CTTC ATATT-CTTC ATATT-CTTC ATATT-CTTC ATATT-CTTC ATATT-CTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC ATATTCTTC	430 428 429 430 420 420 420 420 420 420 420 420 420 42

Figure 5C. (Continued).

		460	470	480	490	50	2	
SUT209	430	TCGCTTTTGC	AGTAGCCCCG	GCGGCTTGCC	GTAAAACCCC	TATATCT	TTAGTGG	483
SUT178	431	TCGCTTTTGC	AGTAGCCCCG	GCGGCTTGCC	GTAAAACCCC	TATATCT	TTAGTGG	484
SUT278	429	TCGCTTTTGC	AGTAGCCCCG	GCGGCTTGCC	GTAAA-CCCC	TATATCT	TTAGGTT	481
SUT039	429	TCGCTTTTGC	AGTAGCCCCG	GCGGCTTGCC	GTAAA-CCCC	TATATCT	TTAGTGG	481
AY616684	430	TCGCTTTTGC	AGTAGCACCG	GCGGCTTGCC	GTAAAACCCC	T		470
SUT168	431	TCGCTTTTGC	AGTAGCCCCG	GCGGCTTGCC	GTAAAACCCC	TATATCT	TTAGTGG	484
SUT322	431	TCG-TTTTGC	AGTAGCCCCG	GCGGCTTGCC	GTAAAACCCC	TATATCT	TTAGTGG	483
SUT085	421	TTGCTTTTGC	AGTAACCCCG	GCGG-TTGCC	GTAAACC			456
AY616682	425	TCGCTTCTGA	GGCCGTTCCG	GTGACTGGCC	GTAAAACCCC	TATACTT	CTAGTGG	478
AY616681	447	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	T		487
AY616682	447	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GT-AAACCCC	T		486
AY616683	447	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	T		487
AF176955	447	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTTT	CTAGTGG	501
AF176958	447	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTTT	CTAGTGG	501
AF176954	447	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTTT	CTAGTGG	501
L1	447	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTTT	CTAGTGG	501
L2	447	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTTT	CTAGTGG	501
AF176957	447	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTTT	CTAGTGG	501
AF176969	443	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTT	CTAGTGG	496
AF176968	443	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTT	CTAGTGG	496
AF176967	443	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTT	CTAGTGG	496
AY315403	444	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTT	CTAGTGG	497
AF176982	443	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTT	CTAGTGG	
AF176975	444	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTAAACCCCC	TATATTTTTT	CTAGTGG	500
AF176974	444	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTAAACCCCC	TATATTTTT	CTAGTGG	500
AF176973	444	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTAAACCCCC	TATATTTTTT	CTAGTGG	500
AF163022	441	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTT	CTAGTGG	494
AF163023	419	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTT	CTAGTGG	472
AF163021	420			GCGGCTTGCC	the second s			473
AF176981	443	TCGCTTCTGT	AGTTGTCCTG	GCGGCTTGCC	GTTAAACCCC	TATATTT	CTAGTGG	496

Figure 5C. (Continued).

		10		30	40	50	60	70	80	90	100	110	120	130			160	50
	4														see level.			955
SUT242	1																CTGAGGG	14.
SUT244	1						the second s						the second s		CCCTTCGGGG			14.
SUT251	1														CCCTTCGGGG			14
SUT081	1														CCCTTCGGGG			14:
SUT285	1										12.1						CTGAGGG	14:
SUT231	1														TCCTTCGGGC		-CTGTGAAGG	15
SUT058	1	CTGAGTTATC	AAAAACTCCA	ACCCTTTGTG	AACCT-ACCT	ATGTTTCCTC	CEGCETTCCE	CTTTAGCCTA	CCCACAGGGC	TCCCCTAAGG	GEGEGETTCTC	CTGGGGAGGT	GCCTGAGTGC	TACCTA-	TCCTTCGGGG	TACGGTTAGT	GCAGTGAAGG	15
SUT243	1	CTGAGTTATC	AAAAACTCCA	ACCCTTTGTG	AACCT-ACCT	ATGTTTCCTC	CEGCETTCCE	CTTTAGCCTA	CCCACAGGGC	TCCCCTAAGG	GGGGGGTTCTG	CTGGGGGAGGT	GCCTGAGTGC	TACCTA-	TCCTTCGGGG	TACGGTTAGT	GCAGTGAAGG	15:
AJ390409	1	CTGAGTTATC	AAAAACTCCA	ACCCTTTGTG	AACCT-ACCT	ATGTTTCCTC	CGGCGTACCG	CTTTAGCCTA	CCCACAGGGC	TCCCCTAAGG	GGGGGGTTCTG	CTGGGGAGGT	GCCTGAGTGC	TACCTA-	TCCTTCGGGG	TACGGTTAGT	GCAGTGAAGG	15!
SUT009	1	CTGAGTTATC	AAAAACTCCA	ACCCTTTGTG	AACCT-ACCT	ATGTTTCCTC	CGGCGTACCG	CTGTAGCCTA	CCCGCAGGGC	TCCCCCTTAG	GGGGGGTTTTG	CTGGGGAGGT	GCCTGAGTGC	TACCTA-	TCCTTCGGGG	TACGGTTAGT	GCAGTGAAGG	15
SUT010	1	CTGAGTTATC	AAAAACTCCA	ACCCTTTGTG	AACCT-ACCT	ATGTTTCCTC	CGGCGTACCG	CTGTAGCCTA	CCCGCAGGGC	TCCCCCTTAG	GGGGGGTTTTG	CTGGGGAGGT	GCCTGAGTGC	TACCTA-	TCCTTCGGGG	TACGGTTAGT	GCAGTGAAGG	15!
AJ390397	1	CTGAGTTATC	AAAAACTCCA	ACCCTTTGTG	AACCT-ACCT	ATGTTTCCTC	CGGCGTACCG	CTGTAGCCTA	CCCGCAGGGC	TCCCCCTTAG	GGGGGGTTTTG	CTGGGGAGGT	GCCTGAGTGC	TACCTA-	TCCTTCGGGG	TACGGTTAGT	GCAGTGAAGG	15!
SUT098 H.urceolatum	1	CTGAGTTTAC	CARARCTCCA	ACCCTTTGTG	AACCT-ACTA	CTGTTTCCTC	CGGCGTAACG	CTTTAGCCTA	CCTACAGGGC	ATTCTTTTGG	GGATGTTCTG	CTAGGGAGGT	GCCCGAAGCA	C	-CCTTC	TTAA-		12:
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SUT244	142															GCCTTTCTTC		291
SUT251		TGCCG-CTAA	GGCCGGCCGC	AGCGCCGT	TAAACTGTTC	CAAAATACTT	TGTCCAACTC	TACCCTATAG	AACTAATCGT	TCGAATCTCT	TATCTCGAGG	CTTTTCTTT	TGCCTTGAGG	CTGAGGCTTT	TCCTTGCCTT	GCCTTTCTTC GCCTTTCTTC	CAGTTCAAAT	291 291
	142	TGCCG-CTAA TGCCG-CTAA	GGCCGGCCGC GGCCGGCCGC	AGCGCCGT AGCGCCGT	TAAACTGTTC TAAACTGTTC	CAAAATACTT CAAAATACTT	TGTCCAACTC TGTCCAACTC	TACCCTATAG TACCCTATAG	AACTAATCGT AACCAATCGT	TCGAATCTCT TCGAATCTCT	TATCTCGAGG TATCTCGAGG	CTTTTCTTTT CTTTTCTTTT	TGCCTTGAGG TGCCTTGAGG	CTGAGGCTTT CTGAGGCTTT	TCCTTGCCTT	GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC	CAGTTCAAAT CAGTTCAAAT	291 291 291
SUT251	142 142	TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA	GGCCGGCCGC GGCCGGCCGC GGCCGGCCGC	AGCGCCGT AGCGCCGT AGCGCCGT	TAAACTGTTC TAAACTGTTC TAAACTGTTC	СААААТАСТТ СААААТАСТТ СААААТАСТТ	TGTCCAACTC TGTCCAACTC TGTCCAACTC	ТАСССТАТАС ТАСССТАТАС ТАСССТАТАС	AACTAATCGT AACCAATCGT AACCAATCGT	TCGAATCTCT TCGAATCTCT TCGAATCTCT	TATCTCGAGG TATCTCGAGG TATCTCGAGG	CTTTTCTTT CTTTTCTTTT CTTTTCTTTT	TGCCTTGAGG TGCCTTGAGG TGCCTTGAGG	CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT	TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT	GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC	CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT	291 291 291 291
SUT251 SUT081	142 142 142	TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA	GGCCGGCCGC GGCCGGCCGC GGCCGGCCGC GGCCGGCCGC	AGCGCCGT AGCGCCGT AGCGCCGT AGCGCCGT	TAAACTGTTC TAAACTGTTC TAAACTGTTC TAAACTGTTC	СААААТАСТТ СААААТАСТТ СААААТАСТТ СААААТАСТТ	TGTCCAACTC TGTCCAACTC TGTCCAACTC TGTCCAACTC	ТАСССТАТАЯ ТАСССТАТАЯ ТАСССТАТАЯ ТАСССТАТАЯ	AACTAATCGT AACCAATCGT AACCAATCGT AACTAATCGT	TCGAATCTCT TCGAATCTCT TCGAATCTCT TCGAATCTCT	TATCTCGAGG TATCTCGAGG TATCTCGAGG TATCTCGAGG	CTTTTCTTT CTTTTCTTTT CTTTTCTTTT CTTTTCTTTT	TGCCTTGAGG TGCCTTGAGG TGCCTTGAGG TGCCTTGAGG	CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT	TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT	GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC	САСТТСАААТ САСТТСАААТ САСТТСАААТ САСТТСАААТ	291 291 291 291 291 31
SUT251 SUT081 SUT285	142 142 142 158	ТСССС-СТАА ТСССС-СТАА ТСССС-СТАА ТСССС-СТАА ТСССС-СТАА	GCCGGCCGC GCCGGCCGC GCCGGCCGC GCCGGCCGC GCCGGCCGC GCCCT-CGCT	AGCGCCGT AGCGCCGT AGCGCCGT AGCGCCGT AGCGCCCGAG	TAAACTGTTC TAAACTGTTC TAAACTGTTC TAAACTGTTC AGGACCGCTC	СААААТАСТТ СААААТАСТТ СААААТАСТТ СААААТАСТТ СААААТАСТТ СААССТСТТТ	TGTCCAACTC TGTCCAACTC TGTCCAACTC TGTCCAACTC TTTCCAACTC	ТАСССТАТАС ТАСССТАТАС ТАСССТАТАС ТАСССТАТАС ТТССТАСААС	ААСТААТССТ ААССААТССТ ААССААТССТ ААСТААТССТ АААСССТТАТ	TCGAATCTCT TCGAATCTCT TCGAATCTCT TCGAATCTCT -CGAAGGAAC	TATCTCGAGG TATCTCGAGG TATCTCGAGG TATCTCGAGG TATCTCGAGG	CTTTTCTTT CTTTTCTTTT CTTTTCTTTT CTTTTCTTTT AAAACCACTC	TGCCTTGAGG TGCCTTGAGG TGCCTTGAGG TGCCTTGAGG GAAAAAAAA	CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT TCGAAGAAAA	TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT	GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC AACTATCGAA	САСТТСАААТ САСТТСАААТ САСТТСАААТ САСТТСАААТ САСТТСАААТ СААТТСААТ	291 291 291 291 291 311 271
SUT251 SUT081 SUT285 SUT231	142 142 142 158 156	TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCGACCAA TGCTGACCAA	GGCCGGCCGC GGCCGGCCGC GGCCGGCCGC GGCCGCC	AGCGCCGT AGCGCCGT AGCGCCGT AGCGCC-CGT AGCGCCCGAG GGCGCCGAGT	TAAACTGTTC TAAACTGTTC TAAACTGTTC TAAACTGTTC AGGACCGCTC AGGACCGCTC	САЛЛАТАСТТ САЛЛАТАСТТ САЛЛАТАСТТ САЛЛАТАСТТ САЛАСТСТТТ САЛАСТТ	TGTCCAACTC TGTCCAACTC TGTCCAACTC TGTCCAACTC TTTCCAACTC	ТАСССТАТАС ТАСССТАТАС ТАСССТАТАС ТАСССТАТАС ТТССТАСААС	ААСТААТССТ ААССААТССТ ААССААТССТ ААСТААТССТ АААСССТТАТ АААСССТТАТ	TCGAATCTCT TCGAATCTCT TCGAATCTCT TCGAATCTCT -CGAACGAAC -TGCA	TATCTCGAGG TATCTCGAGG TATCTCGAGG TATCTCGAGG TATTCCAATA TCCAACC	CTTTTCTTT CTTTTCTTTT CTTTTCTTTT CTTTTCTTTT AAAACCACTC CCGCGT	TGCCTTGAGG TGCCTTGAGG TGCCTTGAGG TGCCTTGAGG GAAAAAAAA TGAACAACTA	CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT TCGAAGAAAA TCGAAAAT	TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TTATCGAAGT	GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC AACTATCGAA TTTTTTTCTT	CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT GAAATTATCG TACGCTA	291 291 291 31
SUT251 SUT081 SUT285 SUT231 SUT058	142 142 142 158 156 156	TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCGACCAA TGCTGACCAA TGCTGACCAA	GGCCGGCCGC GGCCGGCCGC GGCCGGCCGC GGCCGCC	AGCGCCGT AGCGCCGT AGCGCCGT AGCGCC-CGT AGCGCCCGAG GGCGCCGAGT	TARACTGTTC TARACTGTTC TARACTGTTC TARACTGTTC AGGACCGCTC AGGACCGCTC	САЛАЛТАСТТ САЛАЛТАСТТ САЛАЛТАСТТ САЛАЛТАСТТ САЛАСТСТТТ САЛАСТТ САЛАСТТ	TGTCCAACTC TGTCCAACTC TGTCCAACTC TGTCCAACTC TTTCCAACTC	ТАСССТАТАС ТАСССТАТАС ТАСССТАТАС ТАСССТАТАС ТАСССТАТАС ТТССТАСААС	ААСТААТССТ ААССААТССТ ААССААТССТ ААСТААТССТ АААСССТТАТ ААССАССТАС ААССАССТАС	TCGAATCTCT TCGAATCTCT TCGAATCTCT TCGAATCTCT -CGAACGAAC -TGCA -TGCA	TATCTCGAGG TATCTCGAGG TATCTCGAGG TATCTCGAGG TATTCCAATA TCCAACC TCCAACC	CTTTTCTTT CTTTTCTTTT CTTTTCTTTT CTTTTCTTTT AAAACCACTC CCGCGT	TGCCTTGAGG TGCCTTGAGG TGCCTTGAGG TGCCTTGAGG GAAAAAAATA TGAACAACTA TGAACAACTA	CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT TCGAAGAAAA TCGAAAAT TCGAAAAT	TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TTATCGAAGT CTGCTTTTGC	GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC AACTATCGAA TTTTTTTCTT TTTTTTTCTT	CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT GAAATTATCG TACGCTA TACGCTA	291 291 291 311 271
SUT251 SUT261 SUT265 SUT231 SUT058 SUT243	142 142 142 158 156 156	TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCGACCAA TGCTGACCAA TGCTGACCAA	GCCCGCCGC GCCCGCCGC GCCCGCCGC GCCCGCCGC GCCT-CGC GCCT-CGC GCCT-CGC GCCT-CGC	AGCGCCGT AGCGCCGT AGCGCCGT AGCGCCCGAG GGCGCCGAGT GGCGCCGAGT GGCGCCGAGT	TAAACTGTTC TAAACTGTTC TAAACTGTTC TAAACTGTTC AGGACCGCTC AGGACCGCTC AGGACCGCTC	СААААТАСТТ СААААТАСТТ СААААТАСТТ СААААТАСТТ САААСТСТТТ САААСТТ САААСТТ САААСТТ	TGTCCAACTC TGTCCAACTC TGTCCAACTC TGTCCAACTC TTTCCAACTC	TACCOTATAG TACCOTATAG TACCOTATAG TACCOTATAG TTGCTAGAAC	ААСТААТССТ ААССААТССТ ААССААТССТ ААСТААТССТ АААСССТТАТ ААССАССТАС ААССАССТАС ААССАССТАС	TCGAATCTCT TCGAATCTCT TCGAATCTCT TCGAATCTCT -CGAACGAAC -TGCA -TGCA	TATCTCGAGG TATCTCGAGG TATCTCGAGG TATCTCGAGG TATTCCAATA TCCAACC TCCAACC	CTTTTCTTTT CTTTTCTTTT CTTTTCTTTT AMAACCACTC CCGCGT CCGCGT	ТСССТТСАСС ТСССТТСАСС ТСССТТСАСС ТСССТТСАСС СЛАВАЛАЛАТА ТСАВСАВСТА ТСАВСАВСТА ТСАВСАВСТА	CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT CCGAAGAAAA TCGAA-AAT TCGAA-AAT	TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TTATCGAAGT CTGCTTTGC CTGCTTTTGC	GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC AACTATCGAA TTTTTTCTT TTTTTTTCTT	CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT GAAATTATCG TACGCTA TACGCTA TACGCTA	291 291 291 311 271 271
SUT251 SUT285 SUT285 SUT231 SUT058 SUT243 AJ390409	142 142 142 158 156 156 156	TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCGACCAA TGCTGACCAA TGCTGACCAA TGCTGACCAA	GCCCGCCGC GCCCGCCGC GCCCGCCGC GCCCGCCGC GCCT-CGCT GCCT-CGCC GCCT-CGCC GCCT-CGCC GCCT-CGCC	AGCGCCGT AGCGCCGT AGCGCCGT AGCGCCGT AGCGCCCGAG GGCGCCGAGT GGCGCCGAGT GGCGCCGAGT	TAAACTGTTC TAAACTGTTC TAAACTGTTC AGGACCGCTC AGGACCGCTC AGGACCGCTC AGGACCGCTC	САЛАЛТАСТТ САЛАЛТАСТТ САЛАЛТАСТТ САЛАСТСТТ САЛАСТСТТ САЛАСТСТТ САЛАСТТТ САЛАСТТТ САЛАСТТТ САЛАСТТТ	TGTCCAACTC TGTCCAACTC TGTCCAACTC TGTCCAACTC TTTCCAACTC	TACCCTATAG TACCCTATAG TACCCTATAG TACCCTATAG TTGCTAGAAC	ААСТААТССТ ААССААТССТ ААСТААТССТ ААСТААТССТ ААСТААТССТ ААССАСТТАС ААССАССТАС ААССАССТАС ААССАСТАС ААССАСТАС	TCGAATCTCT TCGAATCTCT TCGAATCTCT -CGAATCTCT -TGCA -TGCA -TGCA	TATCTCGAGG TATCTCGAGG TATCTCGAGG TATCTCGAGG TATCCCAAGA TCCAACC TCCAACC TCCAACC	CTTTTCTTTT CTTTTCTTTT CTTTTCTTTT ANAACCACTC CCGCGT CCGCGT CCGCGT	ТСССТТСАСС ТСССТТСАСС ТСССТТСАСС ТСССТТСАСС САЛАЛАЛАТА ТСАЛСЛАСТА ТСАЛСЛАСТА ТСАЛСЛАСТА ТСАЛСЛАСТА	CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT TCGAAGAAAA TCGAA-AAT TCGAA-AAT TCGAA-AAT	TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TTATCGAAGT CTGCTTTTGC CTGCTTTTGC CTGCTTTTGC	GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC AACTATCGAA TTTTTTTCTT TTTTTTTCTT TTTTTTTCTT	CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT GAATTATCG TACGCTA TACGCTA TACGCTA TACGCTA	291 291 291 311 271 271 271
SUT251 SUT285 SUT285 SUT281 SUT058 SUT243 AJ390409 SUT009	142 142 142 158 156 156 156 156	TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCGACCAA TGCTGACCAA TGCTGACCAA TGCTGACCAA TGCCGACCAA	GCCCGCCGC GCCCGCCGC GCCCGCCGC GCCCGCCGC GCCT-CGGC GCCT-CGGC GCCT-CGGC GCCT-CGGC GCCT-CGGC	AGCGCCGT AGCGCCGT AGCGCCGT AGCGCCGT AGCGCCCGAGT GGCGCCGAGT GGCGCCGAGT GGCGCCGAGT GGCGCCGAGT	TAAACTGTTC TAAACTGTTC TAAACTGTTC TAAACTGTTC AGGACCGCTC AGGACCGCTC AGGACCGCTC AGGACCGCTC AGGACCGCTC	САЛАЛТАСТТ САЛАЛТАСТТ САЛАЛТАСТТ САЛАТТАСТТ САЛАСТСТТТ САЛАСТТ САЛАСТТ САЛАСТТ САЛАСТТ САЛАСТТ	TGTCCAACTC TGTCCAACTC TGTCCAACTC TGTCCAACTC TTTCCAACTC	TACCCTATAG TACCCTATAG TACCCTATAG TACCCTATAG TTGCTAGAAC	ААСТААТССТ ААССААТССТ ААССААТССТ ААСТААТССТ ААССАСТТАТ ААССАССТАС ААССАССТАС ААССАССТАС ААССАССТАС ААССАСТТАС ААССАСТТАС	TCGAATCTCT TCGAATCTCT TCGAATCTCT TCGAACGAAC -TGCA -TGCA -TGCA -TGCA	TATCTCGAGG TATCTCGAGG TATCTCGAGG TATCTCGAGG TATCCCAACC TCCAACC TCCAACC TCCAACC TCCAACC	CTTTTCTTT CTTTTCTTTT CTTTTCTTTT CTTTCCTTT ANACCACTC CCGCGT CCGCGT CCGCGT CCGCGT	ТСССТТСАСС ТСССТТСАСС ТСССТТСАСС ТСССТТСАСС СЛАВАЛАЛТА ТСААСЛАСТА ТСААСЛАСТА ТСААСЛАСТА ТСААСЛАСТА ТСААСЛАСТА	CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT TCGAACAAAT TCGAACAAT TCGAACAAT TCGAACAAT TCGAACAAT	TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TTATCGAAGT CTGCTTTGC CTGCTTTTGC CTGCTTTTGC TTGCTTTTGC	GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC AACTATCGAA TTTTTTTCTT TTTTTTTCTT TTTTTTTCTT TTTTTT	CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT GAAATTATCG TACGCTA TACGCTA TACGCTA TACGCTA	291 291 291 311 27: 27: 27: 27: 27:
SUT251 SUT285 SUT285 SUT231 SUT233 SUT243 AJ390409 SUT009 SUT010	142 142 142 158 156 156 156 156 156	TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCG-CTAA TGCCGACCAA TGCTGACCAA TGCTGACCAA TGCCGACCAA TGCCGACCAA	GeccGeccGe GeccGeccGe GeccGeccGe GeccGecc	AGCGCCGT AGCGCCGT AGCGCCGT AGCGCC-CGT AGCGCCCGAGT GGCGCCGAGT GGCGCCGAGT GGCGCCGAGT GGCGCCGAGT	TAAACTGTTC TAAACTGTTC TAAACTGTTC TAAACTGTTC AGGACCGCTC AGGACCGCTC AGGACCGCTC AGGACCGCTC AGGACCGCTC AGGACCGCTC	САЛАЛТАСТТ САЛАЛТАСТТ САЛАТАСТТ САЛАСТССТСТТ САЛАСТСТСТС САЛАСТТ САЛАСТТ САЛАСТТ САЛАСТТ САЛАСТТ САЛАСТТ	TGTCCAACTC TGTCCAACTC TGTCCAACTC TGTCCAACTC TTTCCAACTC	TACCCTATAG TACCCTATAG TACCCTATAG TACCCTATAG TTGCTAGAAC	ААСТААТССТ ААССААТССТ ААССААТССТ ААСТААТССТ ААССССТТАТ ААССАССТАС ААССАССТАС ААССАСТТАС ААССАСТТАС ААССАСТТАС ААССАСТТАС	TCGAATCTCT TCGAATCTCT TCGAATCTCT -CGAACCTCT -CGAACGAC -TGCA -TGCA -TGCA -TGCA	TATCTCGAGG TATCTCGAGG TATCTCGAGG TATCCCAAGA TATTCCAAGA TCCAACC TCCAACC TCCAACC TCCAACC	CTTTTCTTT CTTTTCTTTT CTTTTCTTTT CTTTTCTTTT ANARCCACTC CCGCGT CCGCGT CCGCGT CCGCGT	ТСССТТСАСС ТСССТТСАСС ТСССТТСАСС ТСССТТСАСС СЛАВЛАЛАТА ТСААСААСТА ТСААСААСТА ТСААСААСТА ТСААСААСТА ТСААСААСТА	CTGAGGCTTT CTGAGGCTTT CTGAGGCTTT TCGAAGAAAA TCGAA-AAT TCGAA-AAT TCGAA-AAT TCGAA-AAT	TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TCCTTGCCTT TTATCGAACT CTGCTTTGC CTGCTTTTGC CTGCTTTTGC TTGCTTTTGC TTGCTTTTGC TTGCTTTTGC	GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC GCCTTTCTTC AACTATCGAA TTTTTTCTT TTTTTTCTT TTTTTTCTT TTTTTTCTT TTTTTT	CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT CAGTTCAAAT GAAATTATCG TACGCTA TACGCTA TACGCTA TACGCTA	291 291 311 27: 27: 27: 27: 27: 27: 27: 27: 27:

