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

Nuttika Suwannasai

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การศึกษาอนุกรมวิธานเชิงโมเลกุลของเชื้อรากลุ่ม XYLARIACEAE

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การศึกษาเพื่อการระบุและจัดจำแนกชนิดของเชื้อราในกลุ่ม Xylariaceae โดยใช้ข้อมูลทางชีววิทยาโมเลกุลนี้เพื่อช่วยแก้ปัญหาที่ไม่สามารถระบุและจัดจำแนกชนิดของเชื้อราที่มีลักษณะทางสัณฐานที่ใกล้เคียงกันมากและชนิดที่ไม่สามารถเพาะเลี้ยงได้ โดยได้ศึกษาตัวอย่างเชื้อราจากทั้งแหล่งอ้างอิงจำนวน 31 ตัวอย่าง และแหล่งธรรมชาติจำนวน 338 ตัวอย่าง จาก 14 พื้นที่ใน 11 จังหวัดของประเทศไทย ซึ่งจากการศึกษาลักษณะทางสัณฐานและทางเคมีเพื่อระบุชนิดของเชื้อราจากแหล่งธรรมชาติพบว่ามีความผันแปรสูงของลักษณะทางสัณฐานและมีข้อจำกัดในการศึกษาแผนภูมิของสารทุติยภูมิ ทำให้ไม่สามารถระบุและจัดจำแนกชนิดของตัวอย่างประมาณร้อยละ 30 (จาก 338 ตัวอย่าง) จากนั้นได้ศึกษาโดยใช้เทคนิคทางชีววิทยาโมเลกุลโดยหาลำดับนิวคลีโอไทด์ของ 18S rDNA ซึ่งพบว่ามีขนาดประมาณ 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 เมื่อเปรียบเทียบลำดับนิวคลีโอไทด์ภายในกลุ่มของเชื้อราที่ศึกษาและจากฐานข้อมูล GenBank พบว่าลำดับนิวคลีโอไทด์ของเชื้อราที่ศึกษาแต่ละชนิดมีความแตกต่างกันและสามารถระบุชนิดของตัวอย่างเชื้อราที่มีปัญหาได้อย่างชัดเจน และเมื่อนำลำดับนิวคลีโอไทด์ที่ได้มาจัดแนวความสัมพันธ์ที่เหมาะสม พบความผันแปรสูงที่สุดในส่วน ITS1 ซึ่งเป็นประโยชน์ในการออกแบบ primers และ probes ที่จำเพาะต่อเชื้อ จากการศึกษาความสัมพันธ์ทางพันธุกรรมในรูปของ Phylogenetic tree ของเชื้อกลุ่มที่ศึกษา พบว่าสามารถอธิบายความสัมพันธ์ของเชื้อราแต่ละชนิดได้และสามารถยืนยันผลของการพบเชื้อราชนิดใหม่ได้อย่างชัดเจน ทั้งนี้พบว่าเชื้อราที่ได้จากแหล่งธรรมชาติทั้งสิ้นมี 9 สกุล (*Astrocystis*, *Biscogniauxia*, *Camillea*, *Daldinia*, *Hypoxylon*, *Kretzschmaria*, *Nemania*, *Rosellinia* และ *Xylaria*) 59 ชนิด ซึ่งรวมชนิดใหม่ 9 ชนิด คือ *Biscogniauxia* 1 ชนิด *Hypoxylon* 5 ชนิด และ *Xylaria* 3 ชนิด ลำดับนิวคลีโอไทด์ที่ได้ยังเป็นข้อมูลสำคัญในการสร้างฐานข้อมูลของลำดับนิวคลีโอไทด์ของเชื้อรากลุ่ม Xylariaceae ในประเทศไทย

สาขาวิชาจุลชีววิทยา
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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.

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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*., 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 wood-decay 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 often found fruiting on the living host material	Many species of *Anthostomella*
To invade living stems and remain dormant until the host is stressed	Many species of *Daldinia*, *Biscogniauxia*, *Camillea*, and *Hypoxylon*
To decay living roots and wood then move to living material from dead material	*Kretzschmaria clavas* (Fr.) Sacc., *Rosellinia necatrix* and *Xylaria* spp.
To form fruiting bodies on decayed material, but to be isolated as endophytes from living hosts	Most species of *Xylaria* and *Nemania*
To form fruiting bodies on seed and fruits, and have specific and discrete relationships with their hosts	*Xylaria magnolia* J.D. Rogers found on *Magnolia* fruits, *Xylaria ianthino-velutina* (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 relationships with animals. Many taxa have dormant ascospores, that seem to be achieved via passage through a mammalian digestive tract.	Most species of *Hypocopra*, *Podosordaria*, and *Poronia*
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.) Y.-M. 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.

Table 2. Examples of xylariaceous phytopathogens.

Species	Plant
Canker diseases	
Entoleuca mammata (Wahlenberg: F.) J.D. Rogers & Y.-M. Ju	*Acer*, *Alnus*, *Betula*, *Carpinus*, *Fagus*, *Picea*, *Pyrus*, *Salix*, *Sorbus*, and *Ulnus* (Manion and Griffin, 1986; Whalley, 1996; Edwards *et al*., 2003)
Biscogniauxia mediterranea	Oak (Macara, 1975; Whalley, 1996)
Biscogniauxia nothofagi Whalley, Læssøe & Kile	*Nothofagus cunninghamii* (Whalley, Læssøe, and Kile, 1990; Whalley, 1996)
Camillea punctulata (Berk. & Rev.) Læssøe, J.D. Rogers & Whalley	*Quercus* (Barnett, 1957; Whalley, 1996; Edwards *et al*., 2003)
Root rot diseases	
Rosellinia necatrix 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 *et al*., 1995; Whalley, 1996)
Rosellinia bunodes (Berk. & Broome) Sacc.	Cacao (*Theobroma cacao*), quinine (*Cinchona* spp.), coffee (*Coffea* spp.), rubber (*Hevea brasiliensis*), and tea (*Camellia sinesis*) (Sivanesan and Holliday, 1972; Whalley, 1996; Edwards *et al*., 2003)
Kretzschmaria deusta (Hoffm.: Fr.) P. Martin	Various tree species (Wilkins, 1934; Whalley, 1996; Edwards *et al*., 2003)
Xylaria arbuscula Fr.	Macadamia (Ko and Kunimoto, 1991)

Table 2. (Continued).

Species	Plant
Xylaria mali Fromme and *Xylaria polymorpha* (Pers.: Fr.) Grev.	Apple (Clayton, Julis, and Sutton, 1976; Whalley, 1996; Edwards *et al*., 2003), and *Acer rubrum* (Sivanesan and Holliday, 1972; Whalley, 1996)
Needle blight diseases	
Rosellinia herpotrichioides Hepting & Davidson	Douglas fir (*Pseudotsuga menziesii*) in forest 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 non-selective 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 (Rodriques, 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).

Table 3. The fungal genera within the Xylariaceae family.

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*		
	Collodiscula I.Hino & Katum.	? *Collodiscula*	*Collodiscula*	
	Creosphaeria Theiss.	*Creosphaeria*	*Creosphaeria*	
Daldinia Ces. & De Not.		*Daldinia*	*Daldinia*	
			Discoxylaria Lindquist & J. Wright	
				Emarcea Duong, R. Jeewon & K.D. Hyde (2004)
Engleromyces Henn.	*Engleromyces*	*Engleromyces*	*Engleromyces*	
			Entoleuca Syd.	
Entonaema A. Møller	*Entonaema*	*Entonaema*	*Entonaema*	
	Euepixylon Füisting	? *Euepixylon*	*Euepixylon*	
Fassia Dennis				
Helicogermslita Lodha & D. Hawksw.	*Helicogermslita*	*Helicogermslita*		
	Holttumia Lloyd	? *Holttumia*		
Hypocopra (Fr.) J. Kickx f.	*Hypocopra*	*Hypocopra*	*Hypocopra*	
Hypoxylon Bull.	*Hypoxylon*	*Hypoxylon*	*Hypoxylon*	
Induratia Samuels, E. Mull. & Petrini	*Induratia*	*Induratia*	*Induratia*	
				Jumillera J.D. Rogers, Y.-M. Ju & San Martín (1997)
Kretzschmaria Fr.	*Kretzschmaria*	*Kretzschmaria*	*Kretzschmaria*	
			Kretzschmariella Viégas	
Leprieuria Læssøe, J.D. Rogers&Whalley	*Leprieuria*	*Leprieuria*	*Leprieuria*	
Lopadostoma (Nitschke) Traverso	*Lopadostoma*	*Lopadostoma*	*Lopadostoma*	
	? *Myconeesia* Kirschst.			

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				
				Poroleprieuria M.C. González, Hanlin, Ulloa et E. Aguirre, (2004)
Poronia Willd.	*Poronia*	*Poronia*	*Poronia*	
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.			
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*	*Versiomyces*	
			Vivantia J.D. Rogers, Y.-M. Ju, & Cand.	
Wawelia Namysl.	? *Wawelia*	*Wawelia*	*Wawelia*	
				Whalleya J.D. Rogers, Y.-M. Ju & San Martín (1997)
Xylaria Hill *ex* Schrank	*Xylaria*	*Xylaria*	*Xylaria*	

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).

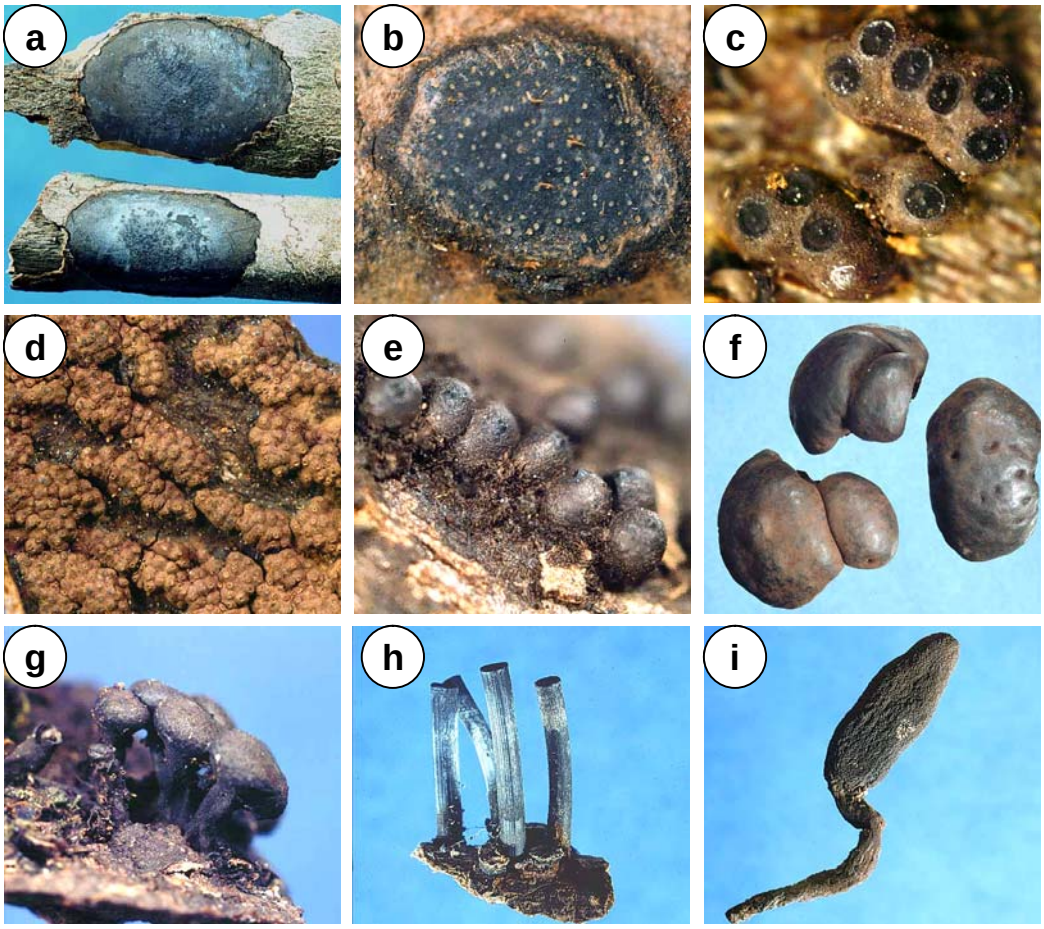

Figure 1. Stromata of the xylariaceous fungi; (a) *Camillea heterostromata* (Mont.) Læssøe, J.D. Rogers & Whalley (applanate form), (b) *Biscogniauxia schweinitzii* Y.-M. Ju & J.D. Rogers (applanate form), (c) *Hypoxylon bovei* Speg. (subglobose to globose form), (d) *Hypoxylon fusoideosporum* Y.-M. 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).