Figure 6C. The ITS1-5.8S-ITS2 sequence alignment of *Hypoxylon* sect. *Annulata*, *Hypoxylon* cf. *nitens* (SUT242, SUT244, SUT251, SUT081, SUT285), *H.* cf. *stygium* (SUT231), *H. stygium* (SUT058, SUT243, AJ390409), *H. atroroseum* (SUT009, SUT010, AJ390397), *H. urceolatum* (SUT098), by using ClustalX and BioEdit programs for phylogenetic tree construction in Figure 90. Arrows indicate the start and the end of 5.8S rDNA sequences.
		33	0 34	0 35	0 36	37(	) 38	0 39	0 40	41	0 42	0 43	0 440	45	0 46	0 47	480	
					11													
SUT242																	TACTGTTTTT	
SUT244 SUT251					TTTACGATGT TTTACGATGT													458
SUT081					TTTACGACGT													458
SUT285					TTTACGATGT													458
SUT231					CTATAATGAT													462
SUT058					TTGGAATTAT													382
SUT243	274	AAACGTC	TTT	CCCGG	TTGGAATTAT	TGCTCGAAAT	AATAATTTCT	TTACCCTGCA	GTCGTTTGTT	TTCAAGCTAC	AATAT			CTGC	TCGAAA	ATTGTTCAAA	GCTCTGAGG-	382
AJ390409		ARACGTC			TTGGAATTAT													382
SUT009					TTGGAATTAT													411
SUT010 AJ390397					TTGGAATTAT TTGGAATTAT													411
SUT098 H.urceolatum					CTAGAAT													322
Soloso_n. alceolatan_	214	TOTOLO	Ch	ACTOL AC	CIAOAA	Adenaceoni	CONNITINGI	00011114444	111010	ALCOARTIT	JIII CAAA		-	ACTING	101100	AATTIAOTOO	Gerranare	262
		49	0 50	0 51	0 521	530	54	0 55	56	57	0 59	0 59	600	61	62	630	640	8
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SUT242					ATTTTTC													615
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SUT251 SUT081		and the second second second	a second s		ATTTTTC ATTTT-C			271 21 21 21 21 21 21 21 21 21 21 21 21 21	Sector Sector Sector Sector		Contraction and the second second			Contraction of the second s		and the second		615
SUT285					ATTTTTC													615
SUT231					ATTAAATCTC													621
SUT058					CAAAAGCCAC													526
SUT243	382	GGTCT	GAATGAATTC	ATAAAATTGG	CAAAAGCCAC	-CTATAAACT	ACGGTT-CTT	AGGGGGTGAT	C-AAACCAAG	GTTTTA	AAAACCA	AAT-ACGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	526
AJ390409	382	GGTCT	GAATGAATTC	ATAAAATTGG	CAAAAGCCAC	-CTATAAACT	ACCCTT-CTT	AGGGGGTGAT	C-AAACCAAG	GTTTTA	AAAACCA	AAT-ACGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	526
SUT009					CAAAAGCCAC													555
SUT010					CAAAAGCCAC													554
AJ390397					CARAAGCCAC													555 446
SUT098_H.urceolatum_	344	T	GAGGGCTATT	CTAGC	GAT	CAUTAGOT	CTUATCUCAT	TAACCCTAAC	TGTTTAA	ATTAAA	CAAATTA	ACTT	AAACTITCAA	CAACUGATCT	CITCOTICIC	GCATCOATGA	AGAACGCAGC	940
		and the second	· · · · · · · · · · · · · · · · · · ·	and so and		and see 1	to a la constitu	and sould	see been	and been li	second second			and seed	and a local l	and south	eres been	
SUT242	616				TTCAGTGAAT													771
SUT244	616	GARATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTOGGCATC	CCTATTCGAG	COTCATTACA	ACCOTTAAGC	CCTGTTGCTT	AGCGTTGGGA	ATCTACGGCT	TAGGCG-	771
SUT251	616	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGCATG	CCTATTCGAG	CGTCATTACA	ACCOTTAAGC	CCTGTTGCTT	AGCGTTGGGA	ATCTACGGCT	TAGGCG-	771
SUT081					TTCAGTGAAT													765
SUT285					TTCAGTGAAT													771
SUT231 SUT058					TTCAGTGAAT TTCAGTGAAT													778
SUT243					TTCAGTGAAT													683
AJ390409					TTCAGTGAAT													686
SUT009					TTCAGTGAAT													715
SUT010	555	GAAATGCGAT	AAGTAATGTG	AATGGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCCC	ATTGCGCCCA	ATAAGAATTT	AGTGGGCATG	CCTATTCGAG	CGTCATTATA	ACCCTTAAGC	CTTGTTGCTT	AACCGTGGGA	ATCTACCCCT	CACTGAGGGG	714
AJ390397					TTCAGTGAAT													
SUT098_H.urceolatum_	447	GAAATGCGAT	AAGTAATGCG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCG	CTAGCATTCT	AGCGGGGCATG	CCTATTCGAG	CGTCATTACG	ACCOTTAAGC	CTTGTCGTTT	AGCGTTGGGA	ATCTGCGGTT	TAGGCCG	603
		81		83	0 84						90 90	0 91	0					
SUT242	772				GCACTCTT													
SUT244	772	TAGTTCCTCA	AAATTAGTGG	CGGAGTTATA	GCACTCTC	GGCGTAGTAA	TTTGCCTCGC	TTCTGAGCTG	-CTGTAGCTG	CCTGCCGT	-AAAACCC-T	ATA-CTTCTA	GT 875					
SUT251	772	TAGTTCCTCA	AAATTAGTGG	CGGAGTTATA	GCACTCTT	AGCGTAGTAA	TTTGCCTCGC	TTCTGAGCTG	-CTGTAGCTG	CCTGCCGT	-AAAACCCCT	ATA-CTTCTA	GT 876					
SUT081					GCACTCTC													
SUT285					GCACTCTC													
SUT231					GCACCCCCTA													
SUT058 SUT243					GCACACTCTA GCACACTCTA													
AJ390409					GCACACTOTA													
SUT009					GCCCACTCTA													
SUT010					GCCCACTCTA													
AJ390397	716	TAGTTCCTTA	AATGTAGTGG	COGGOTTATA	GCACACTCTA	AGCGTAGTAG	TTTAACTCGC	TTTCAGGGAG	GCTGTAGCTG	CTTGCCGT	AAAACCCCTT	ATAACTTATA	GT 825					
SUT098_H.urceolatum_	604	CAGTTCCTTA	AATTCAGTGG	CGGAGTTATA	GCACACCCTA	AGCGTAGTAA	CTTACATCGC	TCCTGGGGAG	TCTATAGCGG	CCTGCCGTTA	AAAAACCCCT	ATA-TTTCTA	GT 714					

Figure 6C. (Continued).

		10	20	30	40	50	60	70	80	90	100	0 110	12	0 130	) 140	150	)
			· · · ·														
SUT103	1	CCGAGTT-AA ACAA															
SUT105	1	CCGAGTT-AA ACAA	AACTCC /	AAA-CCCTTT	GTGAACCTTA	CCAAAGTTGC	CTCGGCGTGA	GCT-GCGG-T	TACCCTGTAG	TTACC	CTGGAGGCGT	CTACCCTGTA	GGTG	C	TTACCCTGGA	GC-TACCTTG	125
ST2333	1	ACGAGTT-AA ACAA	AACTCC 2	AAA-CCCTTT	GTGAACCTTA	CCAAAGTTGC	CTCGGCGTGA	GCT-GCGG-T	TACCCTGTAG	TTACC	CTGAAAGCGT	CTACCCTGTA	GGTG	C	TTACCCTGGA	GC-TACCCTG	125
ST2527	1	ACGAGTT-AA ACAA	AACTCC /	AAA-CCCTTT	GTGAACCTTA	CCAAAGTTGC	CTCGGCGTGA	GCT-GCGG-T	TACCCTGTAG	TTACC	CTGGAAGCGT	TTACCCTATA	GGTGTTTACC	CTATAGTAGC	TTACCCTGGA	GC-TACCCTG	140
ST2336	1	AAGAGTAT AACA	ACTCCC 1	AAACCCAT	<b>GTGAACATAC</b>	CTCATGTTGC	CTCGGCAGGC	CTC-GC		CTC	TCTCGTAGGC	CTTACCCTGT	AAGG	C	ATACCCGGGA	GGCG	103
AJ390395	1	CCGAGTT-AT CACA	ACTCC- 2	AACCCTTT	GTGAACCTTA	CCGTCGTTTC	CTCGGCGCAC	TGC			TGTGGGAGG-	CTACCCTGTA	GCGGTT	GT	TTACCCTACA	GGACG	101
ST2584	1	CTGAGTCC CCCA	AAACTC	CAA-CCCTTT	GTGAACCT-A	CCACAGTTTC	CTCCGCGCAA	ACGCCCTAG-	CCTAACCTAG	GCCTGGGCGC	CGCCGAGAGG	ACAATGCTCC	AACACTTATA	TCCAAC-CCT	ACTACCTAGG	ACACAACCGA	144
SUT001	1	CTGAGTAT CAAA	AACTTC	CAAACCCTTT	GTGAACCT-A	CCGCCGTTGC	CTCGGCGCGC	GCT-GCGGCT	ACCCGCC	CCGG-A	CAGAAGGGCA	GCTGCCTGTG	AGGGCCGCTG	TAAAC	CCTTCCGTCC	AGGTACCGGC	133
SUT004	1	CTGAGTAT CAAA	AACTTC (	CAAACCCTTT	GTGAACCT-A	CCGCCGTTGC	CTCGGCGCGC	GCT-GCGGCT	ACCCGCC	CCGG-A	CAGAAGGGCA	GCTGCCTGTG	AGGGCCGCTG	TAAAC	CCTTCCGTCC	AGGTACCGGC	133
ST2485	1	CTGAGTT-AT CAAA	AACTCC 2	AAAACCCTTT	GTGAACCT-A	CCGCCGTTGC	CTCGGCGCGC	GCT-GCGGCT	ACCCGCC	CCGG-A	CAGAAGGGCA	GCTGCCTGTG	AGGGCCGCTG	TAAAC	CCTTCCGTCC	AGGTACCGGC	134
SUT005	1	CTGAGTAT CAAA	AACTTC	CAAACCCTTT	GTGAACCT-A	CCGCCGTTGC	CTCGGCGCGC	GCT-GCGGCT	ACCCGCC	CCGG-A	CAGAAGGGCA	GCTGCCTGTG	AAGGCCGCTG	TAAAC	CCTTCCGTCC	AGGTACCGGC	133
SUT262	1	CTGAGTT-AT CAAA	AACTCC 2	AAAACCCTTT	GTGAACCT-A	CCGCCGTTGC	CTCGGCGCGC	GCT-GCGGCT	ACCCGCC	CCGG-A	CAGAAGGGCA	GCTGCCTGTG	AGGGCCGCTG	TAAAC	CCTTCCGTCC	AGGTACCGGC	134
ST2448	1	CTGAGTT-AT CAAA	AACTCC 2	AAAACCCTTT	GTGAACCT-A	CCGCCGTTGC	CTCGGCGCGC	GCT-GCGGCT	ACCCGCC	CCGG-A	CAGAAGGGCG	GCTGCCTGTG	AGGGCCGCTG	TAAAC	CCTTCCGTCC	AGGTACCGGC	134
SUT167	1	CTGAGTT-AT CAAA	AACTCC 2	AAAACCCTTT	GTGAACCT-A	CCGCCGTTGC	CTCGGCGCGC	GCT-GCGGCT	ACCCGCC	CCGG-A	CAGAAGGGCA	GCTGCCTGTG	AGGGCCGCTG	TAAAC	CCTTCCGTCC	AGGTACCGGC	134
Ju2	1	GCGAGTTCAT CAAA	AACTCC 2	AAAACCCTTT	GTGAACCTTA	CCGCAGTTGC	CTCGGCGTGC	GCC-GCGGCC	GTTGGGC	CTGCTG	CAGGCCAACG	GCCCCCCGAA	ACGGGGGGCGG	GTGGG	GTTACC-GGC	AGGCCCC-G-	134
ST2579	1	GCGAGTTCAT CAAA	AACTCC 2	AAAACCCTTT	GTGAACCTTA	CCGCAGTTGC	CTCGGCGTGC	GCC-GCGGCC	GTTGGGC	CTGCTG	CAGGCCAACG	GCCCCCCGAA	ACGGGGGGCGG	GTGGG	GTAACC-GGC	AGGCCCCCG-	135
ST2406	1	GCGAGTT-AC CAAA	AACTCC 2	AAAACCCTTT	GTGAACCTTA	CCGCAGTTGC	CTCGGCGTGC	GCC-GCGGCC	GTTGGGC	CTGCTG	CAGCCCACCG	GCCCCCCGAA	ACGGGGGGCGG	GTGGG	GTTACC-GGC	AGGCCCCCG-	134
SUT025	1	GCGAGTTACC ACAG	AACTCC	AAAACCCTTT	GTGAACCTTA	CCGCAGTTGC	CTCGGCGAGT	GCT-GCGGCT	ATATCCC	CTGTCC	C	-CCGCCCGTC	AGGGCTGCGG	G	GC	AGGCTCT-A-	113
SUT220	1	CAGAGTTGTC GGAA	AAACTC	CATACCCTTT	GTGAACCTAC	<b>CTATCGTTGC</b>	CTCCGCCCCC	GCT-GCGGCT	GACGTCCGGA	AGAGCTGCTC	CCCCTCCT	AAGGCCCTGG	AATTCCGGGGG	GGGGC	TTTTCT-TCC	GGGCTTTAG-	140
ST2332	1	GCGAGCTGTC GGAA	AATCTC	CATACCCTTT	GTGAACCTAC	CTATCGTTGC	CTCGGCGCCC	GCTAGCGGCT	GACGTCCGAA	AGAGCTGCTC	CCCCTCCT	ATGGCCCTGG	A-TTCCGGGG	GGGGC	TTTTCT-TCC	AGGCTTTAG-	140
AJ390406	1	CTGAGAGTAA AACA	AAACTC	CAAACCCTTT	GTGAACCTTA	CCTTAGTTGC	CTCGGCGTGC	GCC			GCG	GCTACCCGGG	AGGACCGCTG	TAGGGCG	GTTACCCTGT	AGCC	107
ST2436	1	CTGAGTT-TC TAAC	AACTCC	-AA-CCCTTT	GTCGAACCTA	CCACTGTTTC	CTCGGCGTAC	TGCCGCGGC-				CTCTGG	GCCGC		TGC	AG	82
ST2473	1	CTGAGTT-TC TAAC	AACTCC	CAA-CCCTTT	GTCGAACCTA	CCACTGTTTC	CTCGGCGTAC	TGCCGCGGC-				CTCTGG	GCCGC		TGC	AG	83
Jul	1	CTGAGTT-TT TAAC	AACTCC	-AA-CCCTTT	<b>GTCGAATCTA</b>	CCACTGTTTC	CTCGGCGTAC	TGCCGCGGC-				CTCTGG	GCCGC		<b>T</b> GC	AG	82
ST2313_H.n	1	CTGAGTT-TC TAAC	AACTCC	-AA-CCCTTT	GTCGAACCTA	CCACTGTTTC	CTCGGCGTAC	TGCCGCGGC-				CTCTGG	GCCGC		TGC	AG	82

Figure 7C. The ITS1-5.8S-ITS2 sequence alignment of *Hypoxylon* sect. *Annulata*, *Hypoxylon* cf. archeri (SUT103, SUT105, ST2333, ST2527), *Hypoxylon* sp. ST2336, *H. annulatum* (AJ390395), *H. leptascum* var. *microsporum* (ST2584), *H. purpureonitens* (SUT001, SUT004, SUT005, SUT167, SUT262, ST2485, ST2448), *H. bovei* var. *microspora* (Ju2, ST2579, ST2406, SUT025), *H. moriforme* (SUT220, ST2332), *H. cohaerens* (AJ390406), *H. nitens* (Ju1, ST2436, ST2473, ST2313) by using ClustalX and BioEdit programs for phylogenetic tree construction in Figure 91. Arrows indicate the start and the end of 5.8S rDNA sequences.

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160         170         180         190         200         210         220         230         240         250         260         270	280 290 300
SUT103 126 TAACCGGCTA ACGCCCC-G CCGAAGGACCACTAA ACTCTGTTTT TACCCAAGTG TATCTCTGAA TGCTTCAA CTA-AATAA GTTAAAACTT TCAACAACGG ATCTCTTGGT TCTGG	
SUT105 126 TAACCGGCTA ACGGCCC-G CCGAAGGACCACTAA ACTCTGTTT TACCCAAGTG TATCTCTGAA TGCTTCAA CTA-AATAA GTTAAAACTT TCHACAACGG ATCTCTTGGT TCTGG	SCATCG ATGAAGAACG CAGCGAAATG 264
ST2333 126 TAACCGGCTA ACGGCCCG CCGAAGGACCACTAA ACTCTGTTTT TACCCAAGTG TATCTCTGAA TGCTTCAA CTAAATAA GTTAAAACTT TCAACAACGG ATCTCTTGGT TCTGG	SCATCG ATGAAGAACG CAGCGAAATG 264
ST2527 141 TAACCGGCTA ACGGCCCG CCGAAGGACCACTAA ACTCTGTTT TACCCAAGTG TATCTCTGAA TGCTTCAA CTA-AATAA GTTAAAACTT TCAACAACGG ATCTCTTGGT TCTGG	
ST235 104 CGGCACCCC-TG CCGCGGCGCCCACGAA ACTCTGTCTC -ATCGTT GAGTTCTGAA CCCATAAC -TAAATAA GTTAAAACTT TCAACAACGG ATCTCTTGGT TCTGG	
AJ390395 102 CACCCTGCAG CGCGCGCCTACCAA AGCCTTTTACCCAAGT -ACCTCGAAC AATTTAC-AATA- GTTTAAAGTT TCAACAAGG ACCTCTTGGT TCGG	
ST2584 145 TCGAGGCCTG CGGGCTTAAA TCTTAGGGCT TCTCTAGCGA CCAGTAGGGT CTGAATGGCG TTAAACCTAA C-TGTTTAAA TTAAAACAAA TCAACAACTT TCAACAACGG ATCTTTGGT TCTGG SUT001 134 TAGCCGGCTG AACAGCCC-G CCGAAGGA CCGCTATAAA AACTCTCGCT CCGGCGTGTA TACCTTCTGA A-TCTTCCAA CTTATAATGA GTTAAAACTT TCAACAACGG ATCTTTGGT TCTGG	
SUT01 134 TRECOGOUS ARAGEC-S CORAGE- COCTATAR ARCTICOS CONSULTA ATCENTICA ATCENTICA CITATARISA SITAMACTI TCAACARGE ATCENTICA TOTAS	
ST2485 135 TAGCCGCCCG AACAGCCC-G CCGAAGGA CCGCTGTATAA AACTCTCCGC CCGGCGTGTA TACCTTCGA A-TCTTCCCA CTTATAAGA GTTAAAACTT TCAACAACGG ATCTCTGGT TCTGG	
SUT005 134 TAGCCGGCTG AACAGCCC-G CCGAAGGA CCGCTATAA AACTCTCGCT CCGGCGTGTA TACCTTCGA A-TCTTCCAA CTTATAATGA GTTATAACTT TCAACAACGG ATCTCTTGGT TCTGG	
SUT262 135 TAGCCGGCTG AACAGCCC-G CCGAAGGA CCGCTATAAA AACTCTCGCT CCGGCGTGTA TACCTTCTGA A-TCTTCCAA CTTATAAAGG GTTAAAACTT TCAACAACGG ATCTCTTGGT TCTGG	GCATCG ATGAAGAACG CAGCGAAATG 280
ST2448 135 TAGCCGGCTG AACAGCCC-G CCGAAGGA CCGCTATAAA AACTCTCGCT CCGGCGTGTA TACCTTCTGA A-TCTTCCAA CTTATAATGA GTTAAAACTT TCAACAACGG ATCTCTTGGT TCTGG	SCATCG ATGAAGAACG CAGCGAAATG 280
SUT167 135 TAGCCGGCTG ARCAGCCC-G CCGAAGGA CCGCTATARA AACTCTCGCT CCGGCGTGTA TACCTTCTGA A-TCTTCCAA CTTATARATGA GTTARAACTT TCAACAACGG ATCTCTTGGT TCTGG	
JU2 134GCCGGCAA AACGGCCC-G GCAAAGGA-C CCGCAAACTA AACTCTAAAT T-AACAACGG TACCTTCTGA AAACTTCAAAA CTTTTAATGA GGTAAAACTT TCAACAACGG ATCTCTTGGT TCTGG	
ST2579 135GCCGGCAA AACGGCCC-G CCAAAGGA-C CCGCAAACTA AACTCTAAAT T-AACAACGG TACCTTCTGA AAACTTCAAA CTTTTAATGA GTTAAAACTT TCAACAACGG ATCTCTTGGT TCTGG	
ST2406 134GCCGGCAA AACGGCCC-G CCAARGA-C CCGCAARCTA ARCTCTAAAT T-AACAACG- TACCTTCTGA AAACTTCAAR CTTTAARGA GTTAAARCTT TCAACAACGG ATCCTTGGT TCTGG SUT025 113GCCGATCC ACCAGCCC-G TCAAAGGA-C CCGCTATCTA AACTTCTGAAT T-GACTACG- TAACTTCTGA AAACTTCCAA CTTGAAATAA GTTAAAACTT TCAACAACGG ATCCTTGGT TCTGG	
SUT225 113GCCGATCC ACCAGCCC-5 TCARAGGA-C CCCGATATCA AACTOTGAAT T-GACTACG- TAACTTCTGA AAACTTCCAA CTTGAAATA GTTAAAACTT TCAACAAGG ATCTTTGGT TCTGG SUT220 140CCGGCTAA ACAGCAC-C CCARAGAACAA A-GTCTAATT T-AACTGCG- TACCTTCTGA AAAATATCAA CTTTAATA- TTAAACGA TT	
SIZ23 140CCGCCIAA AACACCAC CCACAAACTA C-CICIAATT T-AACGICC- TACCITCIGA AAAATATCAA CITTAATA- TTAAAACG ATCITIGG CICIGG CICACAAACTA C-CICIAATT T-AACGICC- TACCITCIGA AAAATATCAA	
AJ309406 107GGC CACGGCCC CCGAGGACACTG AACTCTTETT T-ATCACATT GCATCTCTGG TTTAAACTA GTTAAAACTA GTTAAAACTT TCAACAACG ATCTCTTGG TCTGG	
ST2436 82CGCCGG AGGACCGTA CCARACTC	
ST2473 83CGCCCGG ARGACCGTA CCAARCTCTTTTATT TCTCCCCAGT ARAACTCATA TARAATTAT TACAARATA GTTARAACTT TCAACARCGG ATCTCTTGGT TCTGG	GCATCG ATGAAGAACG CAGCGAAATG 214
Jul 82CGGCGG ARG-ACCGTA CCAMACTC	GCATCG ATGAAGAACG CAGCGAAATG 212
ST2313 H.N 82CGCCGG AAGGACCGTA CCAAACTCTTTTATT TCTCCCCAGT AAAATTCATA TAAAATTTAT TACAAAATAA GTTAAAACTT TCAACAACGG ATCTCTTGGT TCTGG	SCATCG ATGAAGAACG CAGCGAAATG 213
310 320 330 340 350 360 370 380 390 40 410 420	430 440 450
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SUT103 265 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG CCCATTAGTA TTCTAGTGGG CATGCCTATT CGAGCGTCAT TTCTACCCTT AAGCCCT-GT AGCTT SUT105 265 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG CCCATTAGTA TTCTAGTGGG CATGCCTATT CGAGCGTCAT TTCTACCCTT AAGCCCT-GT AGCTT	
ST233 265 CREMENTIA TOTALTICA REALTICAT GALATICA ACTIVITICAL COLLETING CULTURE TOTALTOG CHECKINT CORCUTAT TOTAL CULT ARCCUT ARCC	
SI2537 200 CREMENTIA TOTALTOC AGATTACCA ATCITIGAL COLLETING CLARITICS CLARITICS CHARCENET CAGACTAT TRAACCCTT AGCCCT-GT AGCCCT	
ST2336 232 CGATAAGTAA TOTGAATIGC AGAATCAGT GAATCATCGA ATCITTGAAC GCACATIGOG CCCATIAGTA ITCAAGGGG CATGCCTGTI CGAGCGTCAT ITCAACCCTI AGCCCTCGT TGCTT	
AJ390395 236 CGATAAGTAA TOTGAATTGC AGAATTTAGT GAATCATCGA ATCTTTGAAC GCACATTGCG CCCATTAGTA TTCTAGTGGG CATGCCTATT CGAGCGTCAT TTCAACCCTT AAGCCCT-GT TGCTT	
ST2584 294 CGATARGTAN TGCGANTTGC AGANTTCAGT GANCATCGA ATCTTTGANC GCACATTGCG CCCGGCTAGTA TTCTGGCGGG CATGCCTATT CGRGCGTCAT TACAACCCCTT AAGCCCCCTG CGCT	TAGCGT TGGGAGTCTG CGGCTC 439
SUT001 280 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCACGT AACCATTGAAC GCACATTGCG CCCACTAGTA TTCTGGTGGG CATGCCTATT CGAGCGTCAT TTCAACCCCTT AAGCCCCTGT TGCTT	TAGCGT TGGGCGTCTG CGCCGTGCCC 429
SUT004 281 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG CCCACTAGTA TTCTGGTGGG CATGCCTATT CGAGCGTCAT TTCAACCCCTT AGCCCCTGT TGCTT	
ST2485 281 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG CCCACTAGTA TTCTGGTGGG CATGCCTATT CGAGCGTCAT TTCAACCCCTT AAGCCCCTGT TGCT	
SUT005 200 CGATAAGTAA TGTGAATTCAGT GAAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG CCCACTAGTA TTCTGGTGGG CATGCCTATT CGAGCGTCAT TTCAACCCTT AAGCCCCTGT TGCTT	
SUT262 281 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG CCCACTAGTA TTCTGGTGGG CATGCCTATT CGAGCGTCAT TTCAACCCCTT AAGCCCCTGT TGCT	TAGCGT TGGGCGTCTG CGCCGTGCCC 430
ST2448 281 CGATAGGTAA TGTGGAATTCC AGAATTCGG TGTTTGGAC GCACATGCG CCCACTAGTA TTCTGGTGGG CATGCCTAAT CGAGGGTCAT TTCGAACCCTT AGCCCCTG TGCTT	
SUT167 281 CGATAAGTAA TGTGAATTCC AGAATTCAGT GAACATTGGA ATCTTTGAAC GCACATTGCG CCCACTAGTA TTCTGGTGGG CATGCCTATT CGAGCGTCAT TTCAACCCTT AAGCCCCTGT TGCTT	FAGCGT TGGGCGTCTG CGCCGTGCCC 430
SUT167 281 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGCG CCCCCAGTAT TTCTGGTGGG CATGCCTATT CGAGCGTCAT TTCAACCCTT AAGCCCTGT TGCTT JU2 280 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGC GCCACCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAAT TTCAACCCTT AAGCCCTTCG -GCTT	TAGCGT TGGGCGTCTG CGCCGTGCCC430TAGCGT -GGGAGTCCG CGTCCCC424
SUT167 281 CGATAGGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGCG CCCCCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTCAT TTCAACCCTT AAGCCCTGT TGCTT JU2 280 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAAT TTCAACCCTT AAGCCCTTCG -GCTT ST2579 281 CGATAGGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTGGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAAT TTCAACCCTT AAGCCCTTCG -GCTT	TAGGGT         TGGGCGTCTG         CGCCGTGCCC         430           TAGCGT         -GGGAGTCCG         CGTCCCC         424           TAGCGT         TGGGAGTCCG         CGTCCCC         426
SUT167 281 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGC CCACTAGTA TTCTGGTGGG CATGCCTATT CGAGCGTCAT TTCAACCCTT AAGCCCTGT TGCTT JU2 280 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATGGA ATCTTTGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CCGAGCGTAAT TTCAACCCTT AAGCCCTTCG -GCTT ST257 281 CGATAGGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTGGAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CCGAGCGTAAT TTCAACCCTT AAGCCCTTCG -GCTT ST2406 279 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTGAAC GCACATTGCG CCCACGGTA TTCTGGTGGG CATGCCTATT CCGAGCGTAAT TTCAACCCTT AAGCCCTTCG -GCTT	TAGCGT TGGGCGTCTG CGCCGTGCCC430TAGCGT -GGGAGTCCG CGTCCCC424
SUT167 281 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGC CCACCAGTA TTCTGGTGGG CATGCCTATT CGAGGGTCAT TTCAACCCTT AAGCCCTGT TGCTT JU2 280 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGAGGGTAAT TTCAACCCTT AAGCCCTTCG -GCTT ST2579 281 CGATAGGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTGGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGAGGGTAAT TTCAACCCTT AAGCCCTTCG -GCTT ST2579 279 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTGGAAC GCACATGCG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGAGGGTCAT TTCAACCCTT AAGCCCTTCG -GCTT SUT05 258 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGAGGGTCAT TTCGACCCTT AAGCCCTTCG -GCTT	TAGGCGT       TGGGCGTCTG       CGCCGTGCCC       430         TAGCGT       -GGGAGTCCG       CGTCCCC       424         TAGCGT       TGGGAGTCCG       CGTCCCC       426         TAGCGT       TGGGAGTCCG       CGTCCCC       424
SUT167         281         CGATAGTAA TGTGAATTGC AGAATTCAGT GAACATCGA ATCTTTGAAC GCACATTGG CCACTAGTA TTCTGGTGG CATGCTATT CGAGCGTAT TTCAACCCTT AAGCCCTG TGCT           JU2         280         CGATAGTAA TGTGAATTGC AGAATTCAGT GAATCATGGA ATCTTTGAAC GCACATTGCG CCACCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAT TCTAACCCTT AAGCCCTTG GCT           ST2579         281         CGATAGTAA TGTGAATTGC AGAATTCAGT GAATCCGA ATCTTGGAC GCACATTGCG CCCACGATA TTCTGGTGGG CATGCCTATT CGAGCGTAT TTCAACCCTT AAGCCCTTG GCT           ST2579         281         CGATAGTAA TGTGAATTGC AGAATTCGA ATCTGGA GCACATTGCG CCACCAGTA TTCTGGTGGG CATGCCTATT TCGACCGTTA TTCAACCCTT AAGCCCTTG GCT           ST2579         281         CGATAGTAA TGTGAATTGC AGAATTCGA GAATCGCA ATCTTGGAC GCACCAGTA TTCTGGTGGG CATGCCTATT TCGACGGTCAT TTCAACCCTT AAGCCCTTCG GCT           SU0025         282         CGATAAGTAA TGTGAATTGCA GAATTCAGA ATCTTGGA GACATTGGA CCCACTAGTA TTCTGGTGGG CATGCCTATT CGAGCGCAT TTCGACCCTT AAGCCCTCGG GCT           SU125         282         CGATAAGTAA TGCGAATTGCA GAATTCAGA ATCTTTGAC GCACATTGCG CCCACCAGCA TTCTGGTGGG CATGCCTATT CGAGCGCAT TACGACCCTT AAGCCCCCGG GCT           SU126         282         CGATAAGTAA TGCGAATTCCA GAATCATCGA ATCTTGAC GCACATTGCG CCCCCGCACA TTCTGGCGG CATGCCTATT ACGACCCTT AAGCCCCCGG GCT           SU127         282         CGATAAGTAA TGCGAATTCCA GAATCATCGA ATCTTGAC CCACATTGCG CCCCCACACA TTCTGGCGG CATGCCTATT ACGACCCTT AAGCCCCCGG GCT           SU128         282         CGATAAGTAA TGCGAATTCCA GAATCATCGA ATCTTGAC CCACATTGCG CCCCCACACA TTCTGGCGG CATGCCTATT ACGACCCTT AAGCCCCCCGG GCT	TAGGET TGGGGGTCTG         CGCCGTGCCC         430           TAGCGT -GGGAGTCCG         GCTCCCC         424           TAGCGT TGGGAGTCCG         GCTCCCC         424           TAGCGT TGGGAGTCCG         GCTCCCC         424           TAGCGT TGGGAGTCCG         GCTCCCC         424           TAGCGT TGGGAGTCCC         CGTCCCC         424
SUT167281CGATAAGTAA TGTGAATTGC AGAATTCAGT GAACATCGG ATCTTGAAC GCACATGGC CCACCAGTA TTCTGGTGGG CATGCCTATT CGAGGGTCAT TTCAACCCT AAGCCCTG TGCTTJJ2280CGATAAGTAA TGTGAATTCC AGAATTCAGT GAATCACGA ATCTTTGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CCAACGCTAT TTCAACCCTT AAGCCCTTCG -GCTTST2579281CGATAGTAA TGTGAATTGC AGAATTCAGT GAATCACGA ATCTTGGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGAACGTAAT TTCAACCCTT AAGCCCTTCG -GCTTST2579281CGATAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTGGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGACGGTAAT TTCAACCCTT AAGCCCTTCG -GCTTST240279CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTGGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGACGGTCAT TTCGACCCTT AAGCCCTTCG -GCTTSUT025288CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGCG CCCCCAGCA TTCTGGCGGG CATGCCTATT CGACGCTAT TACGACCCTT AAGCCCTGC -GCTTSUT022282CGATAAGTAA TGTGAATGC AGAATTCAGT GAATCATCGA ATCTTTGAC GCACATTGCG CCCGCCAGCA TTCTGGCGGG CATGCCTATC CGACGCTATT TACGACCCTT AAGCCCCTGC -GCTTSUT032284CGATAAGTAA TGCGAATTCC AGAATTCGA GAATCCGA ATCTTTGAC GCACATTGCG CCCGCAGCA TTCTGGCGGG CATGCCTATT CGACGCTAT TACGACCCTT AAGCCCCCGG -GCTTAJ39040242CGATAAGTAA TGTGAATGC GAATTCAGT GAATCATCGA ATCTTGAAC GCACATTGCG CCCACTATATAGTAGT CGACGCTATT CGACGCTAT TACGACCCTT AAGCCCCTGT AGCCCTCT AGCCCCCTT ACGCACCACGA TTCTGGCGG CATGCCTATT CGAGCGCTAT TACGACCCTT AGCACCCTT AGCCCCCCG -GCTT	TAGCGT TGGGCGTCTG CGCCGTGCCC 430         TAGCGT -GGGAGTCCG CGTCCCC 424         TAGCGT TGGGAGTCCG CGTCCCC 426         TAGCGT TGGGAGTCCC CGCCCCCC 424         TAGCGT TGGGAGTCCG CGTCCCC 431         TAGCGT TGGGAGCCTA CGTCCCG 389
SUT167 281 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAACATCGA ATCTTGAAC GCACATGGG CCACTAGTA TCTGGTGGG CATGCCTATT CGAGGGTAAT TTCAACCCTT AAGCCCTGT TGCTT JU2 280 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAAT TTCAACCCTT AAGCCCTTG AGCCCTTG ST2579 281 CGATAGGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCCCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAT TTCAACCCTT AAGCCCTTG AGCCTTG ST250 279 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAT TTCGACCGTT AGCCCTTG AGCCTTG SUT25 258 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCACCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAT TCGACCGTT AAGCCCTTGG GCT SUT25 282 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCACCAGCA TTCTGGCGG CATGCCTATT CGAGCGTAT TACGACCCTT AAGCCCCTGG GCT SUT25 284 CGATAAGTAA TGCGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCGCCAGCA TTCTGGCGGG CATGCCTATT CGAGCGCTAT TACGACCCTT AAGCCCCGGG -GCT SJ390406 242 CGATAAGTAA TGCGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGGG CCCCCATAGTA TTCTGGTGGG CATGCCTATT CGAGCGCTAT TTCGACCGCTA AGCCCCGG -GCT SJ39446 214 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGACG CCCCATAGTA TTCTAGTGGG CATGCCTATT CGAGCGCCAT TTCGACCGCTA AGCCCCGG -GCT SJ39446 214 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGACG CCCCATAGTA TTCTAGTGG CATGCCTATT CGAGCGCCAT TTCGACCGCCTA AGCCCCGG -GCT SJ39446 214 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAACATCGA ATCTTTGACG CCCATTAGTA TTCTAGTGG CATGCCTATT CGAGCGCCAT TTCGACCGCCGT AGCCT SJ3946 214 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAACATCGA ATCTTTGAC GCACATTGG CCCATTAGTA TTCTAGTGGG CATGCCTATT TCGACCGCCAT TACGACCCTAT AGCCCCCGT AGCCT SJ3946 214 CGATAAGTAA TGTGAATTGCA GAACATCGA AATCTTGGAC CCCATTGGA CCCATTAGTA TTCTAGTGGG CATGCCTATT TCGACCGCCAT TACGACCCTAT AGCCCCCGT AGCCT	TAGCGT TGGGAGTCTG CGCCGTGCCC 430 TAGCGT -GGGAGTCCG CGTCCCC 424 TAGCGT TGGGAGTCCG CGTCCCC 426 TAGCGT TGGGAGTCCG CGTCCCC 424 TAGCGT TGGGAGTCCG CGTCT-TCA 429 TAGCGT TGGGAATCCG CGTCT-TCA 431 TAGCGT TGGGAGTCTG CGGCCCA 360
SUT167281CGATAAGTAA TGTGAATTGC AGAATTCAGT GAACATCAGT AACTTTGAAC GCACATGGC CCACTAGTA TCTGGTGGG CATGCCTAT CGAGGGTAT TTCAACCCT AACCCCT ACCCCTG TGCTTJJ2280CGATAAGTAA TGTGAATTGC AGAATTCAGT GAACATCGA ATCTTTGAAC GCACATGGG CCACCAGTA TTCTGGTGGG CATGCCTAT CGAGGGTAT TTCAACCCT AACCCCTTA ACCCCTTAST2579281CGATAAGTAA TGTGAATGC AGAATTCAGT GAACATCGA ATCTTGAAC GCACATGGG CCCCACAGTA TTCTGGTGGG CATGCCTAT CGAGGGTAT TTCAACCCTT AACCCCTTAST2406279CGATAAGTAA TGTGAATGC AGAATTCAGT GAACATCGA ATCTTGGAC GCACATGGG CCCCACAGTA TTCTGGTGGG CATGCCTAT CGAGGGTAT TTCAACCCTT AACCCCTTASU7205282CGATAAGTAA TGTGAATGC AGAATTCAGT GAACATCGA ATCTTTGAC GCACATGGG CCCACAGTA TTCTGGCGG CATGCCTAT CGAGGGTAT TACGACCCTTA AGCCCCTGC -GCTTSU7205282CGATAAGTAA TGCGAATGC AGAATTCAGT GAACATCGA ATCTTGGAC GCACATGGG CCCCACAGTA TTCTGGCGG CATGCCTAT CGAGCGTAT TACGACCCTTA AGCCCCGG -GCTTSU7205282CGATAAGTAA TGCGAATGC AGAATTCAGT GAACATCGA ATCTTTGAC GCACATGGG CCCACAGA TTCTGGCGG CATGCCTAT TCGAGCGTA TACGACCCTT AACGCCCCGG -GCTTSU7205282CGATAAGTAA TGCGAATGC AGAATTCAGT GAACCATCGA ATCTTGGAC GCACATGGG CCCACAGA TTCTGGCGG CATGCCTAT TCGAGCGCTA TACGACCCTT AGCCCCCGG -GCTTSU223284CGATAAGTAA TGCGAATGC AGAATTCAGT GAACATCGA ATCTTGAC GCACATGGG CCCCATAGGA TTCTGGCGG CATGCCTAT TCGACCGTA TACGACCCTT AGCCCCCGG -GCTTSU330406242CGATAAGTAA TGTGAATTCC AGAATTCAGT GAACATCGA ATCTTTGAAC GCACATGGG CCCATAGTA TTCTAGTGGG CATGCCTAT TCGACCGTAT TTCGACCCGT AGCCTTST2436214CGATAAGTAA TGTGAATTCC AGAATTCAGT GAACATCGA ATCTTTGAC CCACATGGG CCCATAGTA TTCTAGTGGG CATGCCTAT TCGACGCCAT TACGACCCTT AGCCCCCGT TGCTTST2473215CGATAAGTAA TGTGAATTGC AGAATTCAGT GAACATCGA ACTTTGAC CCCATATGGA CCCATAGTA TTCTAGTGGG CATGCCTAT TC	TAGCGT TGGGCGTCTG CGCCGTGCCC     430       TAGCGT -GGGATCCG CGTCCCC     424       TAGCGT TGGGATCCG CGTCCCC     424       TAGCGT TGGGATCCC CGCCCCACC     406       TAGCGT TGGGATCCC CGTCT-TCA     431       TAGCGT TGGGATCCG CGTCT-TCA     381       TAGCGT TGGGATCCC CGCCCA     360       TAGCGT TGGGATCCC CGCCCCA     361
SUT167 281 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTGAAC GCACATGGG CCACTAGTA TTCTGGTGGG CATGCCTATT CGAGGGTAAT TTCAACCCTT AAGCCCTGT TGCTT JU2 280 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCCCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAAT TTCAACCCTT AAGCCCTTG -GCTT ST257 281 CGATAGGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCCCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAT TTCAACCCTT AAGCCCTTG -GCTT ST250 279 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCCCAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAT TTCAACCCTT AAGCCCTTGG -GCTT SUT25 258 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCACTAGTA TTCTGGTGGG CATGCCTATT CGAGCGTAT TCGACCCTT AAGCCCTCGG -GCTT SUT25 282 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCCCAGAA TTCTGGCGGG CATGCCTATT CGAGCGCTA TACGACCCTT AAGCCCCCGG -GCTT SUT25 284 CGATAAGTAA TGCGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATGGG CCCCCAGAA TTCTGGCGGG CATGCCTAT CGAGCGCTAT TACGACCCTT AAGCCCCCGG -GCTT AJ390406 242 CGATAAGTAA TGCGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGGG CCCCCATAGTA TTCTGGTGGG CATGCCTATT TCGAGCGCAT TTCGACCCCTT AAGCCCCCGG -GCTT ST245 214 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAAC GCACATTGGC CCCCATAGTA TTCTGGCGG CATGCCTATT CGAGCGCCAT TACGACCCTT AAGCCCCCGT -GCTT ST245 214 CGATAAGTAA TGTGAATTGC AGAATTCAGT GAATCATCGA ATCTTTGAC CCCATAGCG CCCATAGTA TTCTAGTGG CATGCCTATT TCGACCGCAT TTCGACCCCGT AGCCTTT AAGCCCCCGT ACGCTATT CGAGCGCAT TTCGACCGCAT TTCGACCGCAGT TTCGACCGCAGT ATCTGGACGCAT TTCGACCGCAGT TTCGACCGCAGT ATCCGACCTCT AAGCCCCCGT AGCCTATT TTCGACCGCAGT ATTCGACGTA TTCGACCGCCGG -GCTT ST245 214 CGATAAGTAA TGTGAATTGCA GAATTCAGT GAATCATCGA ATCTTGGACGG CCCATAGTA TTCTAGTGG CATGCCTATT TCGACCGCAT TTCGACCGCGT ATTCGACCCTT AAGCCCCCGT TACGA	TAGCGT TGGGCGTCTG CGCCCTGCCC       430         TAGCGT -GGGAGTCCG CGTCCCC       424         TAGCGT TGGGAGTCCG CGTCCCC       424         TAGCGT TGGGAGTCCC CGCCCGCACC       406         TAGCGT TGGGAATCCC CGTCT-TCAG       431         TAGCGT TGGGAATCCC CGTCT-TCAG       439         TAGCGT TGGGAATCCC CGTCT-TCAG       431         TAGCGT TGGGAATCCC CGTCT-TCAG       439         TAGCGT TGGGAATCCG CGTCT-TCAG       439         TAGCGT TGGGAATCCG CGCCCA       360         TAGCGT TGGGAGTCTG CGGCCCA       361         TAGCGT TGGGAGTCTG CGCCCCA       361

Figure 7C. (Continued).

		460											,	-
SUT103	409 <b>T</b>	TTAGCGGCT	CCTTAAAGTT	ATTGGCGGAG	TTATAGCGTA	CTCTAAGCGT	AGTAATTT-T	TATCTCG	CTTCTGTAGT	GGCCCTAAC-	TGTTAGCCAT	AAAACCCCTA	TATTTTTCTA	AT 525
SUT105	409 <b>T</b>	TTAGCGGCT	CCTTAAAGTT	ATTGGCGGAG	TTATAGCGTA	CTCTAAGCGT	AGTAATTT-T	TATCTCG	CTTCTGTAGT	GGCCCTAAC-	TGTTAGCCAT	AAAACCCCTA	TATTTTTCTA	AT 525
ST2333	409 <b>T</b>	TTAGCGGTT	CCTTAAAGTT	ATTGGCGGAG	TTATAGCGTA	CTCTAAGCGT	AGTAATTT-T	TATCTCG	CTTCTGTAGT	AGCCCTAAC-	TGTTAGCCAT	AAAACCCCTA	TATTTTTCTA	AT 525
ST2527	423 <b>T</b>	TTAGCGGTT	CCTTAAAGTT	ATTGGCGGAG	TTATAGCGTA	CTCTAAGCGT	AGTAATTT-T	TATCTCG	CTTCTGTAGT	AGCCCTAAC-	TGTTAGCCAT	AAAACCCCTA	TATTTTTCTA	AT 539
ST2336	375 C2	ACCGTAGCT	CCCCAAAGTC	AGTGGCGGAG	TCGGCTCACA	CTCTAGACGT	AGTAATTT	CTCACCT	CGCCTATAGT	TGGACCGGT-	CCCCTGCCGT	AAAACGCCCA	AGTCTTTAAA	<b>A-</b> 489
AJ390395	382 CC	GGCGCAGTT	CCTTAAATTC	ATTGGCGGAG	CTGTGGCACA	CTCTAGGCGT	AGTAGTTTAA	CACCTCG	CCTCTAGAGT	GGCCGCGGT-	TACTGGCCGT	AAAACCCCTA	TATTTCTAGT	497
ST2584	440 AG	GGCCGAGTT	CCTTAAATT-	AGTGGCGGAG	T-ACAGCACA	ACCTAAGCGT	AGTAGGTTA-	CCTCG	CTCCCGGGGA	GTCTGTGGCG	CCTGCGTAAA	AAAAAACCCT	AAACCTTCTA	551
SUT001	430 <b>T</b>	GGCGCAGTG	CCCTAAATCT	ATCGGCGGAG	CCGTAGCACA	CTCTGAGCGT	AGTAATT-AC	AGTT-CCTCG	CTCCTGCAGT	GGCCGCGGC-	GGCTGGC-GT	TAACCCCT	ACAACTTCTA	<b>GT</b> 545
SUT004	431 <b>T</b> C	GGCGCAGTG	CCCTAAATCT	ATCGGCGGAG	CCGTAGCACA	CTCTGAGCGT	AGTAATT-AC	AGTT-CCTCG	CTCCTGCAGT	GGCCGCGGC-	GGCTGGCCGT	TAAACCCCCT	ACAACTTCTA	<b>GT</b> 549
ST2485	431 <b>T</b> C	GGCGCAGTG	CCCTAAATCT	ATCGGCGGAG	CCGTAGCACA	CTCTGAGCGT	AGTAATT-AC	AGTT-CCTCG	CTCCTGCAGT	GGCCGCGGC-	GGCTGGCCGT	TAAACCCCCT	ACAACTTCTA	<b>GT</b> 549
SUT005	430 <b>T</b>	GGCGCAGTG	CCCTAAATCC	ATCGGCGGAG	CCGTAGCACA	CTCTGAGCGT	AGTAATT-AC	AGTT-CCTCG	CTCCTGCAGT	G-CCGCGGC-	GGCTTGGCGT	AACCCCCT	ATA-CTTCTA	<b>GT</b> 544
SUT262	431 <b>T</b>	GGCGCAGTG	CCCTAAATCT	ATCGGCGGAG	CCGTAGCACA	CTCTGAGCGT	AGTAATT-AC	AGTT-CCTCG	CTCCTGCAGT	GGCCGCGGC-	GGCTGGCCGT	TAAACCCCCT	ATAACTTCTA	<b>GT</b> 549
ST2448	431 <b>T</b>	GGCGCAGTG	CCCTAAATC-	ATCGGCGGAG	CCG-AGCACA	CTCTGAGCGT	AGTAATAC	AGTCCTCG	CTCCTGCAGT	GGCCGCGGC-	GGCT-GCCGT	AACCCCT	ATA-CTTCTA	<b>GT</b> 540
SUT167	431 CC	GGCGCAGTG	CCCTAAATCC	ATCGGCGGAG	CCGTAGCACA	CTCTGAGCGT	AGTAATT-AC	AGTT-CCTCG	CTCCTGCAGT	GGCCGCGGC-	GGCT-GCCGT	TAAA-CCCCT	ATAACTTCTA	<b>GT</b> 547
Ju2	424 -0	GGCGCGGT-	CCCCAAAGTC	ATTGGCGGCT	TCGCAGCCCA	CTCTGAGCGT	AGTAATCAAC	TGGT-TCTCG	CTCCTGCAGT	GGCCGGCGG-	AGCCCGCCGT	AAAACCCCCC	СТАТААСТАА	<b>GT</b> 542
ST2579	426 -0	GGCGCGGTT	CCCCAAAGTC	ATTGGCGGCT	T-GCAGCCAA	CTCTGAGCGT	AGTAATCAAC	TGTT-TCTCG	CTCCTGCAGT	GGCCGGCGG-	AGCC-GCCGT	AAAACCCCCC	СТАТААСТАА	<b>GT</b> 543
ST2406	425 CC	GGCGCGGTT	CCCCAAAGTC	ATTGGCGGCT	TCGCAGCCCA	CTCTGAGCGT	AGTAATCAAC	TGTT-TCTCG	CTCCTGCAGT	GGCCGCGGC-	AGCCCGCCGT	AAAACCCCCC	CTATAACTTA	<b>GT</b> 544
SUT025	407 GC	GGCGGGGTT	CCTTAAAGTC	ATTGGCGGCG	TCGCAGCCCA	CTCTGAGCGT	AGTAATCTAC	TGTT-TCTCG	CTCCTGCAGT	GGCCGCGGCT	GGCTTGCCGT	AAAACCCCCT	ATATGTCTGA	<b>G-</b> 526
SUT220	430 GC	GGCGCGGTT	CCCTAAATTC	ATCGGCGGCG	CCGGGGCGTC	TTCTGAGCGT	AGTAATTTAT	TATCTCG	C-CCTGAAGC	TAGCCCCGTA	CGCCCGCCGT	AAAACCCCCC	AACTACCTTG	<b>T</b> - 546
ST2332	432 GC	GGCGCGGTT	CCCTAAATTC	ATCGGCGGCG	CCGGGGCGTC	TTCTGAGCGT	AGTAATTTAT	TATCTCG	C-CCTGAAGC	TAGCTCCGTA	CGCCCGCCGT	AAAACCCCCC	AACTACCGTA	<b>CT</b> 549
AJ390406	390 <b>C</b>	GGCGCAGCT	CCTCAAAGTC	AGTGGCGGAG	TCGGGTCGTG	CTCTGAGCGT	AGTAGTTAAT	ATCTCG	CTTCTGCGGT	GCCCCCGGC-	TGCCTGCCGT	AAAACCCCCC	CCTATACTTT	CG 506
ST2436	361 GG	GCCGCAGTT	CCTCAAAGTC	AGTGGCGGAG	TTGTAGCACA	CTCTAAGCGT	AGTAGTTTTC	CATTGCCTCG	CATGCAGAGC	GGCCTCAGC-	TGCCAGCCGT	AAAGCCCTAT	ACTTCTTAGT	479
ST2473	362 GG	GCCGCAGTT	CCTCAAAGTC	AGTGGCGGAG	TTGTAGCACA	CTCTAAGCGT	AGTAGTTTTC	CATTGCCTCG	CATGCAGAGC	GGCCTCAGC-	TGCCAGCCGT	AAAGCCCTAT	ACTTCTTAGT	480
Jul	360 <b>G</b>	GCCGCAGTT	CCTCAAAGTC	AGTGGCGGAG	TTGTAGCACA	CTCTAAGCGT	AGTAGTTTC	CATTGCCTCG	C-TGCAGAGC	GGCCTCAGC-	TGCCAGCCGT	AAAGCCCTAT	ACTTCT-AGT	476
ST2313 H.n	361 GG	GCCGCAGTT	CCTCAAAGTC	AGTGGCGGAG	TTGTAGCACA	CTCTAAGCGT	AGTAGTTTC	CATTGCCTCG	C-TGCAGAGC	GGCCTCAGC-	TGCCAGCCGT	AAAGCCCTAT	ACTTCTTAGT	478
-														

Figure 7C. (Continued).