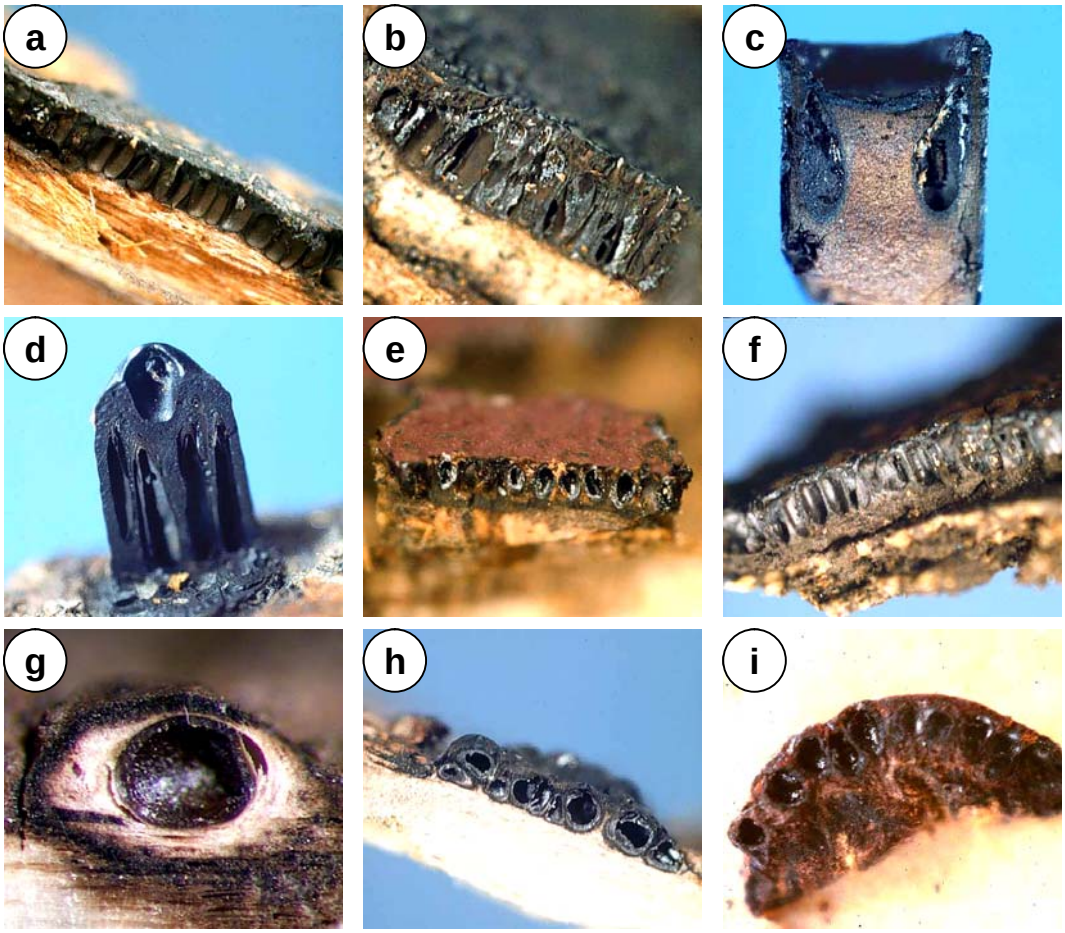

Figure 2. Perithecia of the xylariaceous fungi; (a) *Biscogniauxia schweinitzii* Y.-M. Ju & J.D. Rogers (tubular), (b) *B. dennisii* (Pouzar) Piuzar (tubular), (c) *Camillea leprieurii* Mont. (long spherical), (d) *C. bilabiata* Speg. (tubular), (e) *Hypoxylon chathamense* Y.-M. Ju & J.D. Rogers (spherical), (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.

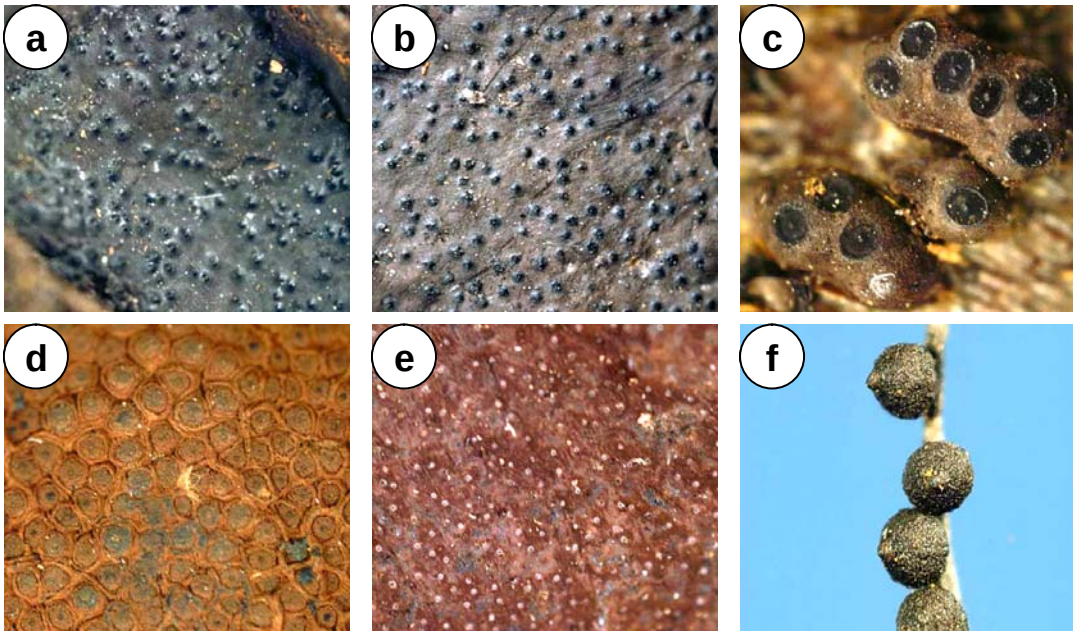

Figure 3. Ostioles of the xylariaceous fungi; (a) *Biscogniauxia dennisii* (Pouzar) Piuzar (papillate), (b) *B. reticulospora* Y.-M. Ju & J.D. Rogers (papillate), (c) *Hypoxylon bovei* Speg. (papillate with disk), (d) *H. kretzschmariodes* Y.-M. Ju & J.D. Rogers (umbilicate), (e) *H. hypomiltum* Mont. (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 diamond-shaped 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).

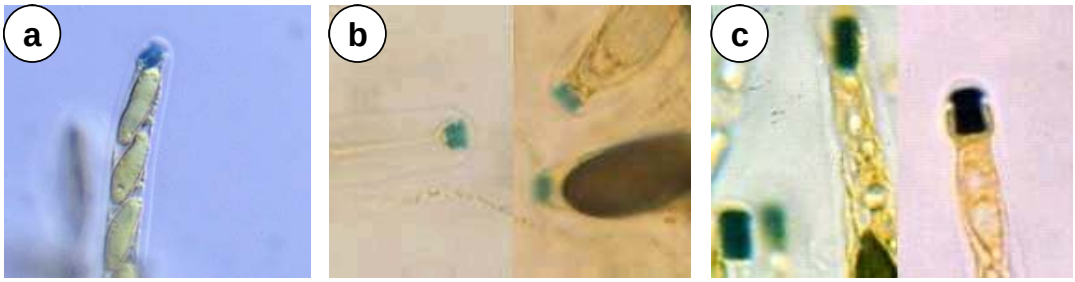

Figure 4. The apical apparatus forms of the xylariaceous fungi; (a) *Nemania aenea* (Nitschke) Pouzar var. *macrospora* (J.H. Miller) Y.-M. Ju & J.D. Rogers (higher than broad), (b) *Hypoxylon rubiginosum* Pers.: Fr. (broader than high), and (c) *Camillea tinctor* (Berk.) Læssøe, J.D. Rogers & Whalley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

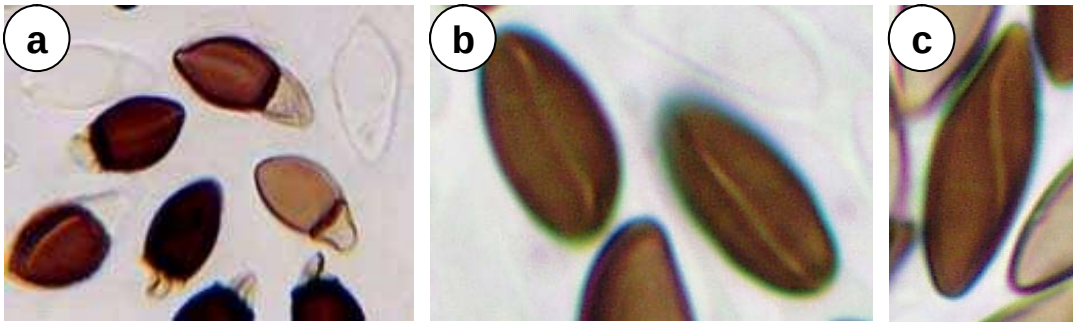

Figure 5. Germ slit characters of the xylariaceous fungi. (a) *Biscogniauxia anceps* (Sacc.) J.D. Rogers, Y.-M. Ju & Cand (straight form) from Ju and Rogers (1997), (b) *Hypoxylon purpureonitens* Y.-M. Ju & J.D. Rogers (SUT004) (straight form), and (c) *Xylaria* sp. SUT155 (spiral form).

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.

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

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
Hypocopra	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. (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 *β*-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).

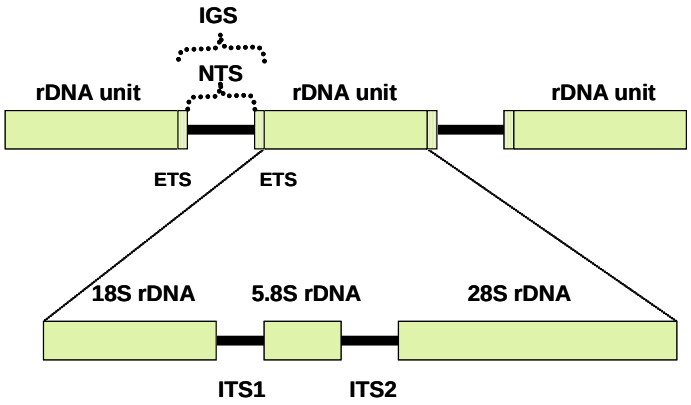

Figure 6. Schematic diagram of a tandem repeat unit of rDNA.