		10	20		40	50	60	70	80	90	100	110		130			16	170	180	5	
Ju2	1	A CONTRACTOR OF	GTAATTTCAA				and the second second second										A CONTRACTOR OF				
ST2579	1		GTAATTICAA																		
ST2406	1	CTATTCGAGC	GTCATTTCAA	CCCTTAAGCC	C-TTCGGCTT	A-GCGTTGGG	AGTCCGCGTC	CCCCGGC	GCGGTTCCCC	AAAGTCATTG	GCGGCTTCGC	AGCCCACTCT	GAGCGTAGTA	ATCAACTGTT	TETEGETEET	GCAGTGGCCG	CGGCAGCCCG	CCGT-AAAAC	CCCCCCTATA	ACTTAGT	181
SUT025	1	CTATTCGAGC	GTCATTTCGA	CCCTTAAGCC	C-TGCGGCTT	A-GCGTTGGG	AGTOCCCCCC	CGCACCGGGC	GEGETTCCTT	ARAGTCATTC	SCCCCTCCC	ACCCCACTCT	GACCOTACTA	ATCTACTOTT	TETEGETEET	GCACTGGCCG	CECCECTTE	CCGT-AAAAC	CCCCTATATC	TCTGAG-	183
ST2333	1	CTATICGAGC	GTCATTTCAA	CCCTTAAGCC	CTGTA-GCTT	A-GCGCTGGG	AGTCCGCTAA	TTTTA	GCGGTTCCTT	AAAGTTATTG	GCGGAGTTAT	AGCGTACTCT	AAGCGTAGTA	ATTTITA	TCTCGCTTCT	GTAGTAGCCC	TAACTGTTAG	CCATAAAA	CCCCTATATT	TTTCTAA	175
ST2527	1	CTATTCGAGC	GTCATTTCAA	CCCTTAAGCC	CTGTA-GCTT	A-GCGCTGGG	AGTCCGCTA-	TTTTA	GCGGTTCCTT	AAAGTTATTG	GCGGAGTTAT	AGCGTACTCT	AAGCGTAGTA	ATTTTTA	TCTCGCTTCT	GTAGTAGCCC	TAACTGTTAG	CCATAAAA	CCCCTATATT	TTTCTAA	174
SUT105	1	CTATICGAGC	GTCATTICAR	CCCTTARGCC	CTGTA-GCTT	A-GCGTTGGG	AGTCCGCTAA	TTTTA	GCGGCTCCTT	ARAGTTATTG	GCGGAGTTAT	AGCGTACTCT	ARGCGTAGTA	ATTTTTA	TCTCGCTTCT	GTAGTGGCCC	TARCTGTTAG	CCATAAAA	CCCCTATATT	TTTCTAA	175
SUT285	1	CTATTCGAGC	GTCATTACAA	CCCTTAAGCC	C-TGTTGCTT	A-GCGTTGGG	AATCTACGGC	TTAGGCG	-TAGTTCCTC	ARAATTAGTG	GCGGAGTTAT	AGCACTCT	CAGCGTAGTA	ATTTG	CCTCGCTTCT	GRGCTG -CTG	TAGCTGCCTG	CCGTAAAA	CCC-TATAC-	TTCTAGT	169
SUT081	1	CTATICGAGC	GTCATTACAA	CCCTTAAGCC	C-TGTTGCTT	A-GCGTTGGG	AATCTACGGC	TTAGGCG	-TAGTTCCTC	ARAATTAGTG	GCGGAGTTAT	AGCACTCT	CAGCGTAGTA	ATTTG	CCTCGCTTCT	GAGCTG-CTG	TAGCTGCCTG	CCGTAAAA	CCCCTATAC-	TTCTAGT	170
SUT244	1	CTATTCGAGC	<b>GTCATTACAR</b>	CCCTTAAGCC	C-TGTTGCTT	A-GCGTTGGG	AATCTACGGC	TTAGGCG	-TAGTTCCTC	ARARTTAGTG	GCGGAGTTAT	AGCACTCT	CGGCGTAGTA	ATTTG	CCTCGCTTCT	GRGCTG-CTG	TAGCTGCCTG	CCGTAAAA	CCC-TATAC-	TTCTAGT	169
SUT251	1	CTATICGACC	GTCATTACAA	CCCTTARGCC	C-TOTTGCTT	A-GCGTTGGG	AATCTACGGC	TTAGGCG	-TAGTICCTC	ARAATTAGTG	GCGGAGTTAT	ACCACTCT	TAGCGTAGTA	ATTTG	CCTCGCTTCT	GAGCTG -CTG	TAGCTGCCTG	CCGTAAAA	CCCCTATAC-	TTCTAGT	170
SUT242	1	CTATICGAGC	GTCATTACAA	CCCTTAAGCC	C-TGTTGCTT	A-GCGTTGGG	AATCTACGGC	TTAGGCG	-TAGTTCCTT	AAAATTAGTG	GCGGAGTTAT	AGCACTCT	TAGCGTAGTA	ATTTG	CCTCGCTTCT	GAGCTG-CTG	TAGCTGCCTG	CCGTAAAA	CCCCTATAC-	TTCTAGT	170
SUT009	1		GTCATTATAA																		
SUT010	1	CTATTCGAGC	<b>GTCATTATAA</b>	CCCTTARGCC	T-TOTTCOTT	A-ACCGTGGG	ARTCTACCCC	TCACTGAGGG	GTAGTTCCTT	ARATGTACTC	GCGGGGGTTAT	ACCCCACTCT	ARCCGTRGTA	GTTTA	ACTOGCTITIC	RECERCECTE	TACCTCCTTC	CCGT-AAAAC	CCCTTATAAC	TTATAGG	179
AJ390397	1	CTATTCGAGC	GTCATTATAA	CCCTTAAGCC	T-TGTTGCTT	A-GCGTTGGG	AATCTACCCC	TCACTGAGGG	GTAGTICCTT	AAATGTAGTG	GCGGGGGTTAT	AGCACACTCT	AAGCGTAGTA	GTTTA	ACTCGCTTTC	AGGGAGGCTG	TAGCTGCTTG	CCGT-AAAAC	CCCTTATAAC	TTATAGT	179
SUT058	1	CTATICGAGC	GTCAT-ACAA	CCCTTAAGCC	T-TGTAGCTT	A-GCGTTGGG	AATCTACCCC	T-ACTGAGGG	-TAGTTCCTT	AAATT-AGTG	GCGGGGGT-AT	AGCACACTCT	AAGCGTAGTA	GTTTA	ACTCGCTTTC	AGGGAGGCTG	TAGCTGCTTG	CCGT-AAAAC	CCCCTATAAC	TTATAGT	174
AJ390409	1	CTATTCGAGC	GTCATTACAA	CCCTTAAGCC	T-TGTAGCTT	A-GCGTTGGG	AATCTACCCC	TCACTGAGGG	GTAGTICCTT	ARATTTAGTG	GCGGGGGTTAT	ACCACACTCT	ARGCGTAGTA	GTTTA	ACTOGCTITIC	AGGGAGGCTG	TRECTECTTE	CCGT-RARAC	CCCCTATAAC	TTATAGT	179
SUT243	1		GTCAT-ACAA							and the second se		and the second se						17,776	Contraction of the second second		
SUT231	1	CTATICGAGC	GTCATAACAA	CCCTTAAGCC	C-TGTAGCTT	A-GCGTTGGG	AACCTACCGC	TTAAGCG	GTAGCTCCTT	AAATTTAGTG	GCGGAGTTAC	AGCACCCCCT	AAGCGTAGTA	AAACTA	CCTCGCTTTC	AGGGAGCCTG	TAGCGGCCTG	CCGTTAAAAA	CCCCTATAA-	TTCTAGT	177
ST2584	1		GTCATTACAA																		173
SUT098	1		GTCATTACGA																		177
ST2436	1		GTCATTTCGA																		
ST2473	1		GTCATTICGA												Contraction of the second						
ST2313	1		GTCATTTCGA						a second s					100 C C C C C C C C C C C C C C C C C C							
Jul	1		GTCATTICGA					100000000000000000000000000000000000000													
AJ390406	1		GTCATTTCGA																		
ST2336	1		GTCATTTCAA				and the second se										and the second second second				
AJ390395	1		GTCATITICAA																		177
SUT001	1		GTCATITCAA																		181
SUT004	1		GTCATTTCAA																		
ST2485	1	The second second second second	GTCATITCAA														The second second second				
SUT262	1		GTCATTTCAA																		
SUT005	1		GTCATTICAA					the second second second second												1.	
ST2448	1		GTCATTTCAR																		
SUT167	1		GTCATTTCAA																		182
SUT220	1		GTCATTACGA																		179
ST2332	1	CTATCCGAGC	GTCATTACGA	CCCTTAAGCC	CCCGG-GCTT	A-GCGTTGGG	AATCCGCGTC	TT-CAGGGGGC	GCGGTTCCCT	ARATICATCG	GCGGCGCCGG	GGCGTCTTCT	GAGCGTAGTA	ATTTATTA	TCTCCC-CCT	GARGCTAGCT	CCGTCGCCCG	CCGT-AAAAC	CCCCCAACTA	CCGTACT	180

Figure 8C. The ITS2 sequence alignment of Hypoxylon sect. Annulata for phylogenetic tree construction in Figure 95 by using

ClustalX and BioEdit programs. Arrows indicate the start and the end of 5.8S rDNA sequences.

		10	) 20							90 90							)
SUT223	1														 	AAAC	104
SUT218	1															AAAC	
SUT233	1															TACCCGG	
SUT240	1															TACCCGG	
SUT066	1															TACTCGG	
SUT068	1									GTAG							113
SUT069	1															TACTCGG	
SUT046	1															TACCCGG	
SUT041	1									GTAGTTACCC							146
SUT063	1															AGGTACCTTG	
SUT020	1									GGAGTTACCC							154
SUT294	1									GGAG							104
SUT256	1																77
SUT154	1																72
SUT070	1																62
SUT237	1																62
SUT108	1																74
SUT082	1																63
SUT080	1																77
SUT116	1																77
SUT042	1																77
SUT164	1																79
SUT293	1																79
SUT292	1																79
SUT159	1															CCTAC	
SUT162	1															CCTAC	
SUT061	1	CAGAGTTATT	CTAAACT	CCAAA-CCCT	ATGTGAAC-T	TACCACTG	TTGCCTTGGC	GTG-TGCC	GCGAG				CTACACTGTA	GTGA	 	CCTAC	84
SUT280	1																61
SUT165	1															CCTAC	85
SUT120	1	CAGAGTTATA	CTAAACT	CCAAA-CCCT	ATGTGAACTT	TACCACTG	TTGCCTTGGC	GTG-TGCC	GCTTG				CTACCCTGTA	GCTC	 	CCTAC	85
SUT016	1															<b>AGG</b>	
SUT180	1															AGG	
ST2324	1	CAGAGTTACC	AAAACT	CCCAA-CCCT	TTGTGTACC-	TACTACCA	TTGCTTCGGC	GGGCTGCG	GCTACCCTGC	AG			CTACCCTGTA	ATTC	 	<b>AGG</b>	89
SUT250	1	CTGAGTTACT	CAAAACT	CCCAA-CCCT	ATGTGAAC-T	TACCATCG	TTTCTTCGGC	GGGCTGCG	GCTACCCTGT	AC			CTACCCTGAA	GCAA	 	AAGA	91
SUT282	1	CAGAGTTACT	AAACT	CCCAA-CCCT	ATGTGAAC-T	TACCGTCG	TTGCTTCGGC	GGGCTGCG	GCTACCCTGT	AC			CTACCCTGTA	CCTACCCTGT	 	AGGGCCC	98
SUT166	1	CAGAGTTACT	ATAAAACT	CCCAA-CCCT	TTGTGAACCT	TACCGTCG	TTGCCTCGGT	GGAAGGTGGT	GTGCGGTGGG	AAG-CTACCC	TGGAG	C	CTACCCTGTA	GATAGC	 	TAC	109
SUT158	1															TAC	
SUT148	1	CAGAGTTATT	AAAAACT	CCCAAACCCT	TTGTGAACGT	TACTGTTG	TTGCCTCGGC	GT	GAGCGAGGG-				CTACCC	GGGAGC	 	TAC	81
SUT187	1															TAC	
SUT182	1															C	
SUT215	1																
SUT221	1	CTGAGTTATC	CAAACT	CCAAAACCCT	TTGTGAACCT	TACCGTCG	TTGCCTCGGC	GTGAGCTG	CGGCTACCCG	GTAG			CTACCCTGTA	G	 		87
AJ390400	1															cctac	
													-	-			

**Figure 9C.** The ITS1-5.8S-ITS2 multiple sequence alignment of *Hypoxylon* sect. *Hypoxylon* for phylogenetic tree construction in Figure 94 by using ClustalX and BioEdit programs. Arrows indicate the start and the end of 5.8S rDNA sequences.

SUT223	105 GETAGGGETE TEGGEGEGEC CECCECCECTE CECCECCECTE CEGECEGECE CECCECCECTE CARACTATE CAR
SUT218	105 GGCAGGGGCT TTGGGCGCCC CGCCCGCACC CGCCCGCCC GTGGACCAC CCAACTOTT CAAAT-ATT GTGGAACTCT GAAA-TATA AAATAAACC AATCAAAACT TTCAACAACG GATCTTTGGCATC GATGAAGAAC GCAGCGAAAT 260
SUT233	87 TAGCTA CCCTGTAGCT G-GCCCA-CG GCCCGCCG CAGGACCGCT ANACTCTTGT TTTTACCA CTGTATCTCT GATTGTT AACTGAAAAT AGTTAAAACT TTCAACAACA GATCTCTTGG TTCTGGCATC GATGAAAAA GCAGGAAAAC GCACCGAAAT 234
SUT240	87 TAGCTA CCCTGTAGCT G-GCCCA-CG GCCCGCCG CAGGACCGCT AAACTCTTGT TTTTACCA CTGTATCTCT GAAT-TGT AACTGAAATA AGTTAAAACT TTCAACAACG GATCTCTTGG TTCTGGCATC GATGAAAAAC GCAGCGAAAT 234
SUT066	114 CAGOTS COCTOTAGEC G-GACGA-CG GCCCGCCG GAGGACTGCT AAACTETTGT TITTTACCA CTGTATCTCT GATTCT AACTGAAAAC CGTAAAACT TICAACAACG GATCTCTTGG TICTGGCATC GATGAAAAAC GCACGGAAAT 263
SUT068	114 CAGOTS COCTOTAGEC G-GACGA-CG GCCCGCCG GAGGACTGCT AAACTETTST TITTITACCA CTGTATCTCT GATTETT AACTGAAAAT CGTTAAAACT TICAACAACG GATCTETTGG TETGGCATC GATGAAAAAC GCAGGGAAAT 263
SUT069	114 CAGOTG COCTOTAGEC G-GACGA-CG GCCCGCCG GAGGACTGCT ANACTETTGT TITTITACCA CTGTATCTCT GATTETT ANEGGAATA CGTTATACAT TICAACAACG GATCTETTGG TETGGCATC GATGAAAAAC GCAGGAAAAC 263
SUT046	114 TAGCTA CCCTGTAGCC G-GACCA-CG GCCCGCCG GAGGACTGCT ANACTCTTGT TITTT-ACCA CTGTATCTCT GAAT-TCTT AACTGAAATA CGTTAAAACT TICAACAACG GATCTCTTGG TICTGGCATC GATGAAAAAC GCAGGGAAAC 262
SUT041	146GAGCTA COCTOTAGAC G-GCTTA-TG GCCCGCCG AAGGACCGCT AAGCTCTTGT TITTATTG CTGTTATTCT GAATTATA AACTAAAATA AGTTAAAACT TTCAACAACG GATCTCTTGG TTCTGGCATC GATGAAAAAC GCAGCGAAAT 294
SUT063	136AAGCTA CCCTGTAGAC G-GCTTA-TG GCCCGCCG AAGCACCGCT AAGCTCTTGT TTTTATTG CTGGTATTCT GAATTATA AACTAAAATA AGTTAAAACT TTCAACAACG GATCTCTTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 284
SUT020	155 TAAGGAGCTA CCCTGGAGTT GCACTCA-CG CTCCGCCG ATGGACCAGT AAACTCT-GT TTTTT-ATAG -TGTATCTCT GAATTCTT TAACAAAATT CGTTAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAGAAAC GCAGCGAAAT 30(
SUT294	104GAGCTA CCCTGGAGTT GCGCCTAACG CTCCGCTG GCGGACCACC AAACTCT-GT TTTAC-A-AG -TGTATCTCT GAGTATAT AACCAAAATA CGTTAAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATTAAGAAC GCAGCCAAAT 252
SUT256	77G CCAGTGGGGCC C-ATG-AACT GG-ACTCTGTTTA GCTGCCACT GCAGCGAATG -TGAATATCT GAACGGCCCTT AACTGRAATA CGTTARAACT TTCAACAACG GATCTCTTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 218
SUT154	72G CCGGGGGGCCACT AA-ACTC TGTTATA CCTACT
SUT070	62CCT C-GCC-GGCG GACCAC
SUT237	62CCC C-GCC-GGCG GACCAC
SUT108	74A COGGACGGCC C-GCC-CGAG GA-CCCC
SUT082	63GCAGCC C-GCC-GGCG GACCACTANAC TCTGT TTTTA CAGCATCTCT GAATGATA ACTTANAT AGTTARAACT TTCAACAACG GATCTCTTGG TTCTGGCATC GATGAGAAAC GCAGCGAAAT 184
SUT080	77 -CTGTAGCTA CCCGGGAACA C-ATT-CCAA GCTCGCCAGAGGACC TACCAACTCT GTATTATACT GTATCTCT GAACTTTATA AGTTAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAGAAAC GCAGCGAAAT 225
SUT116	77 -CTGTAGCTA CCCGGGAACA C-ATT-CCAA GCTCGCCAGAGGACC TACCAACTCT GTTTTATACT GTATCTCT GAACTTTATA ACTT-AAATA AGTTAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 226
SUT042	77 -CTGTAGCTA CCCGGGTACA C-ATT-CCAA GCTCGCCAGAGGACC TACCAACTCT GTATTATACT GTATCATCT AACTTTATAA CTRAATA AGTTAARACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAGAAAC GCAGCGAAAT 224
SUT164	79 -CTGTAGCTG CCGCGTAGCA C-GCA-CATG GCCCGCCG GTGGACAG-C TAAACTCTT- GTATGTACAC AAGTATGTCT GATTGCTT AAATAAAATA AGTCAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 230
SUT293	79 -CTGTAGCTG CCGCGTAGCA C-GCA-CATG GCCCGCCG GTGGACAG-C TARACTCTT- GTATGTACAC AAGTATGTCT GATTGCTT AAATAAAATA AGTCAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 23(
SUT292	79 -CTGTAGCTG CCGCGTAGCA C-GCA-CATG GCCCGCCG GTGGACAG-C TAAACTCTT- GTATGTACAC AAGTATGTCT GATTGCTT AAATAAAATA AGTCAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 23(
SUT159	85 CCGGGAGCTA CCCTGTAGTG C-GCA-TACG GCCCGCCG AAGGACTAAC TAAACTCTTT GTCTT-ACTG TG-AATATCT GAATGCTT CAACTAATA AGTTAAAAACT TTCAACAACG GATCTCTTGG TTCTGGCATC GATGAGAAAC GCAGCGAAAT 236
SUT162	85 CCGGGAGCTA CCCTGTAGTG C-GCA-TACG GCCCGCCG AAGGACTAAC TAAACTCTTT GTCTT-ACTG TG-AATATCT GAATGCTT CAACTAATA AGTTAAAAACT TTCAACAACG GATCTCTTGG TTCTGGCATC GATGAGAAAC GCAGCGAAAT 236
SUT061	85 CCGGGAGCTA CCCTGTAGTG C-GCA-TATG GCCCGCCG AAGACTAAC TAAACTCTTT GTCTT-ACTG TG-AATATCT GAATGCTT CAACTAATA AGTTAAA-CT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAAC GCAGCGAAAT 235
SUT280	61GGAGCTA CCCTGTAGTG C-GCA-TATG GCC-GCCG AAGGACTAAC TAAACTCTTT GTCTT-ACTG TG-AATATCT GAATGCTT CAACTAATA AGTTAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 205
SUT165	86 CCGGGAGCTA CCCTGTAGTG C-GCA-TACG GCCCGCCG AAGACTA-C TAAACTCTTT GTCTT-ACTG TG-AATATCT GAATGCTT CAACTAATA AGTTAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 236
SUT120	86 CCTGGAGCTA CCCTGTAGCA C-GCA-CACG GCCCGCCA AAGGACCA-C TAAACTCTTT ATTTTACTG TG-AATATCT GAATGCTT CAACTTAATA AGTTAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 237
SUT016	89 - GTGGGCTTG CCTGGTAGCT C-GCGCGAAG GCCCGTCA GAGGACCA-T TAAACTCTTG TTACCCTGTA CGTCATATCT GAATGCTT CAACTAATA AGTTAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 242
SUT180	89 - GTGGGCTTG CCTGGTAGCT C-GCGCGAAG GCCCGTNA GAGGACCA-T TAAACTCTTG TTACCCTGTA CGTTATATC GAATGCTT CAACTAATA AGTTAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAAC GCAGCGAAAT 242
ST2324	89 - GTGGGCTTG CCTGGTAGCT C-GCGCGAAG GCCCGTCA GAGGACCA-T TAAACTCTTG TTACCCTGTA CTTAATATCT GAATGCTT CAACTAATA AGTTAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAAC GCAGCGAAAT 242
SUT250	92 GGGGGGGCTG CACGGTAGCT T-GCCATAAG GCCCGTCA GAGGACCA-T TAAACTCGTG TTACCCTGTA CGTAA-ATCT GAATACTT CAACTAATA AGTTAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 244
SUT282	99 GGGGGGGTTT CCTGGTAGCT T-GCGCTAAG GCCCGTCA GAGGACCA-T CAAACTCATG TTACCCTGTA CGTACTATCT GAATACTT CAACTAATA AGTTAAAACT TTCAACAACG GATCTCTGG TTCTGGCATC GATGAAGAAAC GCAGCGAAAT 252
SUT166	110 CCTGGAGCTA CCCTGAAAAT ACGCCCCCCC CCAGCCGCCC AAGGACTACT AAACTCTTGT TTT-ACTG -TGTCTCTCT GAATA-ATGA AACAAAAATT CGTTAAAACT TTCAACAACG GATCTTTGG TTCTGGCATC GATGAAGAAA 264
SUT158	86 CCTGGAGCTG CAAACT ACGCCCGCCG GAGGACCACT AAACTCTTGT TTTTACCA -TGTATTTCT GAATG-CTTC AACTATAAAT AGTTAAAACT TTCAACAACG GATCTTTGG TTCTGGCATC GATGAAGAAAC GCAGCGAAAT 227
SUT148	82 CCTGTAGCTA CCCT-GTAAC CCGTTGTAAG CCCGCCG GAGGACCACT AAACTCTGGT TTATTACTG -TGTATCTCT GAATG-CTTC AACTGAAAACT TTCAACAACG GATCTCTTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 235
SUT187	82 CCTGTAGCTA CCCT-GTAAC CCGTTGTAAG CCCGCCG GAGGACCACT AAACTCTGGT TTATTACTG -TGTATCTCT GAATG-CTTC AACTGAAAACT TTCAACAACG GATCTCTTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 235
SUT182	98 GGGGCGACCT ACCCTGTAGT TACACCTAC GCT-CCGCCG GTGGACCACT AAACTCTGTT TTTA-ACCA CTGTATCTCT GAAATACTTA ACGAAATA CGTTATACACG GATCTCTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 252
SUT215	87CTA COCTGTAGEC GGTTCACG GCCCGCCG AAGGACAGCT AAACTCTTGT TAATT-ACCA CTGTATCTCT GAATTGTC AACT-AAATA AGTTAAAACT TTCAACAACG GATCTTTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 232
SUT221	87CTA COCTGTAGCC GGTTCACG GCCCGCCG AAGGACAGCT AAACTCTTGT TAATT-ACCA CTGTATCTCT GAATTGTC AACT-AAATA AGTTAAAACT TTCAACAACG GATCTTTGG TTCTGGCATC GATGAAGAAC GCAGCGAAAT 232

Figure 9C. (Continued).