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 β" 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

	Kingdom	Phylum	Class	Order	Family	Genus	Species	Population
Nuclear rDNA								
SSU (16-18S)	———	———	———	———	.....			
LSU (23-28S)			———	———	———	.....		
5.8S	———	———	———	.....				
IGS							———	———
ITS						———	———	———

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.

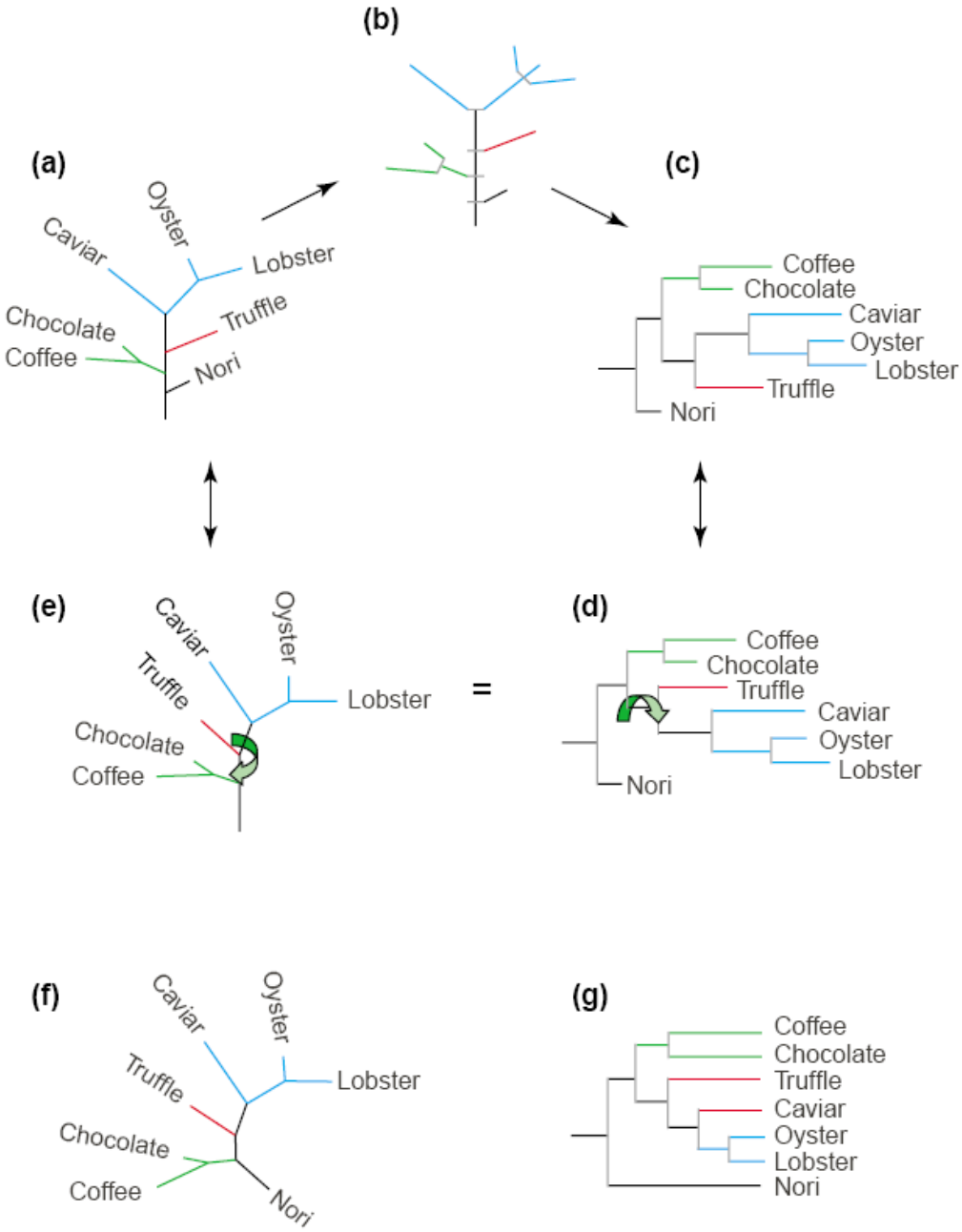

Figure 7. Phylogenetic tree styles. All these trees have identical branching patterns.

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), neighbour-joining, 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, *Hpa*II, *Hae*III, and *Taq*I. 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 μm, chiefly 11-13 x 5-7 μm…" 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, dome-shaped 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 µm in length and 3.5 µm 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 *Nodulisporium*-like 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 (Rodriques 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 *Xylaria* indicate that a combination of morphological characters and biochemical analyses might enable satisfactory identifications to be made (Brunner and Petrini, 1992; Rodriques, 1992; Rodriques, 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.

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

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

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 one-tenth 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 ^{TM} 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_{260} = 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 µL mixture containing 50 ng of fungal DNA, 5 µL of 10X reaction buffer (10 mM KCL, 20 mM Tris-HCl pH 8.8, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100), 200 µM of each dATP, dCTP, dGTP, and dTTP, 1 µM of each primer, 1.0 unit of *Taq* DNA polymerase (Sigma), and adjusted volume to 50 µL 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.).

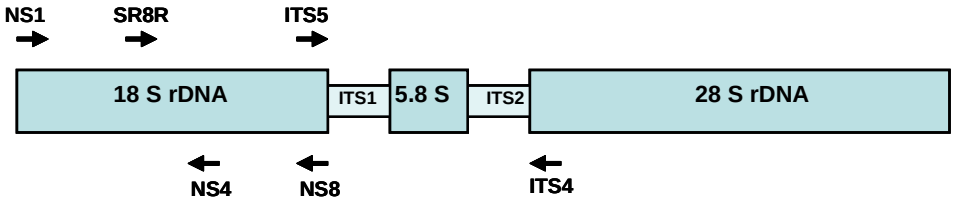

Figure 8. The map of oligonucleotide primers for 18S rDNA and ITS region amplification. The arrowheads represent the 3´ end of each primer.
Source: Vilgalys, www (1999)

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

Name	Sequence (5´- 3´)	Target region[a]	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 *et al*. (1990)

[a] *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 µL mixture containing 10 ng of fungal DNA, 5 µL of 10X reaction buffer (10 mM KCL,

20 mM Tris-HCl pH 8.8, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100), 200 µM of each dATP, dCTP, dGTP, dTTP, 2.5 µM of each primer, 1.0 unit of *Taq* DNA polymerase, and adjusted volume to 50 µL 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 µg/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 µL cycle sequencing reaction mixture contained 80-200 ng DNA, 4 µL 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 μL of deionized water and 64 μL 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 μL 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 μL 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.

Table 8. Xylariaceous collections from 14 locations of Thailand in years 2002 and 2003.

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. (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:

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

Genus	No. of specimens	No. of species
Astrocystis Berk. & Broome	8	1
Biscogniauxia Kuntze	6	2
Camillea 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

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 μm cf. (10-)10.6-12.5 x 5.6-6.3 μm (Thienhirun, 1997). However other characters were well matched *A. mirabilis* as described by Ju and Rogers (1990).

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

Character	***A. mirabilis****	***R. procera****
Stromata		
Shape	Subglobose to hemispherical, blackish, each stroma encircled with a more or less stellate to irregular ring of mixed host and stromatic material at the base to midportion	Subglobose to hemispherical, blackish, embedded on a brown cottony subiculum
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 3-4 µm broad	Inverted hat, 2-3 µm high x 3.5-5 µm broad
Germ slit	Straight slightly less than spore length	Straight longitudinal germ slit spore length
Culture	White radiate strands with fimbriate margins, velutinous or floccose, and faintly zonate	Uncultured
Habitat	On bamboo	On wood
Location	Ratchaburi	Ratchaburi, Nakhon Ratchasima
Specimen examined	SUT047, SUT048, SUT049, SUT051, SUT052, SUT054, SUT055, SUT056	SUT102, SUT109, SUT113, SUT114

* More details on collections are given in Appendix B.

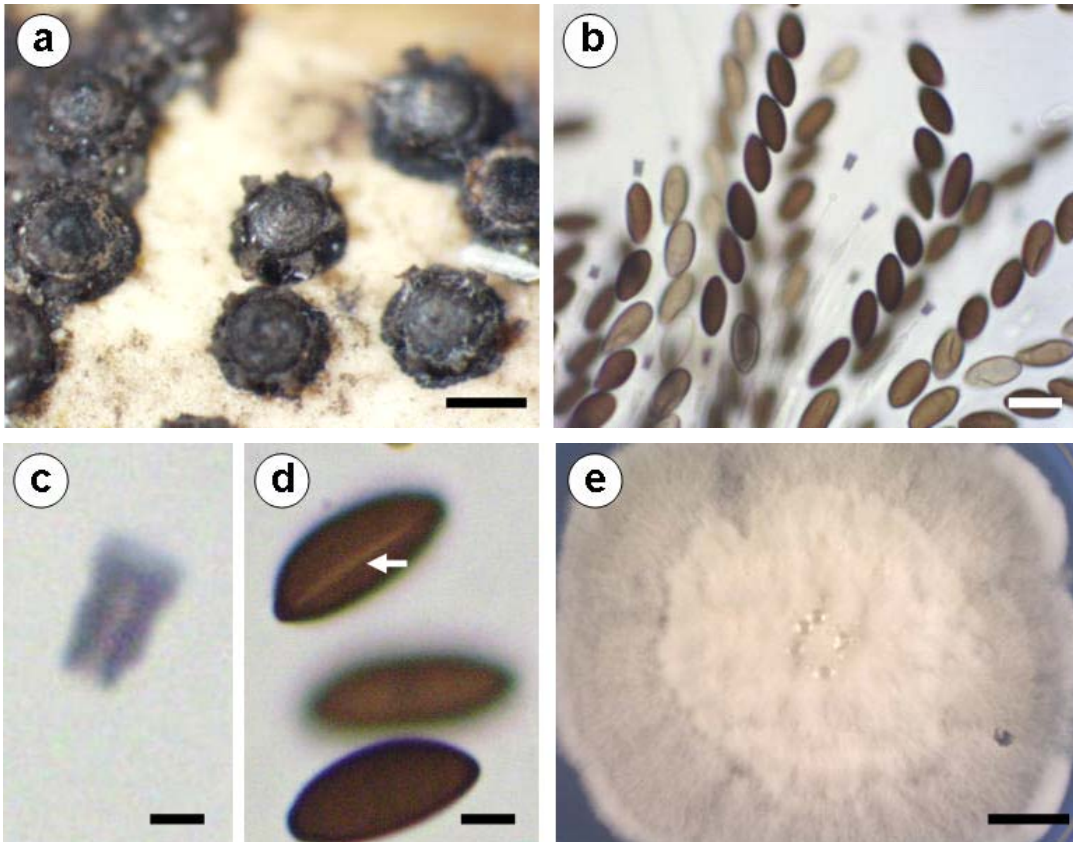

Figure 9. *Astrocystis mirabilis* Berk. & Broome (SUT051); (a) stromatal form (Bar = 1 mm), (b) ascospores with ellipsoid equilateral (Bar = 10 µm), (c) apical apparatus bluing in Melzer's iodine reagent (Bar = 4 µm), (d) germ slit straight nearly spore length (arrowed) (Bar = 2 µm), and (e) cultural 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 µm cf. 75-130 x 15-18 µm (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*.

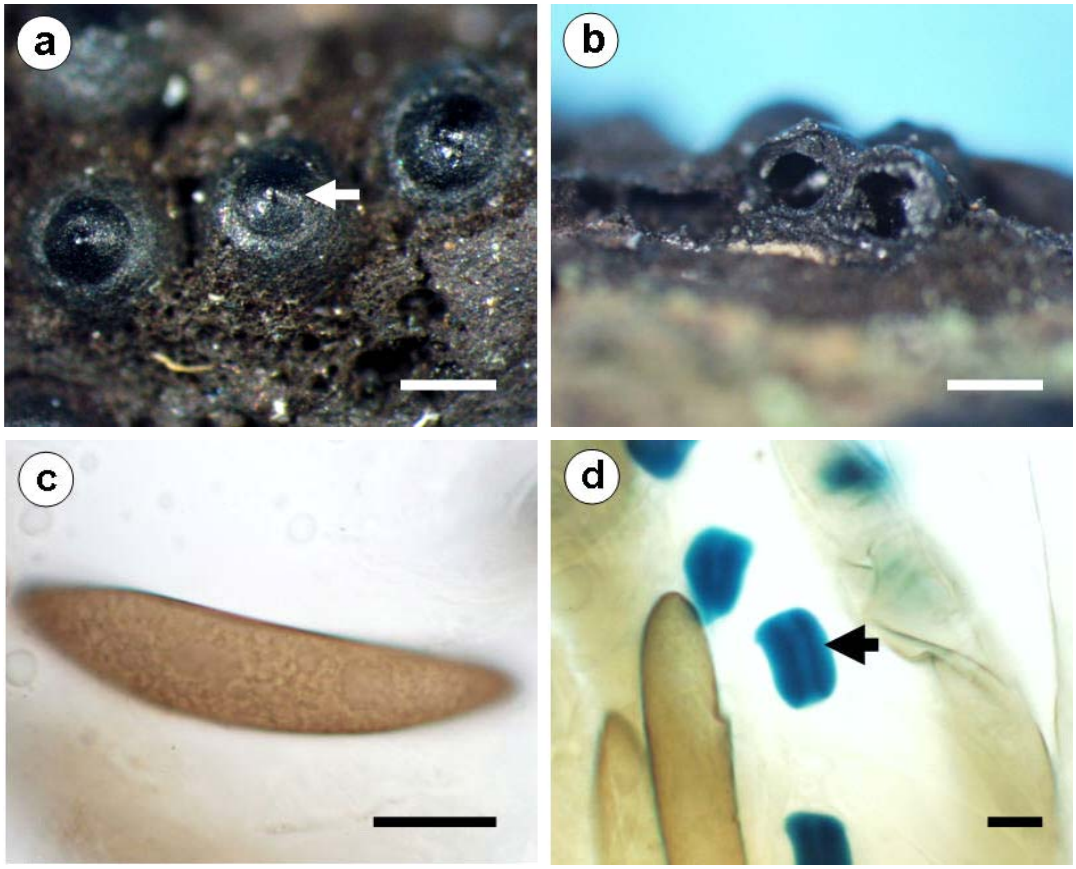

Figure 10. *Rosellinia procera* Syd. (SUT113); (a) stromatal form with conico-papillate of ostiole (arrowed) (Bar = 2 mm), (b) perithecia (Bar = 3 mm), (c) ascospore (Bar = 10 µm), and (d) apical apparatus (Bar = 5 µm).

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.

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

Character	***C. tinctor* (Berk.) Læssøe, J.D. Rogers & Whalley ***	***C. selangorensis* M.A. Whalley, A.J.S. Whalley & E.B.G. Jones.**	***C. leprieurii* Mont.**
Stromata			
Shape	Applanate with a slightly raised center, elongate elliptic	Circular to orbicular, or elongated, with applanate to convex apex, surrounded by a slightly raised rim	Erumpent through bark, cylindrical, seated on slightly broader disc
Color	Externally black, mat or shiny, internally dark brown, surface smooth	Black	Black
Perithecia			
Shape	Deeply immersed, cylindrical to slightly elongate	Deeply immersed, brittle entostroma, basally seated, cylindrical, individually erumpent	Elongate ovoid
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 SEM	Ellipsoid inequilateral, minutely warted by light microscopy, strongly verrucose by SEM	Elongate with upper end acute wedge shaped and 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	

* More details on collections are given in Appendix B.

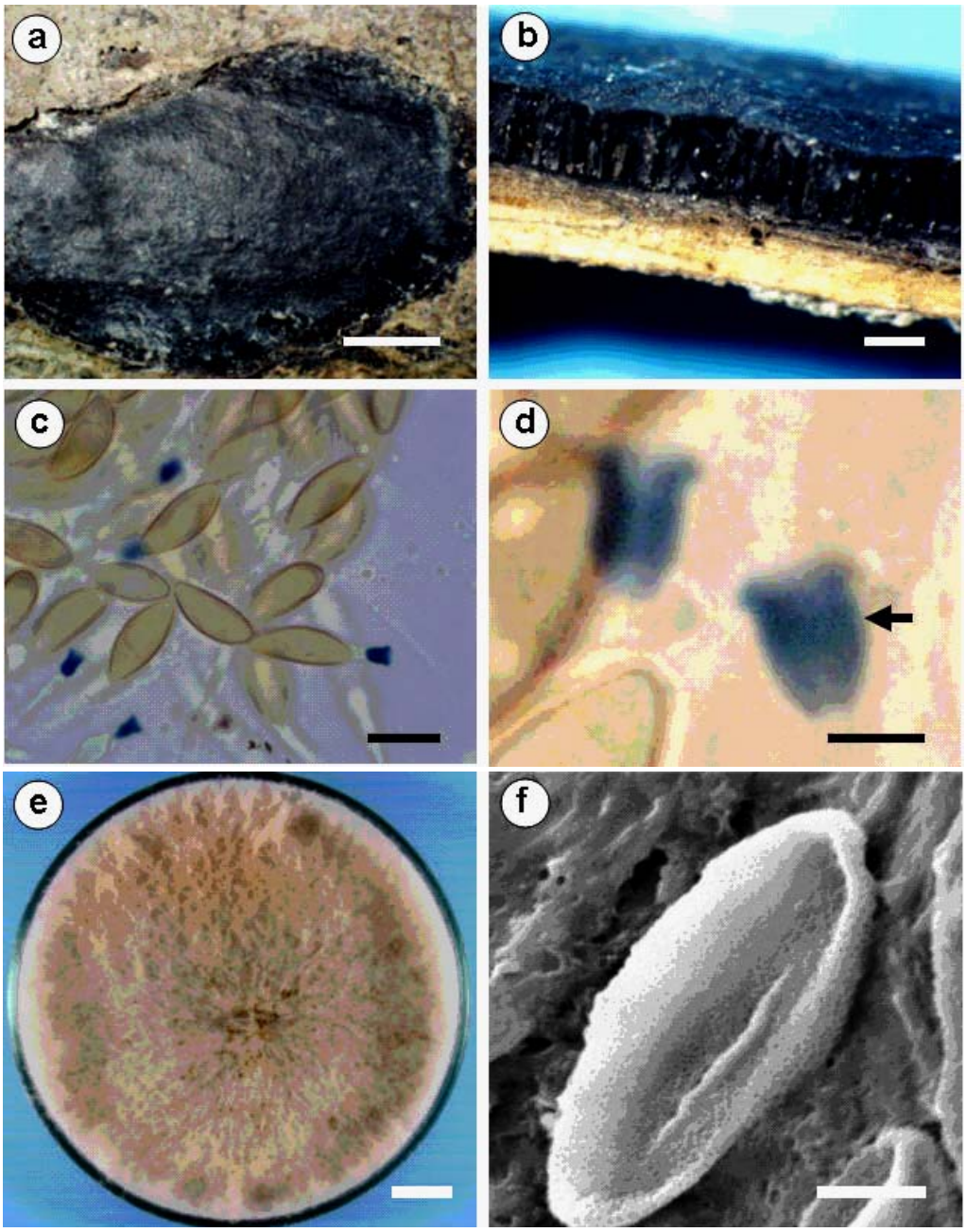

Figure 11. *Camillea tinctor* (Berk.) Læssøe, J.D. Rogers & Whalley (SUT260); (a) stromatal form (Bar = 1 mm), (b) perithecia (Bar = 0.5 mm), (c) ascospores (Bar = 15 µm), (d) apical apparatus bluing in Melzer's iodine reagent (Bar = 4 µm), (e) cultural characteristics on PDA cultured at 25°C after 4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

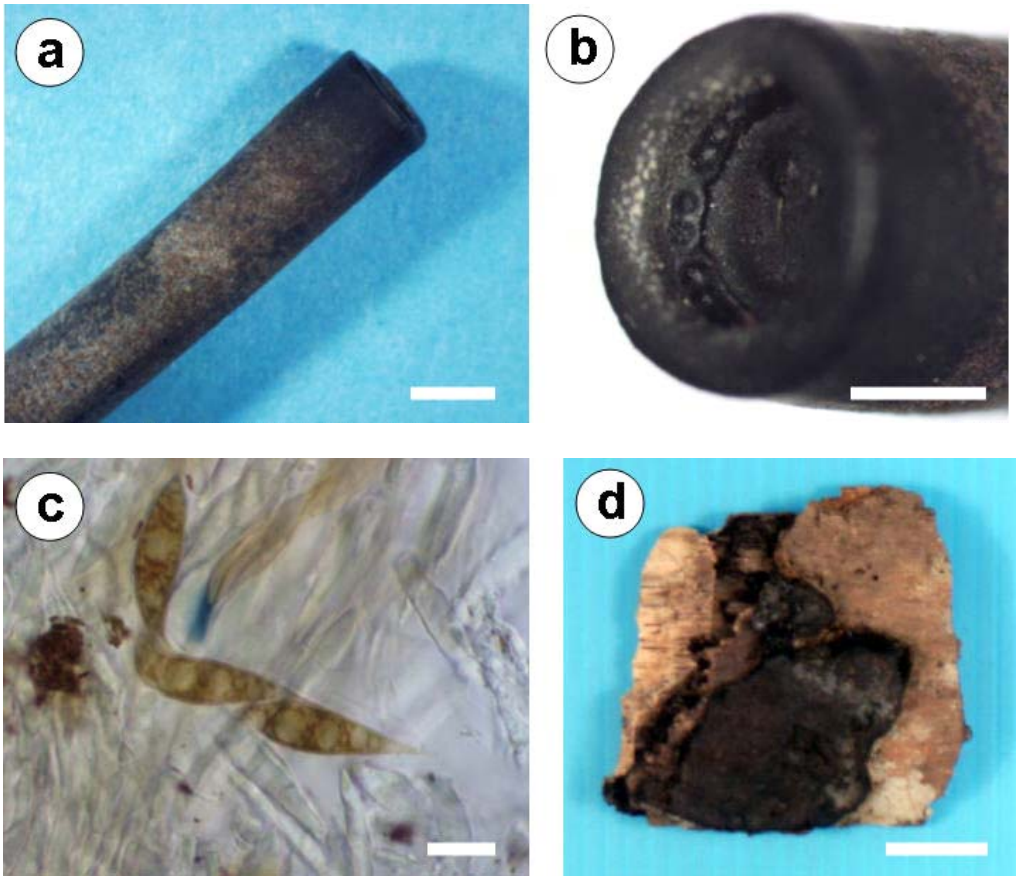

Figure 12. *Camillea selangorensis* M.A. Whalley, A.J.S. Whalley & E.B.G. Jones (KS15), and *C. leprieurii* (Mont.) Mont; (a) and (b) stromatal form of *C. leprieurii* (Bar = 0.5 cm and 0.2 cm respectively), (c) ascospores (Bar = 6 µm), and (d) stromatal form of *C. selangorensis* (Bar = 1 cm).