	330 340	350 360 370			420 430		460 470 480
SUT223							STTGGGACTC TAGCCCTGCC ATAGGCTAGT 419
SUT218							STTGGGACTC TAGCCCTGCC ATAGGCTAGT 419
SUT233							STTGGGAGTC TACGGCTT CGGCGTA-GC 389
SUT240							STTGGGAGTC TACGGCTT CGGCGTA-GC 389
SUT066							STTGGGAGTC TACGTCTT-A CGGCGTA-GT 420
SUT068	264 GCGATAAGTA ATGTGAATTG CAGAA						
SUT069							GTTGGGAGTC TACGTCTT-A CGGCGTA-GT 420
SUT046							GTTGGGAGTC TACGTCTT-A CGGCGTAAGT 421
SUT041							GTTGGGAATC TACGGCGTA-GT 444
SUT063	285 GCGATAAGCA ATGTGAATTG CAGAA	ATTCAG TGAATCATCG AATCTTTGAA	CGCACATTGC GCCCATTAGT	ATTCTAGTGG GCATGCCTAT T	CGAGCGTGA TTTCGACCCC	TAAGCCCCTG NTGCTTA-GC	GTTGGGAATC TACGGCGTA-GT 434
SUT020	307 GCGATAAGTA ATGTGAATTG CAGAA	ATTCAG TGAATCATCG AATCTTTGAA	CGCATATTGC GCCCAGTAGT	ATTCTACTGG GCATGCCTAT T	CGAGCGTCA TTTCAACCCT	TATGCCC-TG TAGCATA-GT	GTTGGGGGCTC TACC-G AAAGGTA-GT 458
SUT294	253 GCGATACGTA ATGTGAATTG CAGAA	ATTCAG TGAATCATCG AATCTTTGAA	CGCATATTGC GCCCATTAGT	ATTCTAGTGG GCATGCCTAT T	CGAGCGTCA TTTCAACCCT	TAAGCCC-TG TAGCTTA-GC	GTTGGGACTC TACTCCTC-C GGGTGTA-GT 408
SUT256	219 GCGATAAGTA ATGTGAATTG CAGAA	ATTCAG TGAACCATCG AATCTTTGAA	CGCACATTGC GCCCACTAGC	ATTCTAGTGG GCATGCCTAT C	CGAGCGTCA TTTCAACCCT	AAGGCCCTTG CGGCTAACC-	GTTGGAAGCC TGTGGCTG CAGCGCAGCT 375
SUT154	192 GCGATAAGTA ATGTGAATTG CAGAA	ATTCAG TGAATCATCG AATCTTTGAA	CGCACATTGC GCCCATTAGT	ATTCTAGTGG GCATGCCTAT T	CGAGCGTCA TTTCAACCCT	TAAGCCCCTG TTGCTTAGT-	STTGGGAATC TGCGTTA CGGCGCAG-T 346
SUT070	186 GCGATAAGTA ATGTGAATTG CAGAA	ATTCAG TGAATCATCG AATCTTTGAA	CGCACATTGC GCCCATTAGT	ATTCTAGTGG GCATGCCTAT T	CGAGCGTCA TTTCAACCCT	TACGCCCT-G TAGCGTAGT-	GTTGGGAATC TACCTATAGGTAG 336
SUT237	186 GCGATAAGTA ATGTGAATTG CAGAA	ATTCAG TGAATCATCG AATCTTTGAA	CGCACATTGC GCCCATTAGT	ATTCTAGTGG GCATGCCTAT T	CCACCGTCA TTTCAACCCT	TACGCCCT-G TAGCGTAGC-	GTTGGGAATC TACCTGCGGGTAG 336
SUT108	204 GCGATAAGTA ATGTGAATTG CAGAA	ATTCAG TGAATCATCG AATCTTTGAA	CGCACATTGC GCCCGGCAGT	ATTCTGGCGG GCATGCCTGT T	CGAGCGTCA TTTCAACCCT	CAAGCTCAGCTTGGT-	GTTGGGACTC GCGGTAACCCGCG 351
SUT082	185 GCGATAAGTA ATGTGAATTG CAGAA	ATTCAG TGAATCATCG AATCTTTGAA	CGCACATTGC GCCCATTAGT	ATTCTAGTGG GCATGCCTAT T	CGAGCGTCA TTTCAACCCT	TAAGCCCCTG TTGCTTAGC-	GTTGGGAATC TACCTCCTTC GGGGGGCGTAG 343
SUT080	226 GCGATAAGTA ATGTGAATTG CAGAA	ATTCAG TGAATCATCG AATCTTTGAA	CGCACATTGC GCCCATTAGT	ATTCTAGTGG GCATGCCTAT T	CGAGCGTCA TTTCAACCCT	TAAGCCTCAG TTGCTTAGC-	GTTGGGACTC TACGACCTAT TATAGCGTAG 384
SUT116	227 GCGATAAGTA ATGTGAATTG CAGAA	ATTCAG TGAATCATCG AATCTTTGAA	CGCACATTGC GCCCATTAGT	ATTCTAGTGG GCATGCCTAT T	CGAGCGTCA TTTCAACCCT	TAAGCCTCAG TTGCTTAGC-	GTTGGGACTC TACGACCTAT TATAGCGTAG 385
SUT042							GTTGGGACTC TACGACCTAT TATATCGTAG 383
SUT164							GTTGGGAGCC TACGTC TACAGCGTAT 385
SUT293							GTTGGGAGCC TACGTC TACAGCGTAT 385
SUT292							GTTGGGAGCC TACGTC TACAGCGTAT 385
SUT159							GTTGGGAATC AGCGTCTTCA CGGCGCTG-T 393
SUT162							GTTGGGAATC AGCGTCTTCA CGGCGCTG-T 393
SUT061							GTTGGGAATC AGCGTCTTCA CGGCGCTG-T 392
SUT280	210 GCGATAAGTA ATGTGAATTG CAGAA						
SUT165							GTTGGGAATC AGCGTCTTCA CGGCGCTG-T 393
SUT120							GTTGGGAATC AGCGTCTTCT CGGCGCTG-T 394
SUT016 SUT180							GTTGGGACCC TGCGGCGT-A CGGCGCAG-G 398 GTTGGGACCC TGCGGCGT-A CGGCGCAG-G 398
ST2324							GTTGGGACCC TGCGGCGT-A CGGCGCAG-G 398 GTTGGGACCC TGCGGCGT-G CGGCGCAG-G 398
SUT250							STTGGGAATC TACGGCTT-A CCCTGTAG-T 401
SUT282							STTGGGAATC TACGGCTT-A CCCTGTAG-T 401
SUT166							STTGGGAGTC TGC-GCC TTGCCGCAGT 405
SUT158							STTGGGAGTC TACCGCC TGGCGGTAGT 382
SUT148							STTGGGAGCC TACGTCTG CGGGCGTAGT 392
SUT187	236 GCGATAAGTA ATGTGAATTG CAGAA						
SUT182	253 GCGATAAGTA ATGTGAATTG CAGAA						
SUT215	233 GCGATAAGTA ATGTGAATTG CAGAA						
SUT221							STTGGGAGCA TACCCTCCCC GGGGGGGTATC 39(
AJ390400							gttgggaatc tacgtt acggcgtagt 422
	see gegacaayoa abyoyaabby bayaa	oguatoutog autottogaa	-generating goodabbagb	array and a good at the			

Figure 9C. (Continued).

		490												
	400													500
SUT223 SUT218									ACCCCTAGGC					
SUT218 SUT233									ACCCCTAGGC G-GCCCTGGC					
SUT233 SUT240									G-GCCCTGGC					
SUT066									G-TCCCTGGC					
SUT068									G-TCCCTGGC					
SUT069									G-TCCCTGGC					
SUT046									G-TCCCTGGC					
SUT041									G-GCCTTGGC					
SUT063									G-GCCCTGGC					
SUT020									G-GCCTGAAT					
SUT294	409	TCCCTAAAAC	CA-GTGGCGG	TGTTAGG-TA	CCCTCATAGC	GTAGTAAAT-	CTTTTCTCGC	TTC-TGCAGT	G-TGTCTAGC	TACCTGCCGT	TAAACCCCCC	TATTTTTCTA	GT	515
SUT256	376	TCCTTAAAGT	CAGT-GGCGG	GGTTGGGGCCG	CGCCTTCAGC	GTAGTAGTT-	-CTATGTCGT	TGT-TGCGGC	GGCCGAAC	TTGCGGCCGT	AAAG-CCCGT	GATGCTTTTA	A-	479
SUT154	347	TCCTTAAAGT	GATTTGGCGG	AGCTAGTGCA	TACTCTAGGC	GTAGTAAATA	CCATTCTCGC	TTT-TGTAGT	A-GGCCTGGC	GGCTTGCCGT	AAAA-CCCCT	-ATACTTCTA	GT	454
SUT070	337	TTCCTCAAAT	CGATTGGCGG	AGTTAGCACA	TACTCTAGGC	GTAGTAACA-	CCATTCTCGC	TTC-GGTAGT	AAGTGCTGGC	GGCTAGCCAC	TAAA-CCCCC	TATACTTCTA	GT	445
SUT237	337	TTCCTCAAAT	CGATTGGCGG	AGTTAGCGCA	TACTCTAGGC	GTAGTAATA-	CCATTCTCGC	TTC-TGTAGT	A-GTGCTAGC	GGCTAGCCAT	TAAA-CCCCC	TATATTTCTA	GT	444
SUT108	352	TTCCCCAAAT	CGATTGGCGG	TCACGTCGAG	CTTCCATAGC	GTAGTAATC-	ATACAC-CTC	GTT-ACTGGT	AATCGTCGCG	GCCACGCCGT	TAAA-CCCCA	ACTTCTG	AA	456
SUT082	344	TTCCTGAAAG	TGATTGGCGG	AGTTAGAGCA	TACTCTAGGC	GTAGTAACA-	TACCTCTCGC	TTC-TGCAGT	A-GCCCTGGC	GACCTGCCGT	AAAA-CCCCC	TATACTTCTA	GT	451
SUT080	385	TTCCTTAAAG	GTAGTGGCGG	AGTTATAGCA	CACTCTAAGC	GTAGTAATT-	-CTCTCTCGC	TTCTTGTAGT	G-GCTATAAT	TGCTAGCCAT	AAAA-CACCC	CCTATTTTAA	т-	491
SUT116	386	TTCCTTAAAG	TTAGTGGCGG	AGTTATAGCA	CACTCTAAGC	GTAGTAATT-	-CTCTCTCGC	TTCTTGTAGT	G-GTTATAGT	TGCTAGCCAT	AAAA-CACCC	CCTATTTTAA	$\mathbf{T}^{-}$	492
SUT042	384	TTCCTTAAAG	TTAGTGGCGG	AGTTATAGCA	CACTCTAAGC	GTAGTAATT-	-CTTTCTCGC	TTCATGTAGT	G-GTTATAGT	CGCTAGCCAT	AAAA-CACCC	CTTATTTTAA	$\mathbf{T}^{-}$	490
SUT164		CTCCTCAAAG							G-GCCCCTGC					
SUT293									G-GCCCCTGC					
SUT292		CTCCTCAAAG							GCCCCTGC					490
SUT159									T-GCCTTGAT					
SUT162		TCCTTAAATT							T-GCCTTGAT			-TATTTTCTA		
SUT061									T-GCCTTGAT			-TATTTTCTA		
SUT280									T-CCCTTCAT					
SUT165		TCCTTAAATT							T-GCCTTGAT			-TATTATCTA		
SUT120									C-GCCCTGAC					
SUT016 SUT180		TCCTTAAATT							A-CGCCTAGC			-TATTTTTA		
SUT180 ST2324									A-CGCCTAGC A-CGCCTAGC					
SUT250									A-TGCCTAGC					
SUT282									A-AGCCCGGC					
SUT166									GGTCCTGAAC					
SUT158									GGTTCTGG-C					
SUT148		TCCTGAAAGT							AGT-CTAA-C					
SUT187									AGTTCTAA-C					
SUT182									G-GCCCGAAT					514
SUT215									G-GCCCTGGC					
SUT221									GGGGCCTGCT					
AJ390400									t-actgtgac					
			5 55 55				- 9 -	2 - 2 -		2 . 5 -			-	

Figure 9C. (Continued).

		10 20	30	40	50	60	70			10									
	12	····I····I ····I····I ··																	1000
SUT032_	1	CAGAGTTC TATTACTCCC AAL																	16.
SUT142	1	CAGAGTTC TATAACTCCC AA																	16
SUT076	1	CAGAGTTC -AT-ACTCCC AA																	15
ST2417	1	CAGAGTTC TATCACTCCC AA																	16
SUT207	1	AAGAGTTC TATAACTCCC AA																	15
AF163033	1	CTCCC AA																	14.
AF163026	1	AAGAGTTCTA TAACTCCCTA AAJ																	15
AF163030	1																		13
AF163039	1	AAGAGTTCTA TAACTCCC AA																	
AF163031	1	AAGAGTTATT ATAAACTCCC AA																	16
AF163034	1	AAGAGTTCTA TAACTCCC AA																	15
SUT123	1	AAGAGT-GTA TACTCCC AA																	15
ST2027	1	CTGAGTTATC CAAAACTCCC AA																	15
ST2326	1	CTARATT-CC CARARC-CTT AN																	16
SUT090	1	CTGAGTTATC TAAA-CTCCA AA																	15
AY787733	1	CAGAGTTATC TAACTCCC AN																	16
SUT201	1	AAGAGTTTAT TAACTCCC AA																	13
ST2349	1	AAGAGTTTAT TAACTCCC AA																	13:
SUT203	1	AAGAGTTATT A-CAACTCCC AA																	16
SUT177	1	AAGAGTTATT A-CAACTCCC AA	ACCCATGT G	AACCTTACC	ATTTGTTGCC	TCGGCAGGTC	-GCA	GCTTACCC	TGTGAGAT-C	CTACC-CTGT	AGG-GCCTCA	CCTGGTAGTT	GCGGGGTA-AT	CCTGCCGGTG	GTCTA-C-CA	AACTCTGT	TTACTATG	TTATTCTGAA	16
SUT138	1	AAGAGTTATT A-CAACTCCC AA																	16
SUT139	1	AAGAGTTATT A-CAACTCCC AA	ACCCATGT G	AACCTTACC	ATTTGTTGCC	TCGGCAGGTC	-GCAA	GCTTACCC	TGTGCGAC-C	CTACC-CTGT	AGG-GCCTCA	CCTGGTAGTT	GCGGGTA-AT	CCTGCCGGTG	GTCTA-C-CA	AACTCTGT	TTACTATG	TTATTCTGAA	16
SUT129	1	AAGAGTTT A-CAACTCCC AA																	15
ST2372	1	AAGAGTTAAA A-CAACTCCT AA																	16
SUT192	1	AAGAGTTAAA AACAACTCCT AA																	16.
SUT088	1	AAGAGTTATACAACTCCT AA	ACCCATGT G	AA-CCTACC	-TTTGTTGCC	TCGGCAGGTC	TGCA	GCCTACCC	TGTGAGGG-C	CTACC-CTGT	AGG-ATCTTA	CGCGGTAGTT	GCAGGTTCAA	CCTGCCGCTG	GTCTA-C-CA	AACTCTGT	TTT-ACCATG	TTATTCTGAA	16
SUT140	1	ANGAGTTATA CAACTCCT AND																	16
AF163027	1	AAGAGTTATT A-CAACTCCC AA	ACCCATGT G	AA-CATACC	TTCTGTTGCC	TCGGCAGGTC	TGCA	GCCTACCC	TGTAAGCC-C	CTACC-CTGT	AGGGACCTTA	CCCGGTAGTT	GCGGGGTAAAG	CCTGCCGGTG	GTCTA-C-TC	AACTCTGT	TTATTATG	TTATTCTGAA	16
ST2348	1	AAGAGTT-AT TACAACTCCC AA	ACCCATGT G	AA-CTTACC	TTCTGTTGCC	TCGGCAGGTC	-GCG	ACCTACCC	TGTGAGGC-C	TTACC-CTGT	AGG-GCCCTA	CTTGGTAGTC	GCGGGTA-CG	CCTGCCGGTG	GCCCA-T-GA	AACTCTGT	TTATTCT-TG	TTATTCTGAA	16
ST2363	1	AAGAGT AT TACAACTCCC AAL	ACCCATGT G	AA-CTTACC	TTCTGTTGCC	TCGGCAGGTC	-GCG	ACCTACCC	TGTGAGGC-C	CTACC-CTGT	AGG-GCCCTA	CCTGGTAGTC	GCGGGTA-CG	CCTGCCGGTG	GCCCA-T-GA	AACTCTGT	TTATTCT-TG	TTATTCTGAA	15
AF163037	1	AAGAGTT-AT TACAACTCCC AA	ACCCATGT G	AA-CTTACC	TTCTGTTGCC	TCGGCAGGTC	-GTG	ACCTACCC	TGTGAGGC-C	CTACC-CTGT	AGG-GCCCTA	CCCGGTAGTC	GCGGGTA-CG	CCTGCCGGTG	GCCCA-T-GA	AACTCTGT	TTATTCA-TG	TCATTCTGAA	16
AF163040	1	AAGAGTT-TT GATAACTCCC AA	ACCCATGT G	AA-CTTACC	TTCTGTTGCC	TCGGCAGGTC	-GCG	TCTACCC	TGTG-GCA-C	CTACC-CTGT	AGG-ACCCGA	CCTGGTGGTC	GCGGTCA-TG	CCTGCCGGTG	GCCCT-T-TA	AACTTTCTGT	GTATTCTATG	TTATTCTGAG	16
SUT078	1	CAGAGTTTGA ACGAACTCC- AA	ACCCATGT G	AA-CTTACC	TTCTGTTGCC	TCGGCAGGGT	CGCG	CCTACCG	TGTGAGGC-C	CTACCACTGT	AGG-GCCCTA	CGCGGTGCGT	GCGGGGCAGC-	CCTGCCGGCG	GCCCGTG	AAA-TTCTGT	TTG-ACTACG	TTATTCTGAA	16
SUT028	1	AAGAGTTTGA AC-AACTCC- AA	ACCCATGT G	AA-CTN-CC	TTCTGTTGCC	TCGGCAGGGT	CGCG	CCTACCG	TGTGAGGC-C	CTACCACTGT	AGG-GCCCTA	CCCCGTCCCT	GCGGGGCAGC-	CCTGCCGGCG	GCCCGG	AAA-TTCTGT	TTG-ACTACG	TTATTCTGAA	15
SUT124	1	AAGAGTAT AC-AACTCC- AA	ACCCATGT G	AA-CTTACC	GTACGTTGCC	TCGGCAGG-T	CGCG	CTCACCC	CGTAACAC-C	CTACCACG-T	AGG-GGCCTA	CTCGGTGGCC	GCGGACTAAG	CCTGTCGGTG	GCCCAAC-CA	AACTCTGTCA	GTG-ATTGTG	TCTTCTGAAC	16
SUT125	1	AAGAGTAT AC-AACTCC- AA	ACCCATGT G	AA-CTTACC	GTACGTTGCC	TCGGCAGG-T	CGCG	CTCACCC	CGTAGCAC-C	CTACCACG-T	AGG-GGCCTA	CTCGGTGGCC	GCGGACTAAG	CCTGTCGGTG	GCCCAAC-CA	AACTCTGTCA	GTG-ATTGTG	TCTTCTGAAC	16
SUT074	1	CCGAGT-T AC-AACTCCC AA	ACCCATGT G	AA-CATACC	TACTGTTGCT	TCGGCGGGAT	TGCC	CCGGGGCG	CCTCGTGT-G	CCCCGGAT-C	AGG-CGCCCG	CCTAG-GAAC	TTGAACT	CTTGTTTT	ATTTTG	AATCTTCTGA	GTA-GTT		13
SUT027	1	AAGAGTT TATAACTCCC AA	ACCCATGT G	AA-CATACC	TAACGTTGCC	TCGGCGGGTC	GTAC	CTACCC	TGTAGTGCAC	TTACCTGT	AAG-TGCCTA	CCCGGTAGGC	ACGGGTA-AG	CCCGCCGGCG	CCCCATTA	AACTCTGT	TTAATTACTG	GATATCTGAA	15
SUT198	1	AAGAGTT TATAACTCCC AA	ACCCATGT G	AA-CATACC	TAACGTTGCC	TCGGCGGGTC	GTAC	CTACCC	TGTAGTGCAC	TTACCTGT	AAG-TGCCTA	CCCGGTAGGC	ACGGGTA-AG	CCCGCCGGCG	CCCCATTA	AACTCTGT	TTAATTACTG	GATATCTGAA	15
SUT155	1	AAGAGTT TATAACTCCC AAL	ACCCATGT G	AA-CATACC	TAACGTTGCC	TCGGCGGGTC	GTAC	CTACCC	TGTAGTGCAC	TTACCTGT	AAG-TGCCTA	CCCGGTAGGC	ACGGGTA-AG	CCCGCCGGCG	CCCCATTA	AACTCTGT	TTAATTACTG	GATATCTGAA	15
SUT200	1	AAGAGTT TATAACTCCC AA	ACCCATGT G	AA-CATACC	TAACGTTGCC	TCGGCGGGTC	GTAC	CTACCC	TGTAGTGCAC	TTACCTGT	AAG-TGCCTA	CCCGGTAGGC	ACGGGTA-AG	CCCGCCGGCG	CCCCATTA	AACTCTGT	TTAATTACTG	GATATCTGAA	15
ST2298	1	AAGAGTT TATAACTCCC AA	ACCCATGT G	AA-CATACC	TAACGTTGCC	TCGGCGGGGTC	GTAC	CTACCC	TGTAGTGCAC	TTACCTGT	AAG-TGCCTA	CCCGGTAGGC	ACCCCTA-AC	CCCGCCGGCG	CCCCATTA	AACTCTGT	TTAATTACTG	GATATCTGAA	15
SUT127	1	AAGAGTT CTATACTCCC AAL	ACCCATGT G	AA-CATACC	GTATGTTGCC	TCGGCAGGTC	GTGT	CTACCC	TGTGGTGCCT	TACCCTGT	AGGGCCTA	CCTGGTAGAT	CCGGATAG	CCTGCCGACG	GCCCCTCA	AACTCTGT	TTAAT-AGTG	AATCTCTGAA	15
SUT195	1	AAGAGTT CTATACTCCC AA	ACCCATGT G	AA-CATACC	GTATGTTGCC	TCGGCAGGTC	GTGT	CTATCC	TGTGGTGCCC	TACCCTGT	AGGGCCTA	CCTGGTAGAT	CCGGATAG	CCTGCCGACG	GCCCCTCA	AACTCTGT	TTAAT-AGTG	AATCTCTGAA	15
SUT130	1	AAGAGTT CTATACTCCC AA	ACCCATGT G	AA-CATACC	GTATGTTGCC	TCGGCAGGTC	GTGT	CTACCC	TGTGGTGTCC	TACCCTGT	AAGGCCTA	CCTGGTAGAT	CCGGATAG	CCTGCCGACG	GCCCCTCA	AACTCTGT	TTAAT-AATG	AATCTCTGAA	15
AF163042	1	AAGAGTTT TATAACTCCC AA	ACCOATGT G	AA-CATACC	GTACGTTGCC	TCGGTGCGTC	TCCCCGTGAG	G-ACCTACCC	TGTAGGAC-G	CTACG-CTGT	AAGGCTTA	TCGGGAAGAT	GCACTAA-AG	CCTGCCGGCG	GCCCATTA	AACTCTGT	TTA-TTTTTG	AATT-CTGAG	16
ST2382	1	AAGAGTTC TATGACTCCC AA	ACCCATGT G	AA-CATACC	GTACGTTGCC	TCGGCGCGTC	TACCCTGTAG	CACCOTACCC	TGTAAGAC-C	CTACC-CTGT	AGGAGACCTA	CCCGGCAGAC	GNGGGTA-AG	CCEGCCGGCG	GCCCA-CGCA	AACTCTGT	TTTGGCAATG	TAATTCTGAA	17
SUT092	1	GCAGGTTGCG CCTACTTCGT GG	CCACATGT G	CGGCCTACA	TTGTAGGA	GCTATGGACT	ATTCCTGGTA	G-ACCTACCC	TGTAGTAGAC	GTACC-TTGT	AGATATCATA	CCTGGTAGAC	GCGGGGTA-AG	CCTGCCGGTG	GCCCATT	AACACTCTGT	TTA-GCGTTG	TGTTTCTGAG	17

Figure 10C. The ITS1-5.8S-ITS2 sequence alignment of *Xylaria* specimens for phylogenetic construction in Figure 97 by using ClustalX

and BioEdit programs. Arrows indicate the start and the end of 5.8S rDNA sequences.

		19		21													0 34	0 35	360	5
SUT032 Xba	1.62	C-ATATACCA																		34
SUT142		C-ATATACCA																		
SUT076		C-ATATACCA																		
ST2417		C-ATATACCA																		
SUT207		C-CTGTAACA																		
AF163033		CA-TATAACT																		31
AF163026		CC-TATAACT																		
AF163030		CC-TATAACT																		
AF163039	156	CC-TATAACT	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTOT	AGTGGGCATG	CCTGTTCGAG	CGTCATTTCA	33.
AF163031	161	CC-TATAACT	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTEGECATE	CCTGTTCGAG	CGTCATTTCA	33
AF163034	157	AA-CATAACT	AAATACGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGAT-A	AGAACGGAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGGCATG	CCTGTTCGAG	CGTCATTTCA	33.
SUT123	156	CANTATAACT	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	ANGTANTOTO	ANTTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGCATG	CCTGTTCGAG	COTCATTICA	33
ST2027	151	TTTTATAACT	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGGACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGGCATG	CCTATTCGAG	CGTCATTTCA	33
ST2326	168	TGCTTCAACT	TAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGGCATG	CCTATTCGAG	CGTCATTTCA	34
SUT090	160	TTGAAACT	GAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGGCATG	CCTATTCGAG	CGTCATTTCA	33
AY787733	168	TGTTTATACT	TAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGAT-A	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGGCATG	CCTGTTCGAG	CGTCATTTCA	34
SUT201	133	GTTATATA	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTTAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGGCATG	CCTGTTCGAG	CGTCATTTCA	31
ST2349		GTTACATA																		
SUT203		TATTATAACT																		
SUT177		TATTATAACT																		
SUT138		TATTATAACT																		
SUT139		TATTATAACT																		
SUT129		TATTATAACT																		33
ST2372		TAATATAACT																		
SUT192		TAATATAACT																		
SUTOBB		TAATATAACT																		
SUT140		TAGTATAACT																		
AF163027		ТАСТАТААСТ Т-СТАТААСТ																		
ST2348 ST2363	1000	T-CTATAACT													and the second se					
AF163037		T-CTATAACT																		
AF163040		T-TOGCAACT																		
SUT078		T-ACATAACA																		
SUT028		T-ACATAACA																		33
SUT124	1.000	T-TGACAACG																		
SUT125		T-TGACAACG																		
SUT074		T-TTACAAAT																		
SUT027		TTACAACT																		
SUT198		TTACAACT																		
SUT155	159	TTACAACT	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGCATG	CCTGTTCGAG	CGTCATTTCA	33
SUT200	159	TTACAACT	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGGCATG	CCTGTTCGAG	CGTCATTTCA	33
ST2298	159	TTACAACT	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTEGECATE	CCTGTTCGAG	CGTCATTTCA	33
SUT127	156	CTATAACT	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGGCATG	CCTGTTCGAG	CGTCATTTCA	33
SUT195	155	CTATAACT	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGCATG	CCTGTTCGAG	CGTCATTTCA	33:
SUT130	156	CTATAACT	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTATTCT	AGTGGGGCATG	CCTGTTCGAG	CGTCATTTCA	33
AF163042	166	G-CTATAAT-	AAATAAGTTA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTTAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	CTAGTATTCT	AGTGGGCATG	CCTGTTCGAG	CGTCATTTCA	34
572382		TACTATAACT																		35
SUT092	172	T-ATACAACG	AAAGAATTAA	AAACTTTCAA	CAACGGATCT	CTTGGTTCTG	GCATCGATGA	AGAACGCAGC	GAAATGCGAT	AAGTAATGTG	AATTGCAGAA	TTCAGTGAAT	CATCGAATCT	TTGAACGCAC	ATTGCGCCCA	TTAGTACTCT	AGTGGGGCATG	CCTGTTCGAG	CGTCATTTCA	351

Figure 10C. (Continued).

		370											470	480	49					
SUT032_Xba				TTA-CGTTGG																
SUT142 SUT076			The second second	TTAGCGTTGG TTAGCGTTGG		and the second se			and the second se											
572417				TTAGCGTTGG																
SUT207				TTAGTGTTGG																
AF163033				TTAGCGTTGG																
AF163026				TTAGTGTTGG																
AF163030				TTAGTGTTAG																
AF163039				TTAGTGTTGG																
AF163031				TTAGCGTTGG																
AF163034				TTAGCGTTGG																
SUT123				TTAGCGTTGG																
ST2027	331 A	CCCTTAAGC	CT-CAGTOGC	TTAACGTTGG	GACTCTACGA	CCTATTATAG	CGTAGTTCCT	TARAGTTAGT	GGCGGAGTTA	TAGCCCACT-	CTAAGCGTAG	TA-ATTC	CT CTCG	CTTCTT	GTAGTGGTT-	ATAGTTGCTA	GCCATAAAAC	CCCNCTATTT	T	490
ST2326	348 A	CCCTTAAGC	CT-T-GTTGC	TTAGCGTTGG	GAATCAGCGT	CTTTACGG	CGCTGTTCCT	TAAATTTAGT	GGCGGAGTTA	TAGCACACTT	CTAAGCGTAG	TA-AATC	TT CTCG	TTTCTG	G-AGTTGCC-	TTGATTCTTA	GCCGTAAAAC	CCCCCTATTT	TGTAAT	506
SUT090	338 .	CCCTTAAGC	CT-T-GTTGC	TTAGCGTTGG	GAGTCTACGG	CTTCGG	CGTAGCTCCT	GAAAGTTAGT	GGCGGAGTTA	GGGTACACT-	CTCAGCGTAG	TA-ACAC	-T CTCG	CTCGTG !	T-GGTGGCC-	CTGGCTGCTG	GCCGTTAAAC	CCCCATACCT	TTTAGT	492
AY787733	347 A	CCCTCAAGC	CC-TAGCTGC	TTGGTATTGG	GAGCTTGT	CTGCGG	-ACAACTCCT	CAAAAGCATT	GGCG-AGTCG	CGGTG-ACC-	CCAAGCGTAG	TA-ATTC	T-T CTCG	CTTAGG	TGTGTTAACG	CTGGCGTTCG	GCCACTAA	CCCCCTATTT	TCTAGT	497
SUT201	311 🗚	CCCTTAAGC	CT-TTGTTGC	TTAGCGTTGG	GAGCCTACGG	TAG	COTACCTCCT	CAAAATCAGT	GGCGGAGTCG	GTTCACACT-	CTAGACGTAG	TACATT	TAT CTCG	TCTGTG	AGTTGGG-	CTCCTCCCCT	GCCGTAAAA-	CCCCTAATTT	TTAAA-	462
ST2349	311 A	CCCTTAAGC	CT-TTGTTGC	TTAGTGTTGG	GAGCCTACGG	TAA	CGTAGCTCCT	TAAAATTAGT	GGCGGAGTCG	GTTCACACT-	CTAGACGTAG	TACATTT	TAT CTCG	TCTGTG	AGTTGGG-	CTGGTCCCCT	GCCGTAAAA-	CCCCTAATTT	CTAAA-	462
SUT203				TTAGCGTTGG																
SUT177				TTAGCGTTGG																
SUT138		and the second	The second s	TTAGCGTTGG													and the second			
SUT139				TTAGCGTTGG																
SUT129	1000			TTAGCGTTGG																
ST2372				TTAGCGTTGG																
SUT192				TTAGCGTTGG																
SUT088				TTAGCGTTGG																
SUT140 AF163027				TTAGCGTTGG TTAGTGTTGG										-						
ST2348				TTAGCGTTGG									1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1					A CONTRACTOR OF A CONTRACTOR OFTA CONTRACTOR O		
ST2340				TTAGCGTTGG																
AF163037				TTAATGTTGG																
AF163040				TTAGTGTTGG																
SUT078				TTAGCGTTGG																
SUT028				TTAGCGTTGG																
SUT124				TTACTOTTCC																
SUT125	340 A	CCCTTAAGC	CC-CTGTTGC	TTAGTGTTGG	GAGCCTAC	AGCGAT	G-TAGCTCCT	CAAAGTTAGT	GGCGGAGTCG	GTTACACACT	CTAGGCGCAG	TAAACTC	TT ATCT	C-GTCT	ACGGTTGTGG	CCGGTCCCTT	GCCGTAAAAC	CCCC-CAATT	TCTCAA	495
SUT074	315 A	CCCTCATGC	CC-CTAGGGC	GTGGTGTTGG	GAGCCCGCG-	ACCGAC	Geocesecce	TAAATCTAGT	GGCGGACCCG	TCGTGGCCTC	CCCTGCGAAG	TAGTH	AT ATTO	C-GCAT	CGGAGAGCGA	CGAG-CCCCT	GCCGTTAAAC	CCCCAACT	TTCCAA	467
SUT027	337 A	CCCTTAAGC	CTTCTGTAGC	TTAGCGTTAG	GGGCCTACC-	GTATGGC	GGTAGCCCCT	TAAAATTAGT	GGCGGAGTCG	GTCACACT	CTAGACGTAG	TAAT	TT ATCT	CGCCTA	T-AGTTGGAC	CGGTCCT	GCCGTAAA	CCTAA-TT	ATA	481
SUT198	337 A	CCCTTAAGC	CTTCTGTTGC	TTAGCGTTGG	GGGCCTACC-	GTATCCC	GGTAGCCCCT	TAAAATTAGT	GGCGGAGTCG	GTCACACT	CTAGACGTAG	TAAT	TT ATCT	CCCCTA	T-AGTTGGAC	CGGTCCT	GCCGTAAA	CCTATT	ATA	480
SUT155	337 A	CCCTTAAGC	CTTCTGTTGC	TTAGCGTTGG	GGGCCTACC-	GTATGGC	GGTAGCCCCT	TAAAATTAGT	GGCGGAGTCG	GTCACACT	CTAGACGTAG	TAAT	TT ATCT	CGCCTA	T-AGTTGGAC	CGGTCCT	GCCGTAAA	CCTAA-TT	ATA	481
SUT200	337 A	CCCTTAAGC	CTTCTGTTGC	TTAGCGTTCG	GGGCCTACC-	GTATGGC	GGTAGCCCCT	TAAAATTAGT	GGCGGAGTCG	GTCACACT	CTAGACGTAG	TAAT	TT ATCT	CGCCTA	T-AGTTGGAC	CGGTCCT	GCCGTAAA	CCTAA-TT	ATA	481
ST2298	337 A	CCCTTAAGC	CTTCTGTTGC	TTAGCGTTGG	GGGCCTACC-	GTATGGC	GGTAGCCCCT	TAAAATTAGT	GGCGGAGTCG	GT-TCACACT	CTAGACGTAG	TAAAT	TT ATCT	CGCCTA	TTAGTTGGAC	CGGTCCCC-T	GCCGTAAAAC	-CCCTAATTT	ATGCAA	493
SUT127	334 A	CCCTTAAGC	CT-CTGTTGC	TTAGTGTTGG	GAGCCTACG-	GTTCT	AGTAGCTCCT	CANANTTAGT	GGCGGAGTCG	GT-TCACACT	CTAGACGTAG	TAATG	TTT ATCT	CGCCTA	TCAGTTGGAC	COGTCCCCGT	GCCGTAAAAC	-CCCTAATTT	CTCAA-	487
SUT195	334 A	CCCTTAAGC	CT-CTGTTGC	TTAGTGTTGG	GAGCCTACG-	GTTCT	AGTAGCTCCT	CAAAATTAGT	GGCGGAGTCG	GT-TCACACT	CTAGACGTAG	TAATG	TTT ATCT	CGCCTA	TCAGTTGGAC	CGGTCCCC-T	GCCGTAAAAC	-CCCTAATTT	CTCAA-	486
SUT130				TTAGTGTTGG													GCCGTAAAAC	-CCCTAATTT	CTCAA-	486
AF163042				TTAGTGTTGG																427
572382				TTAGTGTTGG																
SUT092	351 A	CCCTTAAGC	CC-TTGTTGC	TTAGCGTTGG	GAGCCTACA-	GCGT	TGTAGCTCCT	TAAATTTAGT	GGCGGAACCG	GT-CCGCCTT	CTAGACGTAG	TAAT-TC	TTT ATTT	CGCCTA	CA-AGTCGTA	CCGGTCCC-T	GCCGTAAAAA	GCGTTAAGAT		498

Figure 10C. (Continued).

		10	20	30	40	50	60	70	80	90	10	11	0 12							9
SUT256							GCCTCGGCGT													73
Ju1	1																			
SUT070	1						GCCTCGGCGA													
SUT237	1																			59
SUT154	1						GCCTCGGCGC													60
SUT082	1																			
SUT074 SUT108	1						GCTTCGGCGG													78
SUT161	î						GCCTCGGCAG													109
SUT260	1																			
572321	1																			
AJ390421	1	GCGAGTTAAT	TACAAACT	CCARACCCA-	TOTGAA-CTT	ACCTGCTGTT	GCCTCGGCAG	GTTGCGCTGC		GGAG	TGCTTACCCT	GGAGTGGC		CTAC	CCTGGAGTAG	CTACCCTGTA	GTGCCTACCC	TGGAGT	AGGCACCC	136
KS15	1						GCCTCGGCGG													98
AJ390411	1																		GCCCGCCC	
SUT290 AF201706	1						GCCTCGGCAG												ACCTACCC	
SUT203	1						GCCTCGGCAG													100
SUT129	1																			98
SUT192	1	AAGAGTTAAA	AACAACTC	CTARACCCA-	TOTGAAACCT	ACC-TTTGTT	GCCTCGGCAG	GTOGCAACTT	ACCC	TGAGGG	GACCTACCCT	GTAGGT					-ACCTTACCC			101
SUT088	1																			97
572348	1						GCCTCGGCAG													99
SUT078	1						GCCTCGGCAG													98
SUT124	-						GCCTCGGCAG													90
AJ390437	1						GCCTCGGCAG													140
AJ390434	1						GCCTCGGCAG													153
ST2325	1	AAGAGTTCT-	-ATAACTC	CCARACCCA-	TOTGAA-CAT	ACCTTACGTT	GCCTCGGCAG	GTCGCGCC	T	ACCTAGTAGC	ACCOTACCCT	GTA					GGGCCTACCC	GG		98
SUT092	1						GCCTCGGCAG													
572382	1						GCCTCGGCAG													124
ST2310 SUT127	1						GCCTCGGCGG													97
SUT123	1																			
SUT051	1																			
SUT056	1	TAGAGTTTC-	-CAA-CTC	C-ARACCCA-	TOTGAA-CAT	ACCAGACOTT	GCCTCGGCAG	GCCGCGTGCC	AACC	TCTCTC	AGGG-GCGGC	GCG				C	-GCAAGGCCT			94
AY541610	1																			
SUT258	1																			
AJ390436 SUT201	1																			
SUT201	1																			
SUT032	1																			
SUT090	1	CTGAGTTATC	TAAACTC	CAAA-CCCTT	GOTGAAC-TT	A-CCGTCGTT	TCCTCGGCGT	-GTTGTGGGG	GTA		TAGCTACC						TACCC	GOTOGC	ATTA	92
SUT233	1						TCCTCGGCGT													
SUT066	1																		70	
SUT063	1						GCCTCGGAGT													
SUT221	-						GCCTCGGCGT													
SUT220	1						GCCTCGGCGC													
SUT001	1						GCCTCGGCGC													
SUT218	1																		CC	
SUT223	1																		cc	
SUT016 SUT282	1																		TC	
SUT166	1																		TA	
SUT158	1																		TA	
SUT294	1	CTGAGTTCTA	CAAAA-CT	CCCAACCCTA	TOTGAATCTT	A-CCACTOTT	GCCTCGGCGC	TGAGCGGCAG	CTA	CCCGG	GAGCTACCCT	GGAGGGAC	07	ACCCTGTAGA	TEGETACCCT	GG	AGCTACCC	TOGAGT	TG	132
SUT182	1																		TA	
SUT148	1																		TA	
SUT187	1																		TA	
SUT116 SUT103	-																		λ	
SUT120	1																		A	
AF616682	1																			
AF616681	1	CCGAGTTATC	TAAACT	CCAACCOTT-	TGTGAAACTT	A-COUTCOTT	GCCTCGGCGG	GCTGCGCTTA	ccc	TG	TAGCTACCCT	GTAG					CTACCC			89
SUT168D	1																			
SUT085D	1																			
AY616684	1																			
572584	1																		CTACTACCTA	

		19	0 20	0 21			0 24			0 27	0 28		0 30	31		0 330	340	35	0 360	0
SUT256	73															TTGGTTCTGG				
Jul																TIGGTICIGG				
SUT070	58															TIGGTICIGG				
SUT237 SUT154	59															TIGGTICIGG TIGGTICIGG				
SUT082	59															TIGGTICIGG				
SUT074	78															TIGGTICIGG				
SUT108	75															TIGGTICIGG				
SUT161 SUT260																TTGGTTCTGG TTGGTTCTGG				
ST2321																TIGGTICIGG				
AJ390421																TIGGTICIGG				
K\$15	98															TTGGTTCTGG				
AJ390411 SUT290																TTGGTTCTGG TTGGTTCTGG				
AF201706																TTGGTTCTGG				
SUT203																TTGGTTCTGG				
SUT129	98															TTGGTTCTGG				
SUT192 SUT088	101															TTGGTTCTGG TTGGTTCTGG				
512348	97															TTGGTTCTGG				
SUT078	98															TIGGTICIGG				
SUT028	96															TTGGTTCTGG				236
SUT124	95				the second s		and the second sec				and the second se					TIGGTICIGG				240
AJ390437 AJ390434	140															TTGGTTCTGG				288
ST2325	98															TTGGTTCTGG				236
SUT092	175															TTGGTTCTGG				
ST2382																TIGGTICIGG				
ST2310 SUT127	97 95															TTGGTTCTGG				
SUT123	95															TIGGTTCTGG				236
SUT051	94	-GCCGGCCGT	ACC	TCCCCGGCGT	CTCGCTGGTG	GGGCCGGCCC	CTGGACGGAG	GCGTCCGCCT	TA-ATTCTTG	AA-TACTGTT	GAATTCTAAA	ATCATAACTA	AATTAGTTAA	AACTITCAAC	AACGGATCTC	TIGGTICIGG	CATCGATGAA	GAACGCAGCG	AAATGCGATA	264
SUT056	94															TTGGTTCTGG				
AY541610 SUT258	84 99															TTGGTTCTGG				
AJ390436	99															TIGGTICIGG				
SUT201	81															TIGGTTCIGG				
SUT207	96															TTGGTTCTGG				
SUT032	98															TIGGTICIGG				
SUT090 SUT233	93															TTGGTTCTGG				
SUT066	120															TTGGTTCTGG				
SUT063																TIGGTICIGG				
SUT221	91															TTGGTTCTGG				
JU2 SUT220																TTGGTTCTGG				
SUT001																TIGGTICIGG				
SUT218	123	CGCCCGCA	CCCGGC	CIGCCGGIGG	ACCAACC	C	7	ACTCTTG-CA	AATATTOT	GGACTCTGAA	AT	ATA-AAAATA	AACGAATCAA	AACTITCAAC	AACGGATCTC	TTGGTTCTGG	CATCGATGAA	GAACGCAGCG	AAATGCGATA	263
SUT223																TIGGTICIGG				
SUT016 SUT282																TTGGTTCTGG				
SUT166																TIGGTICIGG				
SUT158	103	CGCC		-CGCCGGAGG	ACCACTARAC	T	c	TTGTTTTT	ACCA-TG	TATTTCTGAA	TG	CTTCAACT-A	AATA-GTTAA	AACTITCAAC	AACGGATCTC	TIGGTICIGG	CATCGATGAA	GAACGCAGCG	AAATGCGATA	231
SUT294																TIGGTICIGG				
SUT182 SUT148																TTGGTTCTGG				
SUT187																TIGGTICIGG				
SUT116	96	CATTCCAA	GC	TCGCCAGAGG	ACCTACCA			ACCTGTTTTA	T-ACTG	TATCTCTGAA	CT	-TTATAACTA	AATAAGTTAA	AACTITCAAC	AACGGATCTC	TTGGTTCTGG	CATCGATGAA	GAACGCAGCG	AAATGCGATA	229
SUT103	120															TIGGTICIGG				
SUT120 AF616682	96															TTGGTTCTGG TTGGTTCTGG				
AF616681	89															TTGGTTCTGG				
SUT168D	82															TIGGTICIGG				
SUT085D	82	-GCGGCGCGC	TACAGGC	CCGCCGGTGG	ACTGCTAA			AC-TCTGTTA	TATATACG	TATCTCTGAA	TG	-CTTCAACTT	AATAAGTTAA	AACTTTCAAC	AACGGATCTC	TTGGTTCTGG	CATCGATGAA	GAACGCAGCG	ARATGCGATA	224
AY616684	82															TIGGTTCIGG				
SUT164 ST2584	90															TTGGTTCTGG				

Figure 11C. (Continued).

316

	370	380	390	400	0 410	0 42	430										53	0 540	j .
SUT256	223 AGTAATGTGA														TGCAGC				388
Jul	216 AGTAATGTGA																		
SUT070	192 AGTAATGTGA																		357
SUT237 SUT154	192 AGTAATGTGA																		357
SUT082	191 AGTAATGTGA																		364
SUT074	214 AGTAATGTGA																		392
SUT108 SUT161	207 AGTAATGTGA																		369
SUT260	247 AGTAATGTGA																		413
ST2321	265 AGTAATGTGA																		431
AJ390421 KS15	280 AGTAATGTGA																		446
AJ390411	282 AGTAATGTGA																		448
SUT290	280 AGTAATGTGA																		445
AF201706 SUT203	274 AGTAATGTGA																		441
SUT129	240 AGTAATGTGA																		408
SUT192	244 AGTAATGTGA																		414
SUT088 ST2348	242 AGTAATGTGA																		412
SUT078	240 AGTAATGTGA																		408
SUT028	237 AGTAATGTGA																		405
SUT124	241 AGTAATGTGA																		407
AJ390437 AJ390434	289 AGTAATGTGA : 301 AGTAATGTGA :																		462
ST2325	237 AGTAATGTGA																		404
SUT092	318 AGTAATGTGA																		484
ST2382 ST2310	269 AGTAATGTGA																		435
SUT127	235 AGTAATOTGA																		402
SUT123	237 AGTAATGTGA																		402
SUT051	265 AGTAATGTGA																		430
SUT056 AY541610	265 AGTAATGTGA																		427
SUT258	247 AGTAATGTGA																		413
AJ390436	238 AGTAATGTGA																		410
SUT201 SUT207	212 AGTAATGTGA																		378
SUT032	242 AGTAATGTGA																		409
SUT090	238 AGTAATGTGA																		406
SUT233	239 AGTAATGTGA																		407
SUT066 SUT063	268 AGTAATGTGA																		452
SUT221	238 AGTAATGTGA																		409
Ju2	280 AGTAATOTGA																		447
SUT220 SUT001	284 AGTAATGCGA																		455
SUT218	264 AGTAATGTGA																		438
SUT223	264 AGTAATGTGA																		437
SUT282	248 AGTAATGTGA																		417
SUT166	272 AGTAATGTGA																		441
SUT158	232 AGTAATGTGA	ATTGCAGAAT	TCAGTGAATC	ATCGAATCTT	TGAACGCATA	TTGCGCCCAT	TAGTATTCTA	GTGGGGCATGC	CTATTCGAGC	GTCATTTCAA	CCCTTAAG	CCTTGTTGCT	TAGTGTTGGG	AGTCTACC	-GCC	TEGCEGTAGT	TCCTARARGG	TAGTGGCGGT	400
SUT294	270 CGTNATGTGA																		439
SUT182 SUT148	260 AGTAATGTGA : 240 AGTAATGTGA :																		430
SUT187	242 AGTAATGTGA																		409
SUT116	230 AGTAATGTGA																		403
SUT103 SUT120	268 AGTAATGTGA . 244 AGTAATGTGA .																		435
AF616682	234 AGTAATGTGA																		411
AF616681	234 AGTAATGTGA	ATTGCAGAAT	TCAGTGAATC	ATCGAATCTT	TGAACGCACA	TTGCGCCCAT	TAGTATTCTA	GTGGGCATGC	CTATTCGAGC	GTCATTTCAA	CCCTTAAGCC	TT-AGTTGCT	TAGCGTTGGG	AGTCTGCGCT	GTACTTGTT-	ACGGCGCAGT	TCCTCAAAGT	GATTGGCGGA	411
SUT168D SUT085D	225 AGTAATGTGA																		396
SUT085D AY616684	225 AGTAATGTGA																		395
SUT164	237 AGTAATGTGA																		406
ST2584	301 AGTAATGCGA	ATTGCAGAAT	TCAGTGAATC	ATCGAATCTT	TGAACGCACA	TTGCGCCCGC	TAGTATTCTG	GCGGGGCATGC	CTATTCGAGC	GTCATTACAA	CCCTTAAGCC	CC-TGTCGCT	TAGCGTTGGG	AGTCTGCGGC	TCA	GGCCGAGT	TCCTTAAA-T	TAGTGGCGAG	469

Figure 11C. (Continued).