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 and the reference specimen of *D. concentrica*.

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 pigments	Livid purple, dark livid, or vinaceous purple	Livid purple or dark purple
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

* More details on collections are given in Appendix B.

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

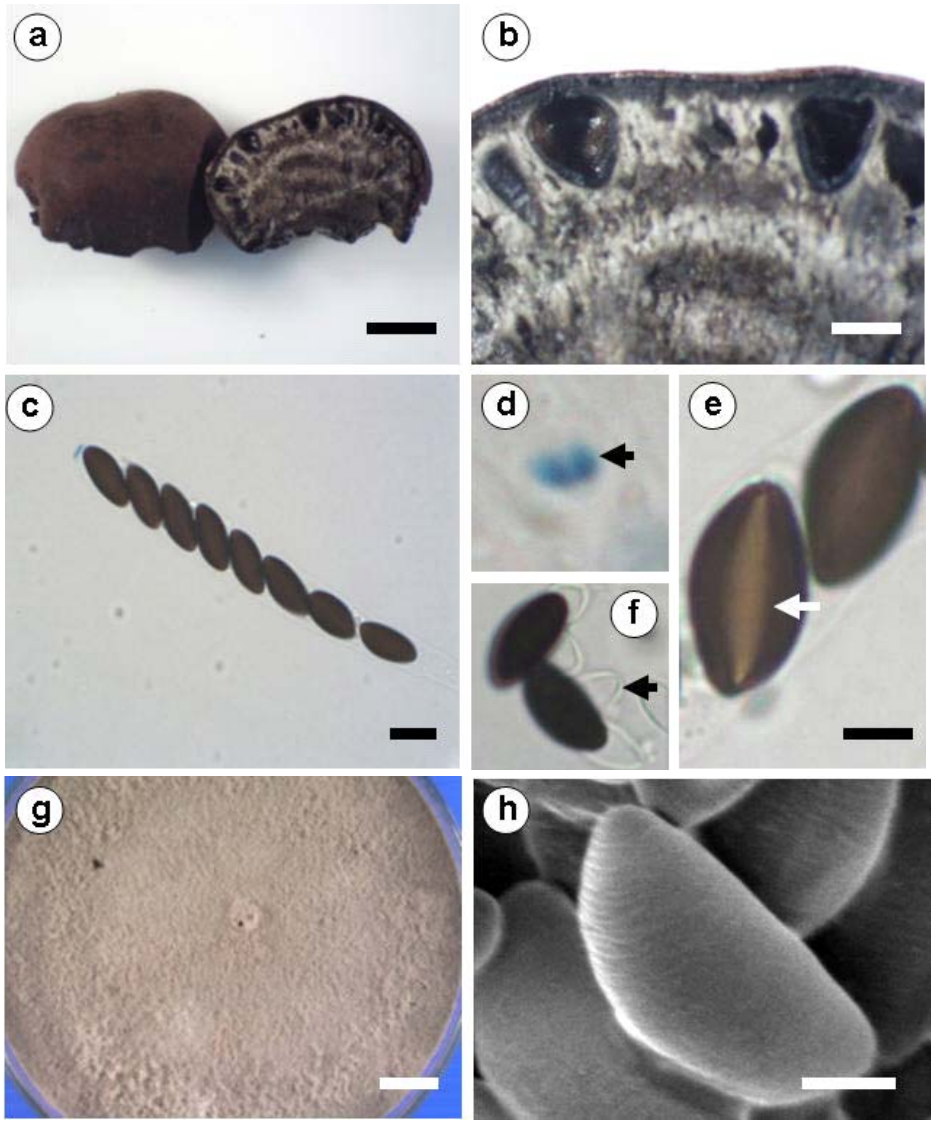

Figure 13. *Daldinia eschscholzii* (Ehrenb.: Fr.) Rehm (SUT039); (a) stromatal form (Bar = 1 cm), (b) perithecia (Bar = 0.5 mm), (c) ascus containing eight ascospores (Bar = 10 µm), (d) apical apparatus bluing in Melzer's iodine reagent (arrowed), (e) germ slit straight nearly spore length (arrowed) (Bar = 2 µm), (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 µm).

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 Y.-M. Ju & J.D., Rogers	12	Ju and Rogers (1996)
H. stygium (Lév.) Sacc.	13	Ju and Rogers (1996)
H. urceolatum (Rehm) Y.-M. 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 disc
Hypoxylon* sect. *Hypoxylon		
H. anthochroum Berk. & Broome	7	Ju and Rogers (1996)
H. brevisporum Y.-M. 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 in germ slit
H. cf. *perforatum* (SUT224)	1	Brown vinaceous in stromatal surface colour, brown vinaceous in granule colour, straight full length in germ slit
H. cf. *perforatum* (SUT294)	1	Reddish brown in stromatal surface colour, reddish brown to black in granule colour, straight full length in germ slit
H. rubiginosum (Pers.: Fr.) Fr., Summa Veg	4	Ju and Rogers (1996)
H. subgilvum Berk. & Broome var. *microsporum* (Abe) Y.-M. 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 *H. lenormandii* except for reddish brown in stromatal colour, smaller in ascospore size, and straight germ slit
H. kanchanapisekii sp. nov.	5	Close to *H. lenormandii* except for stromatal surface color of dull reddish brown not grayish sepia, small ascospores, and having a straight germ slit
*H. suranaree*i 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
Hypoxylon 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.

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

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			
Type	*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

* More details on collections are given in Appendix B.

Table 14. (Continued).

Character	*H. moriforme* Henn.*	*H. purpureonitens* Y.-M. 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

* More details on collections are given in Appendix B.

Table 14. (Continued).

Character	***H. urceolatum* (Rehm) Y.-M. 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		
Type	*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

* More details on collections are given in Appendix B.

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 μm and 5-7 x 2-3 μm 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.

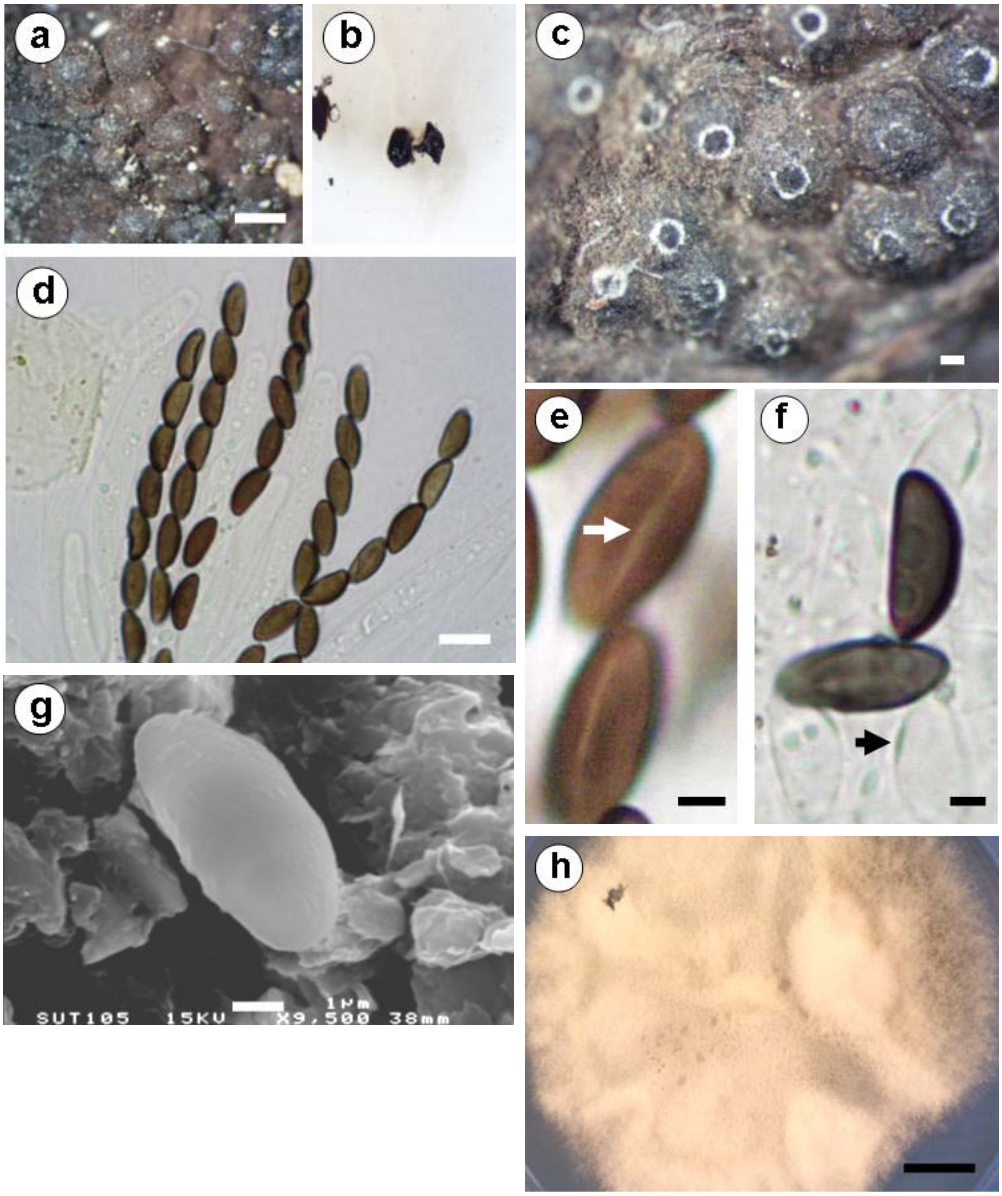

Figure 14. *Hypoxylon* cf. *archeri* (SUT105); (a) stromatal form when immature (Bar = 0.2 mm), (b) KOH-extractable pigment, (c) stromata with white fringe surrounding ostiolar disc (Bar = 0.1 mm), (d) ascospores (Bar = 10 µm), (e) straight germ slit spore length (arrowed) (Bar = 2 µm), (f) the thickening 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).

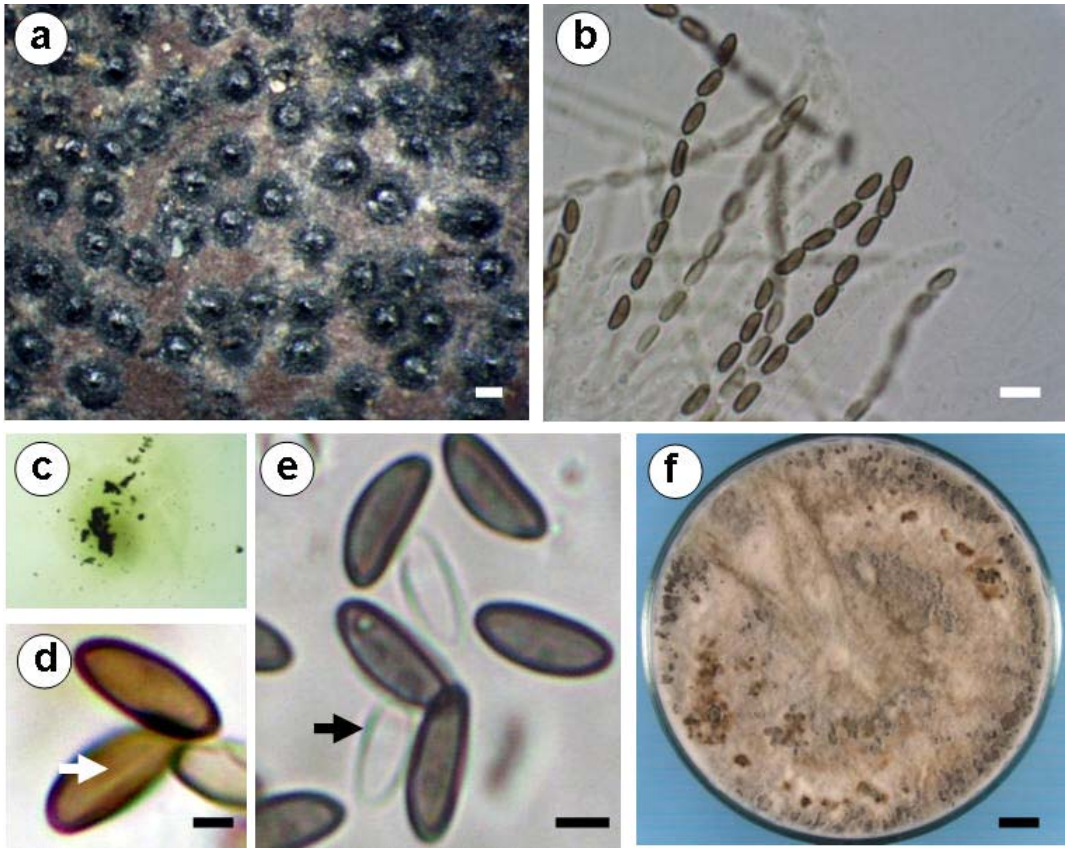

Figure 15. *Hypoxylon atroroseum* J.D. Rogers (SUT009); (a) stromatal form (Bar = 0.1 mm), (b) ascospores (Bar = 10 µm), (c) KOH-extractable pigment greenish olivaceous, (d) straight germ slit spore length (arrowed) (Bar = 2 µm), (e) the thickening on perispore (arrowed) (Bar = 2 µm), and (f) cultural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

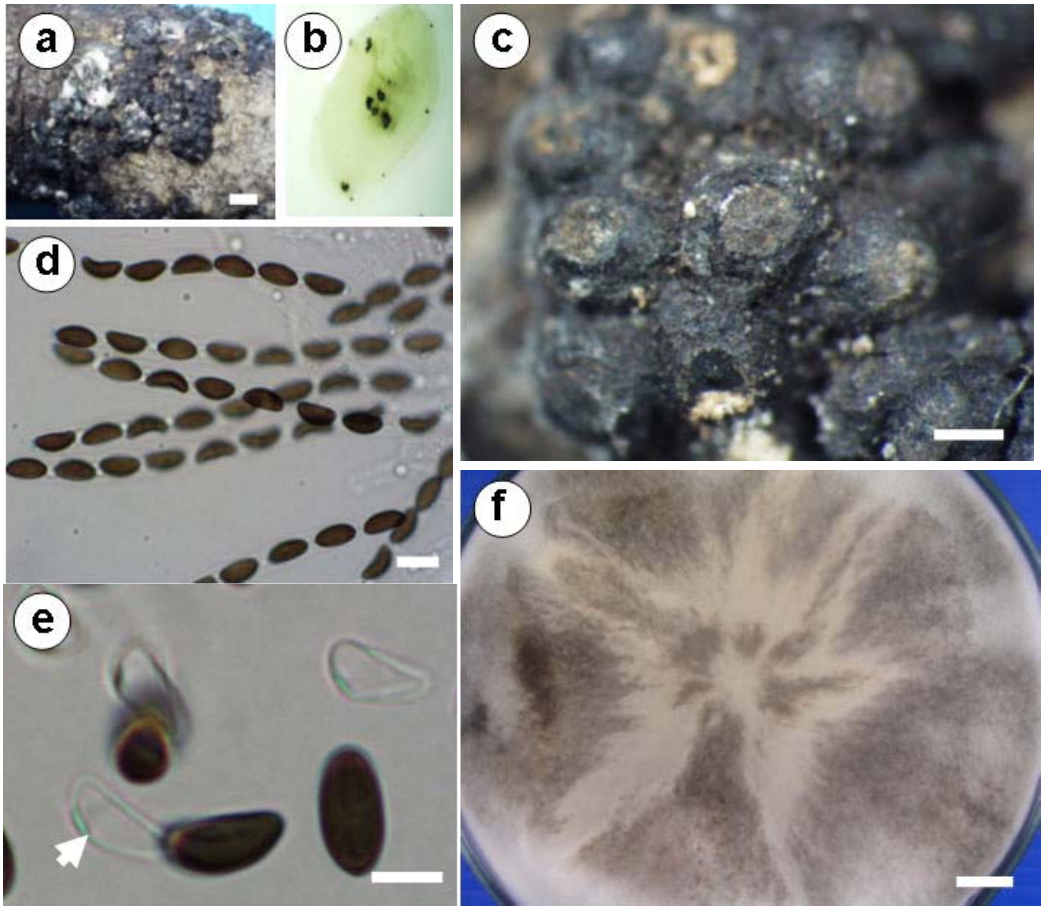

Figure 16. *Hypoxylon bovei* Speg. var. *microspora* J.H. Miller (SUT025); (a) and (c) stromatal form (Bars = 1 cm and 0.5 mm respectively), (b) KOH-extractable pigment, (d) ascospores (Bar = 10 µm), (e) the thickening on perispore (arrowed) (Bar = 5 µm), and (f) cultural characteristics on PDA cultured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

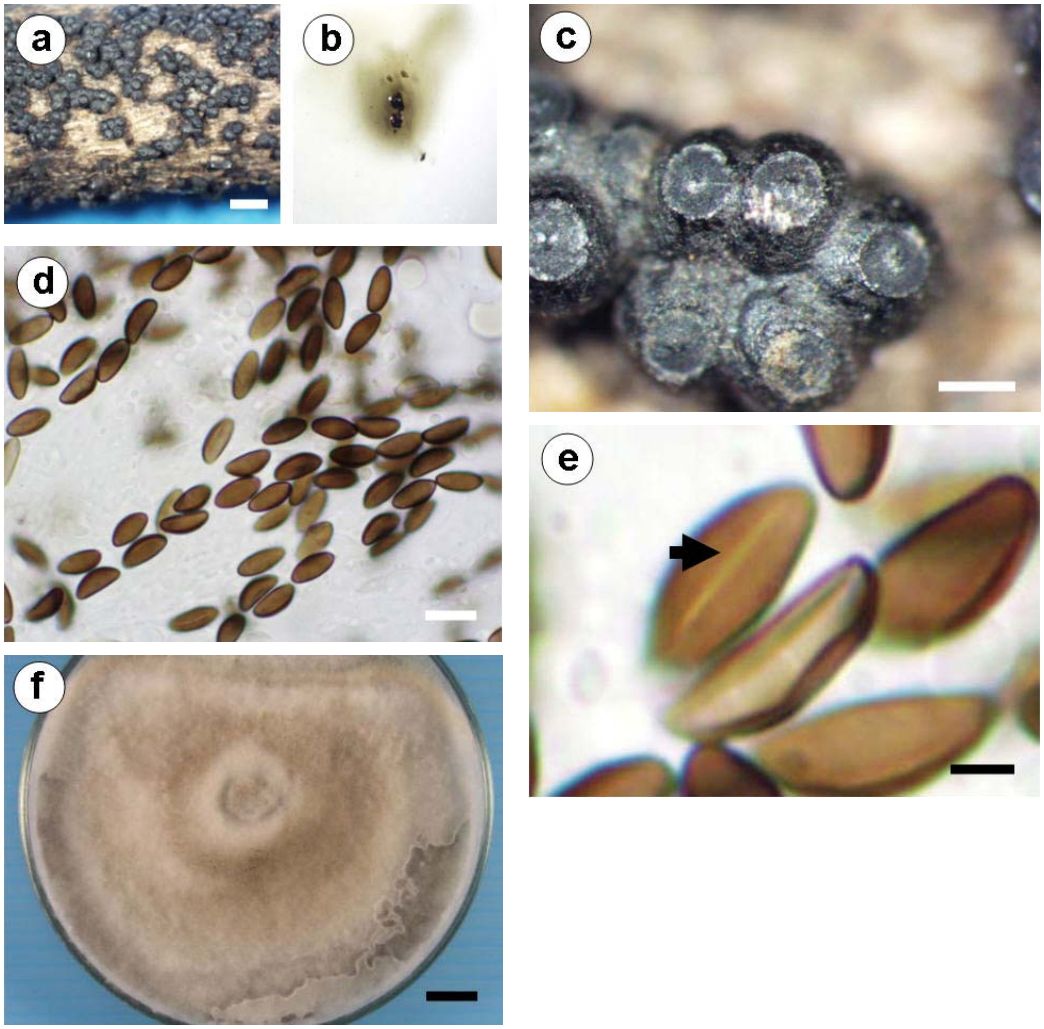

Figure 17. *Hypoxylon moriforme* Henn. (SUT220); (a) and (c) stromatal form (Bars = 1 cm and 0.3 mm respectively), (b) KOH-extractable pigment greenish olivaceous, (d) ascospores (Bar = 10 µm), (e) straight germ slit spore length (arrowed) (Bar = 2 µm), and (f) cultural characteristics on PDA cultured 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 μm), which was smaller than specimens recorded by Ju and Rogers (1996) (9-14(-17) x 3.5-4.5 μm) but it was close to Thai specimens reported by Thienhirun (1997) (8.8-10 x 3-3.8 μm). 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 µm total length x 3.8-5 µm broad, the spore bearing parts 40-65 µm long with stipes 30-55 µm; ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 7.5-9 x 2.8-4.2 µm, 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.