		550				59	600	61				
SUT256	289						-GEGEGECCA					4.6
Jul							-AGCGGCCTC					
SUT070	358	GTTAGCAC-A	TACTCTAGGC	GTAGTAA-CA	CCATTCT	CGCTTCGGT-	AGTAAGTGCT	GGCGGCTAGC	CACTARACCC	CCTATACTTC	TAGT	44
SUT237							AGTA-GTGCT					
SUT154							AGTAGGC-CT					
SUT082							AGTA-GCCCT					
SUT074							-GAGAGCGAC					
SUT108 SUT161							-GTAATCOTC AGCCGGCTTA					
SUT260							AGCCGGCTTA					
ST2321							AGCCGGTTTA					
AJ390421							AGCCGGTCTA					
KS15	406	GTCGGTTCAC	CTCTAGAC	GTAGTAAATA	TTATCT	CGCCTATT-A	GTTGGACCGG	TCCCCTGC	CGTAAAACCC	C-TAATTTTC		48
AJ390411							GGCCGGCCCG					
SUT290							AGCCGGCTAR					
AF201706							AGCTGCCCTA					
SUT203							-GATGAGCCG					
SUT129 SUT192							-GATGAGCCG					
SUT088							-GATGAGCCC					
ST2348							-GATGAGCCC					
SUT078							-GATGCGCTG					
SUT028							-GATGCGCTG					
SUT124							-GTGTGGCCG					
AJ390437	463	GTCGGTTCAC	A-CTCTAGGC	GTAGTAAAGA	TTTTA-TTCT	CGCCTGTA-G	AGATGAGCCG	GTCCCC-TGC	CGTARAACAC	CCCCCTATTT	TT	55
AJ390434							GGATGGACCG					
ST2325							AGTTGACC					
SUT092							AGTCGTACCG					
T2382							TGTTGTGCCCG					
ST2310 SUT127							AGTTGGACCG					
SUT123							GGTCGTGCCG					
SUT051							AGTCGGACCG					
UT056							AGTCGGACCG					
AY541610							-GTCGGGCTA					
SUT258	414	GTCGG-TCAC	A-CTCCAGAC	GTAGTACTCT	TT-CACCT	CGCCTGTA	-GCTGGACCG	GTCCCC-TGC	CGGAAAACAC	CCCAAAATTT		49
AJ390436							GCGCGGGGCGG					
SUT201							AGTTGGGCTG					
SUT207							AGGTAAGCCG					
UT032							AGTAGGACGC					
SUT090 SUT233							GGTGGCCCTG GGTGGCCCTG					
SUT066							GGTGTCCCTG					
SUT063							GGGGGGCCCTG					
SUT221							GGTGGGGGCCT					
Ju2	448	TTCGCAGC-C	CACTCTGAGC	GTAGTAA-TC	AACTGGTTCT	CGCTCCTGC-	AGTGGCCGGC	GGAGCCC-GC	CGTAAAACCC	CCCCTA-TAA	CTAAGT-	53
SUT220	456	GCCGGGGGC-G	TETTETGAGE	GTAGTAA-TT	TATTATCT	CGCCCTGAA-	GCTAGCCCCG	TACGCCC-GC	CGTAAAACCC	CCCAAC-TAC	CTTGT	54
SUTOOL							AGTGGCCGCG					
SUT218							GGTACCCCTA					
SUT223							GGTACCCCTA					
SUT016							AGTACGCCTA AGTAAGCCCG					
SUT282 SUT166							AGTAAGCCCG					
UT158							AGTGGTTCTG					
UT294							AGTGNGTCTA					
UT182							AGTGGCCCGA					
UT148	409	GTTAGGGC-G	TACTCTTA-C	GTAGTAAATC	ACTATT	CGT-ACTG	AGTAGT-CTA	ACTTT-A-GC	CG-GAAACCC	CCATATTTAG	T	48
UT187							AGTTAGGCCG					
UT116							AGTGGTTATA					
UT103							AGTGGCCCTA					
SUT120							GGTCGCCCTG					
F616682							AGTTGTCCTG					
F616681 SUT168D							AGTTGTCCTG AGTAGCCCCG					
SUT168D							AGTAACCCCG					
AY616684							AGTAGCACCG					
SUT164							AGTGGCCCCT					
ST2584							GAGTCTGTGG					

Figure 11C. (Continued).

# Table 1C. The identity matrix of partial 18S rDNA sequences using NS1 and NS4 primers of Astrocystis mirabilis (SUT051, SUT056), Rosellinia sp. (ST2301), R. necatrix (AY083805), and A. cocoes (AY083804) calculated by the BioEdit program.

Species	A. mirabilis (SUT051)	A. mirabilis (SUT056)	A. cocoes (AY083804)	Rosellinia sp. (ST2301)	<i>R. necatrix</i> (AY083805)
A. mirabilis (SUT051)	1.000	1.000	0.982	0.981	0.974
A. mirabilis (SUT056)		1.000	0.982	0.981	0.974
A. cocoes (AY083804)			1.000	0.977	0.970
<i>Rosellinia</i> sp. (ST2301)				1.000	0.983
<i>R. necatrix</i> (AY083805)					1.000

Note: 1.000 means 100% identity.

Table 2C. The identity matrix of 18S rDNA ranging from NS1 and NS8 primers of

Astrocystis mirabilis (SUT056), Rosellinia sp. (ST2301), and R. necatrix

(AB014044) calculated by the BioEdit program.

Species	A. mirabilis (SUT056)	Rosellinia sp. (ST2301)	<i>R. necatrix</i> (AB014044)
A. mirabilis (SUT056)	1.000	0.703	0.687
Rosellinia sp. (ST2301)		1.000	0.673
<i>R. necatrix</i> (AB014044)			1.000

Note: 1.000 means 100% identity.

**Table 3C.** The identity matrix of ITS1-5.8S-ITS2 sequences of Astrocystis mirabilis (SUT056, SUT051), Rosellinia sp. ST2301, R.arcuata (AB017660), R. pepo (AB017659), R. quercina (AB017661), and R. necatrix (AB017657 and AB017658)calculated by the BioEdit program.

Species	AB017660	AB017657	AB017658	AB017659	AB017661	ST2301	SUT051	SUT056
AB017660	1.000	1.000	0.997	0.824	0.851	0.837	0.689	0.689
AB017657		1.000	0.997	0.824	0.851	0.837	0.689	0.689
AB017658			1.000	0.826	0.853	0.839	0.691	0.691
AB017659				1.000	0.772	0.764	0.659	0.659
AB017661					1.000	0.840	0.706	0.706
ST2301						1.000	0.699	0.699
SUT051							1.000	1.000
SUT056								1.000

Note: 1.000 means 100% identity.

Table 4C. The identity matrix of ITS1-5.8S-ITS2 sequences of Camillea tinctor (AJ390421, AJ390422), C. tinctor (ST2321), C.

obularia (AJ390423), C. tinctor (SUT161), C. tinctor (SUT260), and C. selangorensis (KS15) calculated by the BioEdit

program.

Species	AJ390421	AJ390422	ST2321	AJ390423	SUT161	SUT260	KS15
AJ390421	1.000	0.998	0.909	0.867	0.892	0.879	0.716
AJ390422		1.000	0.911	0.869	0.894	0.880	0.718
ST2321			1.000	0.858	0.937	0.920	0.730
AJ390423				1.000	0.863	0.856	0.709
SUT161					1.000	0.978	0.770
SUT260						1.000	0.766
KS15							1.000

Note: 1.000 means 100% identity.

## **APPENDIX D**

# **PHYLOGENETIC TREE CONSTRUCTIONS**



Figure 1D. Phylogenetic tree of *Rosellinia* and *Astrocystis* constructed by the maximum parsimony method using PAUP\* program based on ITS1-5.8S-ITS2 sequences. Tree length = 246; Consistent index (CI) = 0.8984; Homoplasy index (HI) = 0.1016; Retention index (RI) = 0.8663. Numbers of the branches indicate the bootstrap values resulting from 100 bootstrap replications.



Figure 2D. Phylogenetic tree of *Camillea* constructed by the maximum parsimony method using PAUP\* program based on ITS1-5.8S-ITS2 sequences. Tree length = 181; Consistent index (CI) = 0.9337; Homoplasy index (HI) = 0.0663; Retention index (RI) = 0.7600. Numbers of the branches indicate the bootstrap values resulting from 100 bootstrap replications.



Figure 3D. Phylogenetic tree of *Daldinia* constructed by the maximum parsimony method using PAUP\* program based on ITS1-5.8S-ITS2 sequences.
Tree length = 200; Consistent index (CI) = 0.8400; Homoplasy index (HI) = 0.1600; Retention index (RI) = 0.9489. Numbers of the branches indicate the bootstrap values resulting from 100 bootstrap replications.



Figure 4D. Phylogenetic tree of *Hypoxylon* sect. *Annulata* constructed by the maximum parsimony method using PAUP\* program based ITS1-5.8S-ITS2 sequences. Tree length = 613; Consistent index (CI) = 0.9396; Homoplasy index (HI) = 0.0604; Retention index (RI) = 0.9693. Numbers of the branches indicate the bootstrap values resulting from 100 bootstrap replications.



Figure 5D. Phylogenetic tree of *Hypoxylon* sect. *Annulata* constructed by the maximum parsimony method using PAUP\* program based on ITS1-5.8S-ITS2 sequences. Tree length = 867; Consistent index (CI) = 0.6586; Homoplasy index (HI) = 0.3414; Retention index (RI) = 0.8087. Numbers of the branches indicate the bootstrap values resulting from 100 bootstrap replications.



Figure 6D. Phylogenetic tree of *Hypoxylon* sect. *Annulata* constructed by the maximum parsimony method using PAUP\* program based on ITS2 sequences. Tree length = 330; Consistent index (CI) = 0.6242; Homoplasy index (HI) = 0.3758; Retention index (RI) = 0.8545. Numbers of the branches indicate the bootstrap values resulting from 100 bootstrap replications.



Figure 7D. Phylogenetic tree of *Hypoxylon* sect. *Hypoxylon* constructed by the maximum parsimony method using PAUP\* program based on ITS1-5.8S-ITS2 sequences. Tree length = 1437; Consistent index (CI) = 0.4878; Homoplasy index (HI) = 0.5122; Retention index (RI) = 0.7126. Numbers of the branches indicate the bootstrap values resulting from 100 bootstrap replications.



Figure 8D. Phylogenetic tree of *Hypoxylon* constructed by the maximum parsimony method using PAUP\* program based on ITS2 sequences. *Hypoxylon* sect. *Hypoxylon* is black whilst sect. *Annulata* is blue. Tree length = 798; Consistent index (CI) = 0.3797; Homoplasy index (HI) = 0.6203; Retention index (RI) = 0.4620. Numbers of the branches indicate the bootstrap values resulting from 100 bootstrap replications.



**Figure 9D.** Phylogenetic tree of *Xylaria* constructed by the maximum parsimony method using PAUP\* program based on ITS2 sequences. Tree length = 1230; Consistent index (CI) = 0.4886; Homoplasy index (HI) = 0.5114; Retention index (RI) = 0.6921. Numbers of the branches indicate the bootstrap values resulting from 100 bootstrap replications.



Figure 10D. Phylogenetic tree of xylariaceous fungi constructed by the maximum parsimony method using PAUP\* program based on ITS2 sequences. Tree length = 3244; Consistent index (CI) = 0.3203; Homoplasy index (HI) = 0.6797; Retention index (RI) = 0.5708. Numbers of the branches indicate the bootstrap values resulting from 100 bootstrap replications.



Figure 10D. (Continued).

## **APPENDIX E**

# LIST OF PRESENTATIONS

## **Poster Presentation**

- Suwannasai, N., Rodtong, S., Thienhirun, S., and Whalley, A.J.S. (2002). Taxonomic problems of high morphological variation of *Hypoxylon* spp. in Thailand. RGJ-Ph.D. Congress III, April 25-27, 2002, Jomtien Palm Beach Hotel and Resort, Chonburi, Thailand.
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ณัฏฐิกา สุวรรณาศรัย, สุรีลักษณ์ รอดทอง, สุรางค์ เธียรหิรัญ, และ Whalley, A.J.S. (2547). ข้อ

มูลทางชีววิทยาโมเลกุลเพื่อการอนุกรมวิชานของเชื้อราสกุล Hypoxylon (Molecular and Biology data for the Taxonomy Study of Hypoxylon). การประชุมความหลากหลายทางชีวภาพ "งานวิจัยจากอดีตสู่อนาคต", 30 สิงหาคม —

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- Suwannasai, N., Rodtong, S., Thienhirun, S., and Whalley, A.J.S. (2005). **Perispore Ornamentations for the Indication of** *Hypoxylon* **Species**. The 22<sup>nd</sup> Annual Conference of the Microscopy Society of Thailand, Febuary 2-4, 2005, The Tide Resort, Chonburi, Thailand.
- Suwannasai, N., Rodtong, S., Thienhirun, S., and Whalley, A.J.S. (2005). Molecular taxonomic studies of selected members of the Xylariaceae. Annual Scientific Meeting Exploitation of Fungi, September 5-8, 2005, British Mycological Society, Manchester, U.K.

## **Oral Presentation**

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- Suwannasai, N., Rodtong, S., Thienhirun, S., and Whalley, A.J.S. (2004). Relationships within *Hypoxylon* Species Based on Morphological and Molecular Data. (2004). The IV Asia-Pacific Mycological Congress and the IX International Marine and Freshwater Mycology Symposium, November 14-19, 2004, Lotus Hotel Pang Suan Kaew, Chiang Mai, Thailand.
- Suwannasai, N., Rodtong, S., Thienhirun, S., and Whalley, A.J.S. (2005). Nucleotide Sequence Data for the Clarification of Species Complex in

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## Manuscripts

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#### S3-P7

#### Taxonomic Problems of High Morphological Variation of *Hypoxylon* spp. in Thailand

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#### Objective

To identify and classify *Hypoxylon* species using conventional taxonomic methodology.

#### Methods

*Hypoxylon*, Xylariaceae (Ascomycota) specimens, were collected from forest areas as recording by Thienhirun (1). The samples were identified and classified using their macroscopic and microscopic characteristics of teleomorph stage (1,2 and 3). The stromatal and perithecial structures were characterized in size, shape, texture and colour. The colour pigments were extracted using 10% KOH. Asci and ascospores were mounted in Melzer's reagent to detect blue apical rings from amyloid iodine reaction. Cultural features on potato dextrose agar (PDA) were performed. Fungal mycelia were collected for genomic DNA investigations in our future study

#### Results

Form morphological taxonomy, six species of *Hypoxylon: Hypoxylon stygium*, *Hypoxylon nitens*, *Hypoxylon moriforme*, *Hypoxylon purpurconitens*, *Hypoxylon stygium* var. *annulatum* and *Hypoxylon bovei* var. *microspora* were very closely related. Their extracted pigments were green, except *H. purpureonitens* was purple. The stromata, perithecia and disks were also vary in sizes and shapes. They were different from previously reported by Rogers (2). When cultured, they did not form stromatal structure (teleomorph stage) which commonly found in nature. Therefore, other techniques particularly molecular biology techniques (4), for example could help to clarify these problems.

#### Conclusion

Six *Hypoxylon* species were found to be difficult to identify and classify using morphological and culture characteristics, which are frequently used by several investigators. The absence of teleomorphs in their cultures increased the limitation of the conventional method. Nucleic acid techniques will be of our interest for further problem clarification.

Keywords: Hypoxylon, Xylariaceae, taxonomy, culture, teleomorph stage

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## ข้อมูลทางชีววิทยาโมเลกุลเพื่อการศึกษาอนุกรมวิธานของเชื้อราสกุล Hypoxylon

Molecular Biology Data for the Taxonomy Study of Hypoxylon (Fungi)

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#### บทคัดย่อ

Hypoxylon เป็นเชื้อรา Ascomycetes สกุลใหญ่สกุลหนึ่งในวงศ์ Xylariaceae ที่มีบทบาทสำคัญในการย่อยสลาย ซากพืชในระบบนิเวศและบางชนิดยังเป็นสาเหตุของโรคพืช ในการศึกษาอนุกรมวิธานของเชื้อราสกุลนี้อาศัยลักษณะ ทางสัณฐานเป็นหลักได้แก่ ลักษณะรูปร่าง ขนาด และ สี ของ Stroma, Perithecia, Ascospore, Germ slit และ Apical apparatus รวมถึงการเกิดปฏิกิริยาเคมีของ Stroma กับสารละลาย KOH 10% ซึ่งลักษณะทางสัณฐาน ดังกล่าวมีความผันแปรสูงและยากต่อการใช้เพื่อให้ได้ผลการศึกษาอนุกรมวิธานของ *Hypoxylon* ชนิดที่ใกล้เคียงกัน ดังนั้นในการศึกษาครั้งนี้จึงนำเทคนิคทางชีววิทยาโมเลกุลมาศึกษาอนุกรมวิธานของ *Hypoxylon* ชนิดที่ใกล้เคียงกัน ดังนั้นในการศึกษาครั้งนี้จึงนำเทคนิคทางชีววิทยาโมเลกุลมาศึกษาเพื่อให้ได้ข้อมูลลำดับนิวคลีโอไทด์ของดีเอ็นเอ (DNA) ในส่วน Internal transcribed spacer (ITS) regions 1 และ 2 และ 5.8S ribosomal RNA gene ซึ่งพบว่า ITS regions ดังกล่าวมีขนาดประมาณ 500 ถึง 900 คู่เบส และมีลำดับนิวคลีโอไทด์ที่สามารถใช้เพื่อช่วยระบุและจัด จำแนกชนิดของ *Hypoxylon* ได้อย่างชัดเจนและยังเป็นข้อมูลสำคัญที่ใช้เพื่ออธิบายความสัมพันธ์และวิวัฒนาการ ของเชื้อราสกุลนี้ได้ด้วย

#### Abstract

*Hypoxylon*, an Ascomycetes fungus, is one of the large genera of the family Xylariaceae, which plays a major role in wood decomposition in ecosystems, and some are weak plant pathogens. The taxonomy study of *Hypoxylon* species is principally relied on their morphological characteristics of stroma, perithecia, ascospore, germ slit, apical apparatus, and the chemical reaction of stroma with 10% KOH. The high variation of morphological characters among *Hypoxylon* species especially in closely related species is always encountered, which resulted in the taxonomic problems. In this study, the molecular biology technique was applied to obtain data of DNA sequences of internal transcribed spacer (ITS) regions 1 and 2 including 5.8S ribosomal RNA gene. Approximately 500 to 900 base pairs of the ITS sequences were achieved. These sequences could be used for aiding the clear-cut identification and classification of *Hypoxylon* species. The molecular data are also valuable for the explanation of relationships and evolution of the fungus.

คำสำคัญ: Xylariaceae, Hypoxylon, ITS, DNA sequences

#### บทนำ

เชื้อราสกุล *Hypoxylon* เป็นเชื้อราสกุลใหญ่สกุลหนึ่งในวงศ์ Xylariaceae (Ascomycetes) ประกอบด้วยอย่าง น้อย 130 ชนิด (Ju & Rogers, 1996) ซึ่งเป็นที่รู้จักและพบได้ในหลายประเทศโดยเฉพาะในเขตร้อนและเขตอบอุ่น ในประเทศไทยมีรายงานว่าพบเชื้อราสกุลนี้อย่างน้อย 47 ชนิด (Thienhirun, 1997) ซึ่งพบได้บนท่อนไม้และกิ่งไม้ เชื้อราสกุลนี้มีบทบาทสำคัญในการย่อยสลายซึ่งช่วยรักษาสมดุลของระบบนิเวศ และยังพบว่าบางชนิดเป็นสาเหตุ ของโรคพืช เช่น Hypoxylon rubiginosum และ Hypoxylon mammata (คือ Entoleuca mammata ตาม Edward และคณะ (2003) ซึ่งก่อให้เกิดโรค Canker ในการศึกษาอนุกรมวิธานของเชื้อราสกุลนี้อาศัยลักษณะทางสัณฐานเป็น หลักได้แก่ ลักษณะรูปร่าง ขนาด และ สี ของ Stroma, Perithecia, Ascospore, Germ slit และ Apical apparatus รวมถึงการเกิดปฏิกิริยาเคมีของ Stroma กับสารละลาย KOH 10% ซึ่งลักษณะทางสัณฐานดังกล่าวมีความผันแปร สูงโดยเฉพาะอย่างยิ่งชนิดที่ใกล้เคียงกันของ Hypoxylon Section Annulata ซึ่งพบ 1/3 ของเชื้อสกุล Hypoxylon มี ลักษณะเด่นคือ Ostioles อยู่ในระดับที่สูงกว่าผิวของ Stroma และมีลักษณะเป็นแผ่นกลม (Disc) อยู่ข้างบน ซึ่งยาก ต่อการศึกษาเพื่อให้ได้ข้อมูลทางอนุกรมวิธานที่ถูกต้อง ดังนั้นในการศึกษาครั้งนี้จึงได้นำเทคนิคทางชีววิทยาโมเลกุล โดยการหาลำดับนิวคลีโอไทด์ของดีเอ็นเอในส่วน Internal transcribed spacer (ITS) regions 1 และ 2 รวมทั้งส่วน 5.8S ribosomal RNA gene มาช่วยในการระบุและจัดจำแนกชนิดเพื่อเป็นข้อมูลในการศึกษาอนุกรมวิธานของเชื้อ รากลุ่มนี้ได้อย่างเชื่อมั่นและชัดเจนข่อไป

### ระเบียบวิธีวิจัย

1. การศึกษาเพื่อการระบุและจัดจำแนกชนิดของเชื้อราสกุล Hypoxylon ที่พบในประเทศไทย

ศึกษาเพื่อการระบุและจัดจำแนกชนิดตามลักษณะทางสัณฐาน (ลักษณะ รูปร่าง ขนาด และ สี ของ Stromata, Perithecia, Ascospores, Germ slit และ Apical apparatus) รวมทั้งการเกิดปฏิกิริยาของ Stromata กับ KOH 10% ของเซื้อราสกุล *Hypoxylon* Section *Annulata* จำนวน 38 ตัวอย่าง ที่รวบรวมได้จากพื้นที่ในประเทศไทย (ตารางที่ 1)

2. การศึกษาเพื่อให้ได้ข้อมูลทางชีววิทยาโมเลกุลของเชื้อราสกุล Hypoxylon

แยกให้ได้เชื้อบริสุทธิ์จากสปอร์ โดยเพาะเลี้ยงบนอาหาร Potato Dextrose Agar (PDA) และ สกัดดีเอ็นเอ จากเส้นใยโดยใช้วิธีที่ประยุกต์จาก White และ คณะ (1990) จากนั้นใช้เทคนิคพีซีอาร์ (Polymerase Chain Reaction, PCR) เพิ่มจำนวนดีเอ็นเอในส่วน Internal Transcribed Spacer (ITS) regions 1 และ 2 รวมทั้งส่วน 5.8S ribosomal RNA gene โดยใช้ Primers ITS4 (5' TCCTCCGCTTATTGATATGC 3') และ ITS5 (5' GGAAGTAAAAGTCGTAACAAGG 3') (White และคณะ, 1990) ตรวจสอบขนาดของขึ้นดีเอ็นเอที่ได้ (PCR Products) โดยใช้เทคนิคเจลอิเล็กโทรโฟรีซีส (Gel electrophoresis) และหาลำดับ นิวคลีโอไทด์ ด้วยเครื่อง ABI370 Automate DNA Sequencer (Perkin Elmer, USA) จากนั้นวิเคราะห์ข้อมูลที่ได้ด้วยโปรแกรม Chromas และ BioEdit เพื่อ สร้างแผนภูมิความสัมพันธ์ทางพันธุกรรม (Phylogenetic tree) โดยใช้โปรแกรม MagAlign (DNASTAR, USA) เปรียบเทียบกับเชื้ออ้างอิงใน GenBank (AJ390397 และ AJ390409)