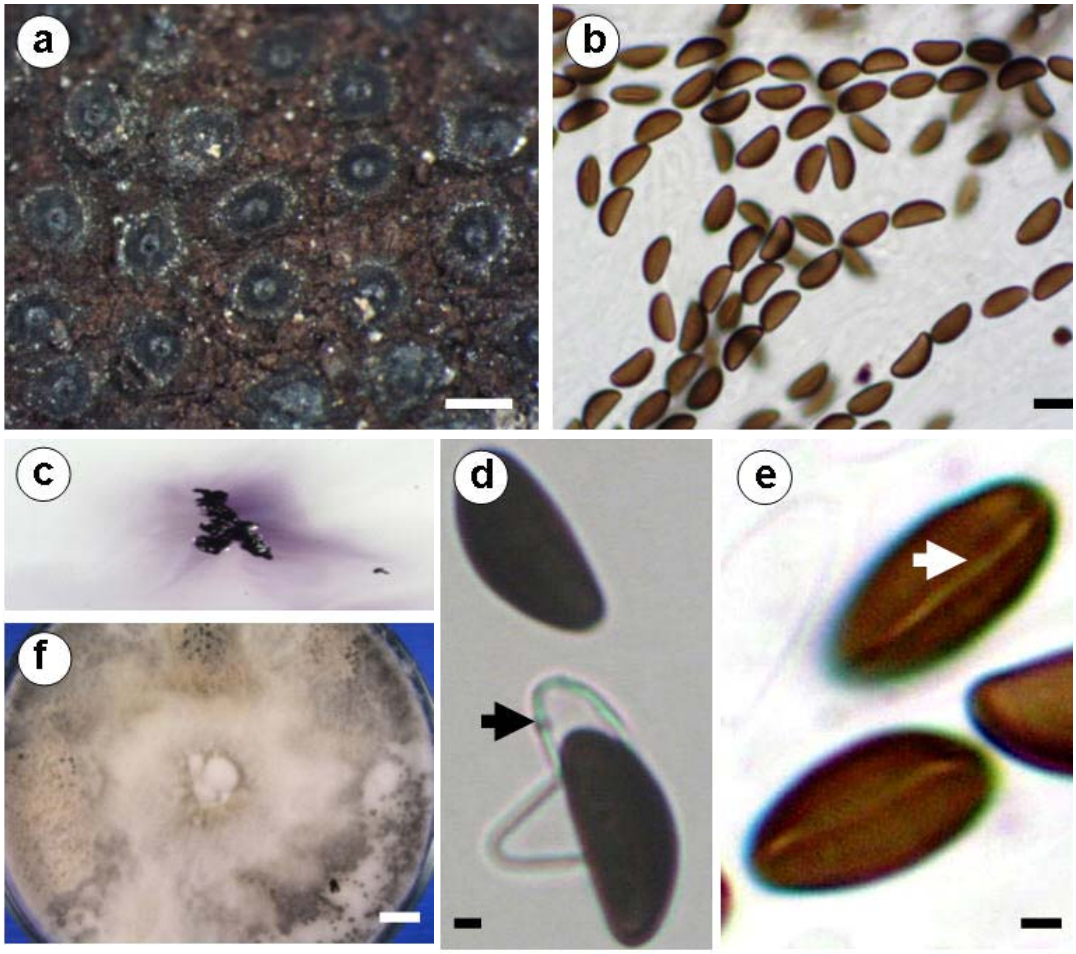

Figure 18. *Hypoxylon purpureonitens* Y.-M. Ju & J.D. Rogers (SUT004); (a) stromatal form (Bar = 0.3 mm), (b) ascospores (Bar = 5 µm), (c) KOH-extractable pigment vinaceous purple, (d) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), (e) straight germ slit spore length (arrowed) (Bar = 2 µm), and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

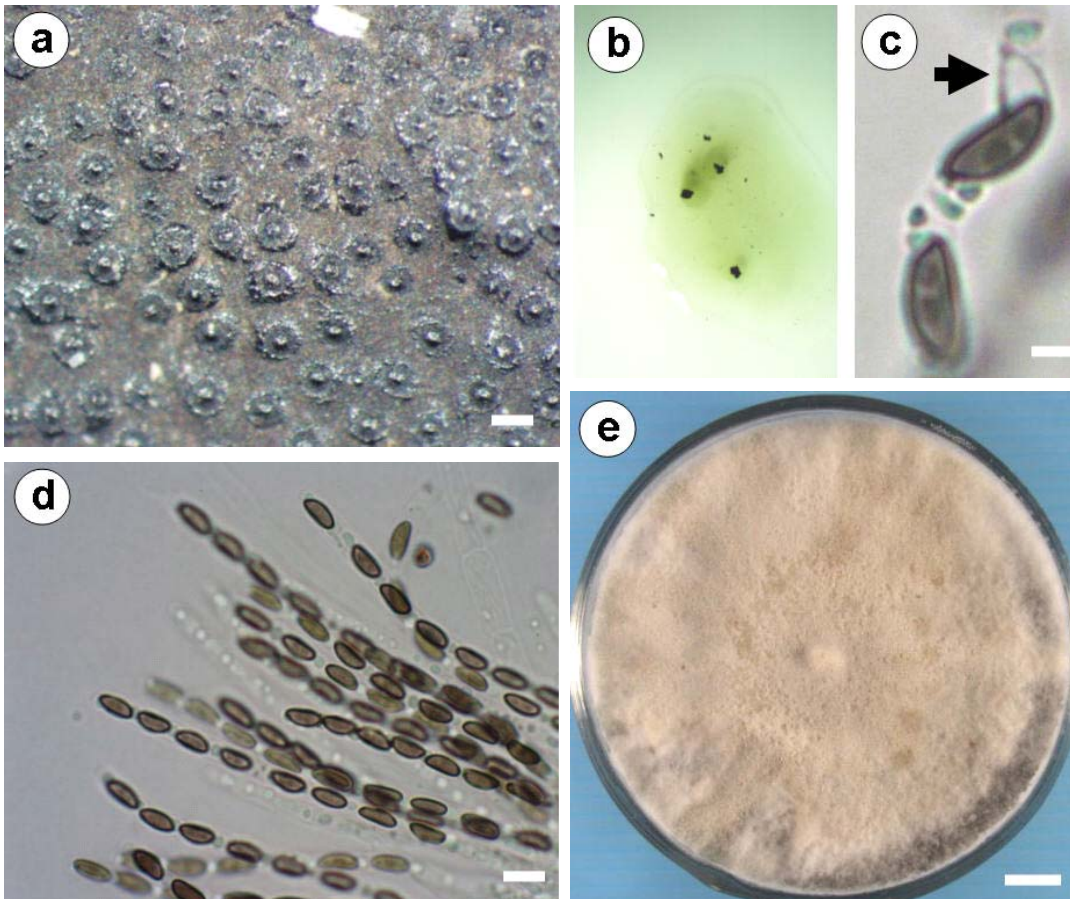

Figure 19. *Hypoxylon stygium* (Lév.) Sacc. (SUT058); (a) stromatal form (Bar = 0.2 mm), (b) KOH-extractable pigment greenish olivaceous, (c) ascospores dehiscent in 10% KOH (arrowed) (Bar = 2 µm), (d) ascospores (Bar = 5 µm), and (e) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar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

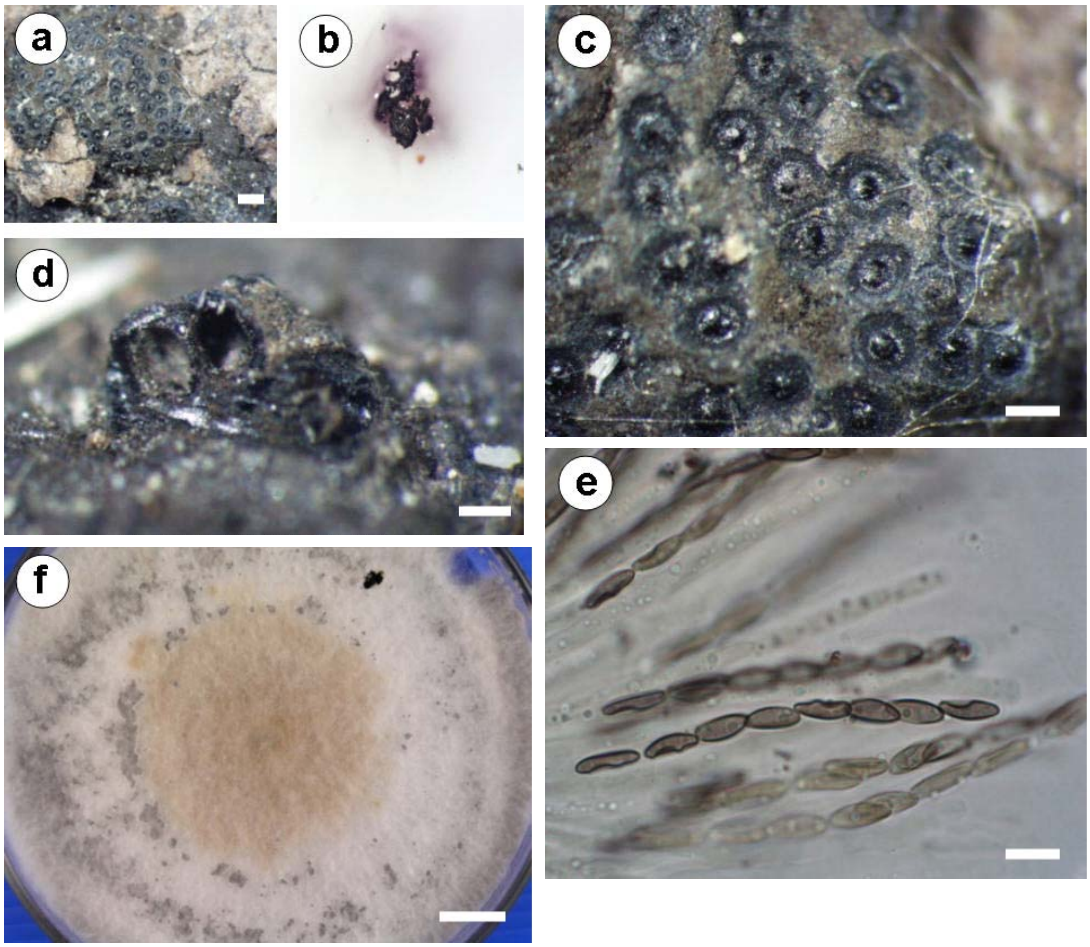

Figure 20. *Hypoxylon urceolatum* (Rehm) Y.-M. Ju & J.D. Rogers (SUT098); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment vinaceous purple, (c) stromatal form (Bar = 0.3 mm), (d) perithecia (Bar = 0.4 mm), (e) ascospores (Bar = 10 µm), and (f) cultural characteristics on PDA cultured at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