## ผลการวิจัย อภิปราย และ สรุปผลการวิจัย

1. การระบุและจัดจำแนกชนิดของเชื้อราสกุล Hypoxylon ตามลักษณะทางสัณฐาน

จากการศึกษาลักษณะทางสัณฐานเพื่อการระบุและจัดจำแนกชนิดของเซื้อรา Hypoxylon ใน Section Annulata สามารถจัดจำแนกได้อย่างน้อย 7 ชนิด (Species) คือ Hypoxylon stygium, H. atroroseum, H. nitens, H. moriforme, H. purpureonitens, H. bovei var. microspora, H. urceolatum และ พบว่าบางตัวอย่างไม่ สามารถจัดจำแนกชนิดได้ เนื่องจากลักษณะทางสัณฐานมีความผันแปรสูงคือสีและขนาดของ Stroma, Ostiolar disc, Ascospores รวมทั้งการเกิดปฏิกิริยาของ Stroma กับ KOH 10% ใกล้เคียงกันมากดังตัวอย่างในตารางที่ 1 และรูปที่ 1

## 2. ข้อมูลทางชีววิทยาโมเลกุลของเชื้อราในสกุล Hypoxylon

ในการเพิ่มจำนวนดีเอ็นเอในส่วน ITS regions ของเชื้อรา Hypoxylon พบว่ามีขนาดแตกต่างกันอยู่ในช่วง 500 ถึง 900 คู่เบส (รูปที่ 2) ซึ่งใช้เพื่อหาลำดับนิวคลีโอไทด์และความสัมพันธ์ เมื่อนำลำดับนิวคลีโอไทด์ที่ได้มาจัด แนวความสัมพันธ์ที่เหมาะสม (Alignment) พบความผันแปรสูงที่สุดในส่วน ITS1 โดยเฉพาะ H. stygium และ H. atroroseum (รูปที่ 3) ซึ่งมีลักษณะทางสัณฐานใกล้เคียงกันมาก แต่พบความต่างของลำดับนิวคลีโอไทด์ที่ได้มาจัด (รูปที่ 3) ส่วน H. nitens, H. bovei var. microspora และ H. moriforme ซึ่งมีลักษณะทางสัณฐานที่คาบเกี่ยวกัน และมีจำนวนตัวอย่างที่หลากหลาย พบว่า H. nitens มีขนาดของส่วน ITS1 ประมาณ 564 คู่เบส ขณะที่ H. bovei var. microspora และ H. moriforme มีขนาดประมาณ 207 และ 181 ตามลำดับ รวมทั้งมีลำดับนิวคลีโอไทด์ที่ แตกต่างกัน ทำให้สามารถแยก Hypoxylon แต่ละชนิดออกจากกันได้อย่างชัดเจน (รูปที่ 4) โดยหาความสัมพันธ์ทาง พันธุกรรมในรูปของ Phylogenetic tree (รูปที่ 5) และเชื้อ Hypoxylon บางตัวอย่างที่ยังไม่สามารถระบุและจัด จำแนกชนิดได้เช่น Hypoxylon sp. SUT103, Hypoxylon sp. ST2345 และ Hypoxylon sp. ST2406 พบว่า สามารถระบุแยกเชื้อราดังกล่าวออกมาจากกลุ่มของ Hypoxylon ชนิดอื่น อาจนำมาซึ่งการค้นพบเชื้อราชนิดใหม่ใน Hypoxylon ก็เป็นได้ โดยลำดับนิวคลีโอไทด์ที่ได้จากการศึกษาครั้งนี้สามารถนำมาใช้ออกแบบ Primers และ/หรือ Probes ที่จำเพาะต่อเชื้อแต่ละชนิดเพื่อช่วยในการศึกษาครั้งนี้สามารถนำมาใช้ออกแบบ Primers และ/หรือ ความสัมพันธ์และวิวัฒนาการของเชื้อราสกูดนี้อีก้วย
ตารางที่ 1 ความหลากหลายของลักษณะทางสัณฐานและการเกิดปฏิกิริยาของ Stroma กับ KOH 10% ของเชื้อราสกุล *Hypoxylon* Section *Annulata* ที่พบใน ประเทศไทย

ชนิดของเชื้อรา	<u></u> ଜି୩	าเมาด	ขนาดของ Ascospores (µm)	สีจากปฏิกิริยาของ	พื้นที่ของจังหวัดที่พบเชื้อ	จำนวนตัวอย่าง
	Stroma	୩୧୯		Stroma ทับ KOH 10%		
		Ostiolar				
		disc				
		(mm/Ø)				F.
Hypoxylon stygium	ด้ามัน	0.1	3.75-6.25 x 2.5-3.75	ធឿខា	ตราด ราชบุรี	10
H. atroroseum	ด้า-ชมพู	0.1	4.6-6.25 x 2.5-3.75	្លឿខាក្	นครราชสีมา	2
H. nitens	ด้ามัน	0.3-0.4	7.6-9.1 x 3.4-4.0	ធើខា	ตราด นครราชสีมา	8
H. bovei var. microspora	ື່ຄຳ	0.3-0.6	7.5-10.0 x 3.75-5.0	ឿេខ	นครราชสีมา	5
H. purpureonitens	ด้ามัน	0.2-0.5	7.5-10.0 × 3.75-5.0	ង់ល្	ตราด นครราชสีมา สงขลา	9
H. urceolatum	ື່ຄ່	0.2-0.3	10.0-12.5 x 2.5-5.0	ม่วง	สรขลา	~
H. moriforme*	ด้า	0.2-0.5	6.0-9.0 x 2.5-4.0	ឿ៩០	218	-
H. sp. SUT103	هٔ	0.1	8.75-10.0-(11.5)x2.5-3.75	เหล็อง-น้ำตาล	<b>ଝଏ</b> ଅଛମ	ю
H. sp. ST2345*	ື່ຄ່	0.1	6.3-7.5 x 3.8	ឿខាភ	I	Ŧ
H. sp. ST2406*	ຄຳ	0.3	7.5-8.8 x 3.8-5	្នោខាភ	ı	-

เชื้อจากสำนักวิจัยการจัดการป่าไม้และผลิตผลป่าไม้ กรมป่าไม้ กรุงเทพฯ (ดร.สุรางค์ เธียรหิรัญ ผู้เก็บและดูแลตัวอย่างเชื้อรา)



รูปที่ 1 ตัวอย่างลักษณะ Stroma (กำลังขยาย 40 เท่า) ของเชื้อราสกุล Hypoxylon ใน Section Annulata (ก) Hypoxylon stygium, (ข) H. atroroseum, (ค) H. nitens, (ง) H. bovei var. microspora, (จ) H. purpureonitens, (ฉ) H. urceolatum, (ซ) Hypoxylon sp. SUT103, (ซ) Hypoxylon sp. SUT251



รูปที่ 2 ผลผลิตจาก PCR ในส่วนของ ITS1-5.8S-ITS2 ของเชื้อรา *Hypoxylon* Section *Annulata* เมื่อตรวจหาด้วย เทคนิคเจลอิเล็กโทรโฟรีซีส (Agarose 1.5%)

ช่อง 1: M, Molecular weight marker (100 base pairs DNA Ladder, Promaga); 1, 5 และ 8, Hypoxylon purpureonitens; 2 และ 4, H. atroroseum; 3, H. bovei var. microspora; 6, 7 และ 9, H. nitens; 10 และ 12, Hypoxylon sp. SUT103; 11, H. moriforme



รูปที่ 3 การจัดแนวความสัมพันธ์ที่เหมาะสมของลำดับนิวคลีโอไทด์ในส่วน ITS1 ของเชื้อ Hypoxylon atroroseum กับ H. stygium (ลูกศรแสดงช่วงของลำดับนิวคลีโอไทด์ที่ต่างกัน) ซึ่งพบความเหมือน (homology) 90 %



รูปที่ 4 การจัดแนวความสัมพันธ์ที่เหมาะสมของลำดับนิวคลีโอไทด์ในส่วน ITS1 ของเชื้อราในสกุล Hypoxylon 10 ชนิด (Hypoxylon atroroseum, H. stygium, H. nitens, Hypoxylon sp. ST2345, H. urceolatum, H. purpureonitens, Hypoxylon sp. SUT103, Hypoxylon sp. ST2338, H. moriforme และ H. bovei var. microspora)

	410	420	430	440	450	460	470	480
SUT0098.atronoseum.seq SUT0588.stygium.seq SUT081.nitens.seq SUT081.nitens.seq SUT0818.urceolatum.seq SUT0048.purpureonitens.s SUT1004.primeronitens.s SUT1004.sp;ST103.seq ST14064.sp;ST238.seq ST14064.sp;ST238.seq ST14064.sp;ST238.seq ST14064.sp;ST238.seq ST14064.sp;ST238.seq	TTTCAASCT TGCTACOCTOTACT TAATOLIATOBAATT TTTCAA -ASCOCSCOSAA -GCOCASCOSCO- CACCCCTS	AC ACTICIOSCI OCPICIIOIA —AACTIAC	GIRGIGIGIC TRANCGAAPI	rcccostrog cracstos pagroggern og cracs	AATATCHGCT AATAATOOCT AATAATOOCT AATAATOOCT AATAATOOCT AATAATOOCT AATOOCT AATOOCT AATOOCT AATOOCT AATOOCT AATOOCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATATCHGCT AATAATTCHGCT AATAATTCHGCT AAT	OGAAAATTGT OGAATATAAT OGAA-ATG	ICAAAOCTOI ICTITTITION ICTITTITION ICTITTITION ICTIA-GODAI	PACIAA 4 PAGING 4 CAGING 3
	490	sdo	slo	520	530	540	530	560
NFT009H.atroroseum.seq UFD58H.stygium.seq UFD58H.stygium.seq TT345M.sp.ST2345.seq UTD98H.urceolatum.seq UTD98H.urceolatum.seq UTD98H.sp.ST103.seq UT03H.sp.ST103.seq TT236H.spST2338.seq TT236H.spST2338.seq TT236H.spST25H.seq UT235H.bovai var.microsp		ANTICATANA PARTICINA CANITATAN CORA ANCICIOSCI ANCICIOSCI SCOSSCOAR	-A-TTOCAA TCTTTCCCCG TOCCTOCAAT TTAAC CCCCC-CTGT TTACCCAACT CCCCCACT CCCCCCCCCCCCCCC	AGOCADOTA PTOGRATITT ATOCTITION CCTA ATROCTTO FIRICICT ARACTTO	TAAACTA COCTOGAG CTTUTAGGAA ACTU ACTU	GITCLATT GITCLATT CORROTACE F T	TAGOGO TTT-TAGAGT FACGTOGAAA TAA-	OTGA 41 OGAAAT 52 OGAAAT 54 
	510	580	590					
NTOCH.atreroseum.seq DTOSH.stygium.seq DTOSH.siygium.seq T2345H.sp.ST2345.seq UTO98H.urceolatum.seq		PITTAAAAAC PITATIAAAA - PIACAAAAA - AAAG	CAAATAOGTT CAACTTATAT CAATACITT-T TAACTT-T	ил 32 4 4 4				

รูปที่ 4 (ต่อเนื่อง)



รูปที่ 5 Phylogenetic tree ของ ITS1-5.8S-ITS2 regions ของเชื้อราชนิดต่างๆในสกุล *Hypoxylon* Section Annulata

**กิตติกรรมประกาศ**: งานวิจัยนี้ได้รับทุนสนันสนุนการวิจัยจากโครงการปริญญาเอกกาญจนาภิเษก สำนักงาน กองทุนสนับสนุนการวิจัย และการสนับสนุนสถานที่และเครื่องมือจากมหาวิทยาลัยเทคโนโลยีสุรนารีและกรมป่าไม้

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#### IV ASIA-PACIFIC MYCOLOGICAL CONGRESS

An improved method for the rapid dereplication of impure chemical ITS sequence heterogenecity of *Xylaria* species and some other Xylariaceous genera

## N. Suwannasai<sup>1</sup>, S. Rodtong<sup>1</sup>, S. Thienhirun<sup>2</sup> and A.J.S. Whalley<sup>3</sup>

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Xylaria was the first described genus of the family Xylariaceae and it is a large and relatively well-known fungus group in most countries in the world. There are also other well-known genera such as Biscogniauxia, Camillea, Daldinia, and Hypoxylon. They occur on wood, leaves, seeds, dung, and soil or in a few cases are associated with insects. Some species are weak phytopathogens and many Xylaria species have been reported as endophytes living inside healthy plant tissue without apparent damage to the host. Recently, endophytes have been widely investigated because of their ability to produce new or interesting secondary metabolites some of which have proved to be bioactive. The conventional taxonomic studies of Xylaria have been based on morphological and cultural characteristics but have in some cases included their metabolite profiles. The major problems concern the high morphological variation among Xylaria species depending on stages of development and localities of collection, and the lack of teleomorph stage in the culture. Therefore, the molecular taxonomic study was undertaken overcome these problems. Nucleotide sequences of ITS1, 5.8S, and ITS2 rDNA of 48 Xylaria isolates as well as some other Xylariaceous genera obtained from different collection locations were analyzed. Alignments of the Xylaria species sequences exhibited the greatest variation in the ITS regions. The 5.8S sequence gave approximately 99% similarity for all isolates tested but ITS sequence comparison results supported a monophyletic group in this genus, which is separated from the genera Biscogniauxia, Camillea, Daldinia, and Hypoxylon. The sequences proved to be valuable for the taxonomic investigation of fungi such as Xylaria with their high morphological variation. In addition, a database of this molecular data would be useful for the designation of specific primers and for the development of species specific probes for the detection of Xylaria in environmental situations.

**AP59** 

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Poster Presentation

# Perispore Ornamentations for the Indication of Hypoxylon Species

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The perispore ornamentation observed by using scanning electron microscope (SEM) has been recently used to indicate new or complex species in the fungal taxonomy. Some species of Hypoxylon have been reported concerning the significance of this characteristic in species indication but many of them have no record. In this study, three complex species of Hypoxylon fendleri, H. retpela, and H. cf. lenormandii collected in Thailand, have been investigated in their morphological characteristics both macroscopic and microscopic methods including perispore ornamentations by SEM. Hypoxylon fendleri and H. retpela are very closely related species. Their SEM micrographs of perispores resulted the same conspicuousness of the coil-like ornamentation which was different from Ju and Rogers (1996) who stated that H. fendleri and H. retpela differ mainly in the conspicuousness of the ornamentation on the perispore. However DNA sequencing results indicated 14 % divergence among both species. In case H. cf. lenormandii SUT065 occurring on bamboo has strange coil-like ornamentation which was different from H. lenormandii occurring on wood that have inconspicuous coil-like ornamentation. This result was supported by DNA sequencing data to separate H. cf. lenormandii to be a new variety.

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Figure 1. The fungus genus Hypoxylon. SEM micrographs of coil-like perispore ornamentation of H. cf. lenormandii (a), H. retpela (b), and H. fendleri (c), germ slit of H. cf. lenormandii (arrow) (d); and ostiole of H. cf. lenormandii (arrow) (d); and ostiole of H. cf. lenormandii (arrow) (e). Light microscope micrographs of H. retpela ascus containing eight ascospores (f), stromatal forms of H. fendleri (g), H. retpela (h), and H. cf. lenormandii (i). Bars equal 1 µm for a, b, c, d, e; and 1 mm for g, h, i.

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Paper/Poster title

Molecular taxonomic studies of selected members of the Xylariaceae

Abstract - maximum 200 words

Representative species of a number of genera belonging to the family Xylariaceae (Ascomycotina) were collected from different regions of Thailand. These were identified using traditional morphological characters of both teleomorph and anamorph stages (when available) and together with selected temperate and non-Thai species were subjected to molecular examination. Nucleotide sequences of ITS1, 5.8S and ITS tDNA were analysed. In general the ITS1 region exhibited the greatest variation among the species studied whereas 5.8S and ITS2 regions were more conserved. The molecular data indicated that *Xylaria* is a monophyletic group which is separated from the genera *Biaognianxia, Camillea, Daldinia* and *Hypoxylan.* The data also supported a clear distinction between *Astropystia* and *Rosellinia* which contrasts with some modern authors views. Furthermore it was found that closely related species groups or genera appeared as separate entities but retained a close similarity. In most cases the molecular data supported the traditional taxonomic grouppings.

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## แนวโน้มการพบเชื้อราชนิดใหม่ในกลุ่ม Xylariaceae ในประเทศไทย

TREND IN THE FINDING OF NEW XYLARIACEOUS FUNGAL SPECIES IN THAILAND ณัฏฐิกา สุวรรณาศรัย<sup>1</sup> สุรัลักษณ์ รอดทอง' สุรางค์ เธียรหิรัญ² และ Anthony Whalley<sup>3</sup>

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บทคัดย่อ: เชื้อราวงศ์ Xylariaceae เป็นเชื้อวงศ์ใหญ่ใน Ascomycetes ประกอบด้วย 45 สกุล (genera) พบได้บน ท่อนไม้ ไบ่ไม้ ผลไม้ มูลสัตว์ และบนดิน และมีบทบาทสำคัญในการย่อยสลายซึ่งช่วยรักษาสมดุลของระบบนิเวศ นอกจากนี้ยังพบว่าบางชนิดเป็นสาเหตุของโรคพีซ และบางชนิดสามารถเจริญอยู่ร่วมกับพืชโดยไม่ก่อให้เกิดโรคและ อาจสร้างสารต้านแมลงศัตรูพืชได้อีกด้วย (3) จากการสำรวจและเก็บตัวอย่างเชื้อรากลุ่ม Xylariaceae ในพื้นที่ 9 จังหวัดในประเทศไทยได้จำนวน 320 ตัวอย่าง สามารถจัดจำแนกตามลักษณะทางสัณฐานและการเจริญได้ 69 ชนิด ใน 9 สกุล คือ Hypoxylon, Xylaria, Daldinia, Astrocystis, Camellea, Biscogniauxia, Rosellinia, Kretzschmaria และ Nemania ทั้งนี้พบเชื้อ Hypoxylon 1 ชนิด ที่มีแนวโน้มว่าจะเป็นขนิดใหม่ คือมีวงแหวนสีขาว อยู่บนผิวของ Stroma ซึ่งเป็นลักษณะเด่นในการระบุว่าเป็น Hypoxylon michelianum ที่พบในแถบยุโรปเท่านั้น (1) แต่เสื้อที่พบนี้มีลักษณะดังกล่าวแต่ขนาดของสปอร์เล็กกว่าประมาณ 1 เท่าซึ่งยังไม่เคยมีการรายงานมาก่อน

ระเบียบวิธีวิจัย: รวบรวมตัวอย่างเชื้อราในกลุ่ม Xylariaceae จากพื้นที่ 9 จังหวัดในประเทศไทยมาศึกษาเพื่อการ ระบุและจัดจำแนกชนิดตามลักษณะทางสัณฐาน (ลักษณะ ขนาด รูปร่าง และรูปแบบของ Stromata, Perithecia, Ascus, Ascospores, Apical apparatus) รวมทั้งการเกิดปฏิกิริยาของ Stromata กับ KOH 10% และศึกษา ลักษณะการเจริญของเส้นใยโดยเพาะเลี้ยงในอาหาร Potato Dextrose Agar (PDA)

ผลการวิจัย อธิปราย และสรุปผลการวิจัย: จากตัวอย่างที่รวบรวมได้จำนวน 320 ตัวอย่าง สามารถจัดอยู่ในสกุล

Hypoxylon 35 ขนิด Xylaria 25 ขนิด Daldinia 1 ขนิด Astrocystis 1 ขนิด Carnellea 1 ชนิด Biscogniauxia 3 ชนิด Rosellinia 1 ชนิด Kretzschmaria 1 ชนิด Nemania 1 ขนิด และพบเชื้อ Hypoxylon 1 ชนิด ที่มีแนวโน้มว่าจะเป็นขนิดใหม่คือมีวงแหวนสี ขาวอยู่บนผิวของ Stroma ซึ่งเป็นลักษณะจำเพาะของ Hypoxylon michelianum ที่ พบในแถบยุโรปเท่านั้น (1) แต่เชื้อที่พบนี้มีขนาดสปอร์เล็กกว่าประมาณ 1 เท่า ซึ่งยัง ไม่เคยมีการรายงานมาก่อน ทั้งนี้การศึกษาลักษณะทางสัณฐานมีข้อจำกัดในการระบุ และจัดจำแนกขนิดได้อย่างเชื่อมั่นและชัดเจน ดังนั้นจึงได้ดำเนินการศึกษาต่อเนื่อง โดยการใช้เทคนิคทางชีวโมเลกุลเพื่อการระบุชนิดที่แน่นอน สร้างฐานข้อมูล และ พัฒนาวิธีการตรวจจับเชื้อแต่ละชนิดต่อไป



รูปที่1. ด้วอย่างเชื่อราในกลุ่ม Xylariaceae

คำนิยม: งานวิจัยนี้ได้รับทุนวิจัยจากโครงการปริญญาเอกกาญจนาภิเษก สำนักงานกองทุนสนับสนุนการวิจัย และ การสนับสนุนสถานที่และเครื่องมือจากมหาวิทยาลัยเทคโนโลยีสุรนารี

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# Relationships within Hypoxylon species based on morphological and molecular data

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Hypoxylon is one of the largest and best known genera of the family Xylariaceae. It has been reported to comprise of at least 130 species and found in most countries but is especially well represented in the tropics and subtropics. Although Hypoxylon species are primarily wood-decay fungi, that play an important role in the natural functions of ecosystems, many species are weak plant pathogens of angiosperms. Some Hypoxylon species seem to be highly host specific but others appear to exhibit wide host ranges. In this study, relationships between Hypoxylon species are revealed based on their morphological and molecular characteristics. One hundred and eighty six Hypoxylon specimens were collected from several forest areas in Thailand and these were then subjected to taxonomic investigation based on their morphological and cultural features and nucleic acid sequences. It was found that there can be considerable variation in morphological characters and furthermore some specimens could not be cultured. This resulted in some identification problems. However, different relationships between the Hypoxylon species were achieved following sequence analysis of ITS1-5.8S-ITS2 rDNA regions. The molecular results showed clearly the relationships of the Hypoxylon species studied and could be used to solve the morphological taxonomic problems. The ITS1 region indicated the highest variation among Hypoxylon species whereas 5.8S and ITS2 regions were more conserved. These molecular data could be applied for distinguishing morphological similar Hypoxylon species which had otherwise proved difficult to separate.

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## Nucleotide Sequence Data for the Clarification of Species Complex in Xylariaceous Fungi

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#### Introduction

Because xylariaceous fungi are high variation in morphological characteristics, an attempt to use the molecular techniques to assist was performed.

#### Objective

To clarify the species complex in xylariaceous fungi using internal transcribed spacer region sequences of rRNA gene.

#### Methods

Two hundreds and fifty one xylariaceous isolates were collected from different forest areas in Thailand and identified to species level based on morphological methods (2). Then, their ascospores were isolated and cultured on potato dextrose agar for DNA extraction (1). The internal transcribed spacer regions (ITS) including 5.8S ribosomal nucleotide sequences was amplified and sequenced. ITS sequences were aligned by ClustalX and manually edited by BioEdit program. The phylogenetic trees were constructed using neighbour joining (NJ) and maximum parsimony (MP) methods by PHYLIP and PAUP software packages respectively.

#### Results

Nine genera of Xylariaceae, Astrocystis, Biscogniauxia. Camillea, Daldinia, Hypoxylon, Kretzschmaria, Nemania, Rosellinia, and Xylaria, were recorded from the whole lot of specimens collected, and identified to fifty species. Hypoxylon and Xylaria were the common genera, and showed high variation in their morphological characters resulting in the difficulty in identification. Their amplification sizes of ITS fragments ranged from 500-900 base pairs, which contained ITS1, ITS2 and 5.8S rDNA regions. Most Hypoxylon section Annulata indicated the extremely long sequences in the ITS1 region, which were the tandem repeat sequences. These repeated sequences could be generated by slipped-strand mispairing or replication slippage. The whole ITS sequence alignments revealed the greatest variation in ITS1 regions, which was suitable to design specific primers and/or probes for these particular strains. The phylogenetic tree showed clearly the relationships of complex species. In this study, at least two new species and one new variety of Hypoxylon were recorded.

#### Conclusion

The nucleotide sequence data based on ITS sequences were proved to be useful for the clarification of species complex in xylariaceous taxonomic investigation. These results are also very useful to create the DNA sequence database of the xylariaceous fungi found in Thailand.

Keywords: Xylariaceae, Nucleotide sequence, Phylogeny, ITS

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