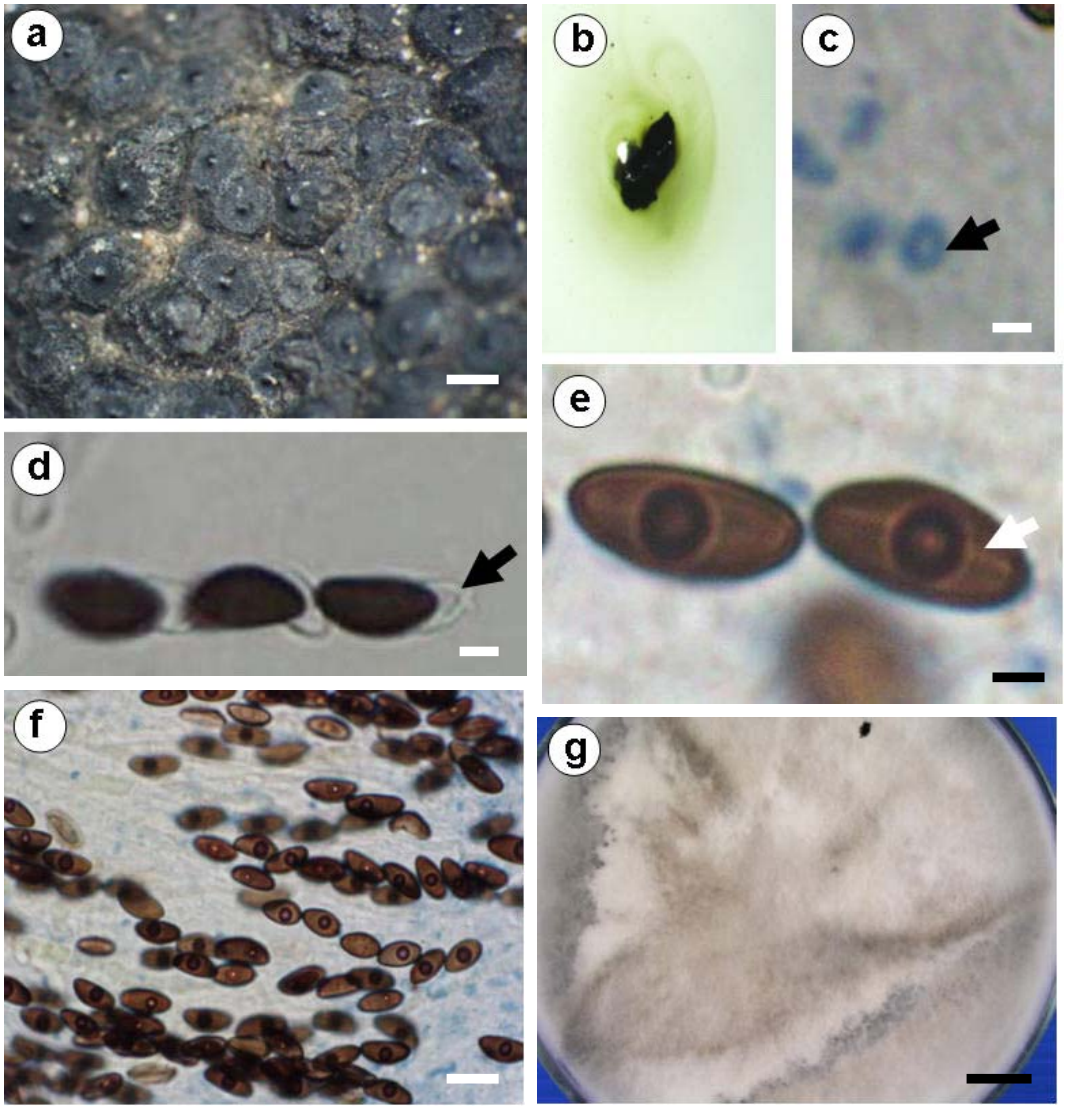

Figure 21. *Hypoxylon* taxonomic species 1 sp. nov. (SUT236); (a) stromatal form (Bar = 0.4 mm), (b) KOH-extractable pigment greenish olivaceous, (c) apical apparatus (arrowed) (Bar = 1 µm), (d) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), (e) straight germ slit spore length (arrowed) (Bar = 2 µm), (f) ascospores (Bar = 10 µm), 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 μm and 9.5-13(-14.5) x 4.5-6.5 μm 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 μm cf. (13.5-)14-17 x 6.5-8(-8.5) μm (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 μm (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 μm cf. 12.5-13.8 x 6.3-7.5 μm (Thienhirun, 1997).

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

Character	***H. anthochroum* Berk. & Broome***	***H. brevisporum* Y.-M. 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

* More details on collections are given in Appendix B.

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-pulvinate	Effused-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

* More details on collections are given in Appendix B.

Table 15. (Continued).

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 surface	Brown vinaceous	Reddish brown	Dull rusty brown
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 high	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
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 broad	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
Ascospores			
Color	Brown to dark brown	Brown to dark brown	Light brown to brown
Shape	Ellipsoid-inequilateral, with narrowly rounded ends	Ellipsoid-inequilateral, with narrowly rounded ends	Nearly equilateral, with nearly acute 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 ornamentation	Dehiscent, smooth	Dehiscent, smooth
Habitat	On wood	On wood	On wood
Location	Trad	Kanchanaburi, Ratchaburi, Yasothorn	Yasothorn
Specimen examined	SUT237	SUT062, SUT064, SUT164, SUT292, and SUT293	SUT166

* More details on collections are given in Appendix B.

Table 15. (Continued).

Character	***H. investiens* (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

* More details on collections are given in Appendix B.

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 μm high x 2 μm 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

* More details on collections are given in Appendix B.

Table 15. (Continued).

Character	*H.* cf. *perforatum* (SUT224)*	*H.* cf. *perforatum* (SUT294)*	*H. rubiginosum* (Pers.: Fr.) Fr., Summa Veg*
Stromata			
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 μm high x 2.5μm broad	Discoid, 0.8-1.5 μm high x 2-3 μm 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

* More details on collections are given in Appendix B.

Table 15. (Continued).

Character	***H. subgilvum* Berk. & Broome var. *microsporum* (Abe) Y.-M. 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 ornamentation	Dehiscent, with inconspicuous coil-like ornamentation	Dehiscent, with inconspicuous coil-like ornamentation
Location	Songkhla	Nakhon Ratchasima	Nakhon Ratchasima, Trad
Specimen examined	SUT095, SUT104, and SUT108	SUT154	SUT187

* More details on collections are given in Appendix B.

Table 15. (Continued).

Character	***H. kanchanapisekii* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley. sp. nov. ***	***H. sublenormandii* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley. sp. nov. ***	***H. suranareei* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley. sp. 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	Reddish brown	Amber or yellowish brown	Yellowish orange
Perithecia			
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 μm high x 2-3μm broad	Discoid, 0.7-1.5 μm high x 2-3μm 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

* More details on collections are given in Appendix B.

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

* More details on collections are given in Appendix B.

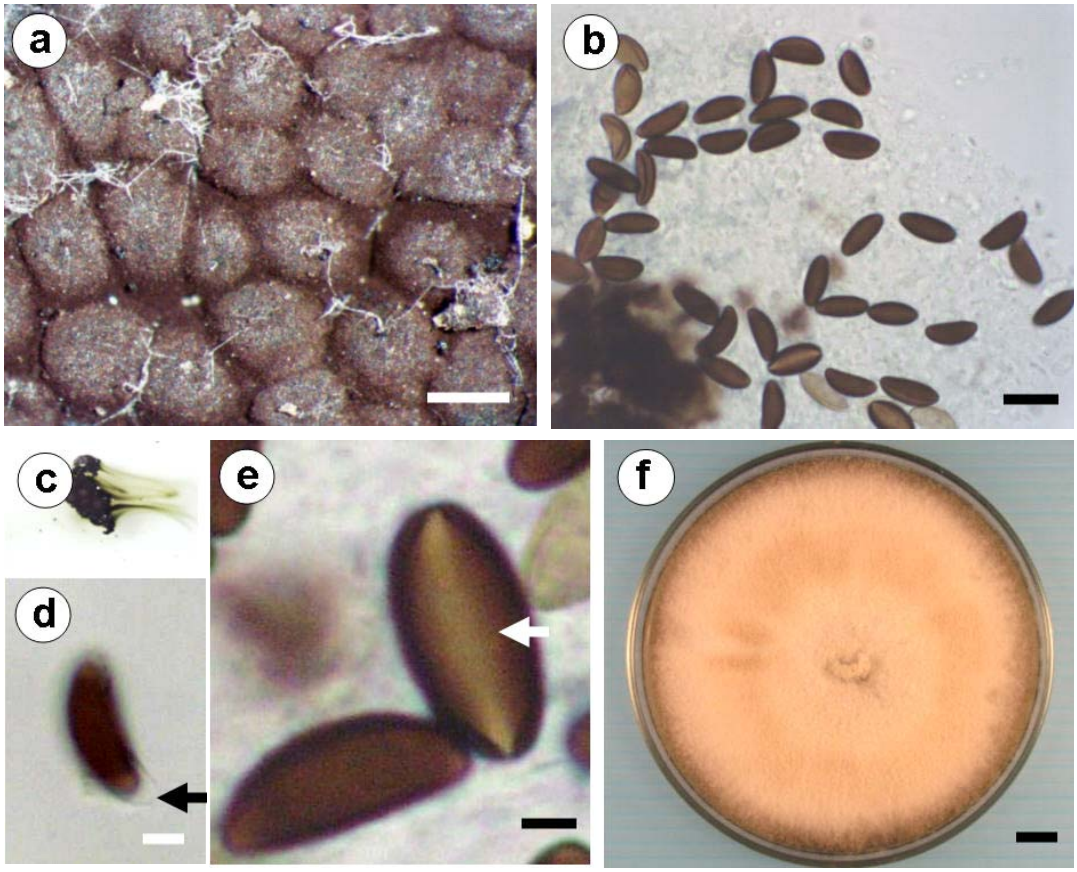

Figure 22. *Hypoxylon anthochroum* Berk. & Broome (SUT233); (a) stromatal form (Bar = 0.3 mm), (b) ascospores (Bar = 12 µm), (c) KOH-extractable pigment olivaceous, (d) ascospore dehiscent in 10% KOH (arrowed) (Bar = 4 µm), (e) straight germ slit spore length (Bar = 2 µm), and (f) cultural characteristics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

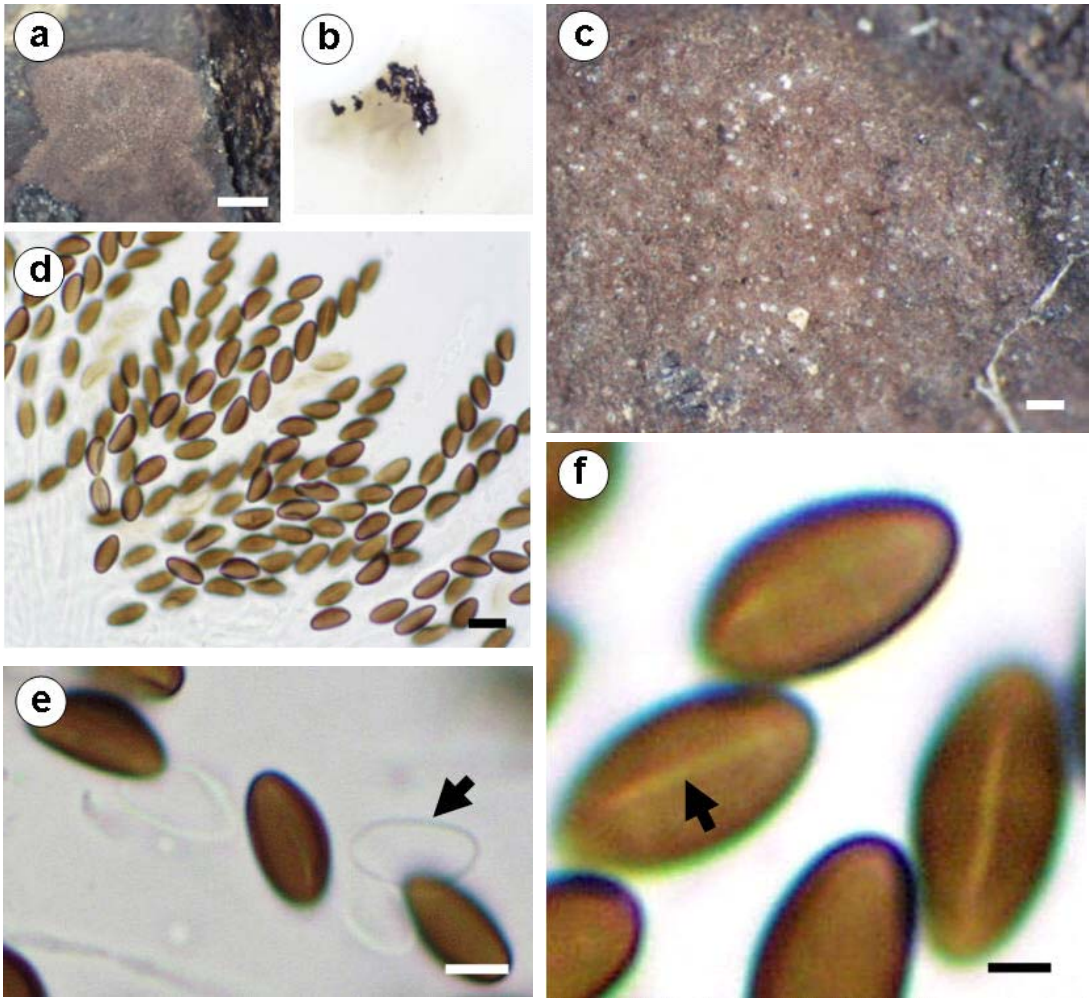

Figure 23. *Hypoxylon brevisporum* Y.-M. Ju & J.D. Rogers (SUT256); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment hazel, (c) stromatal with white substance on the ostioles (Bar = 0.2 mm), (d) ascospores (Bar = 6 µm), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 4 µm), and (f) straight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

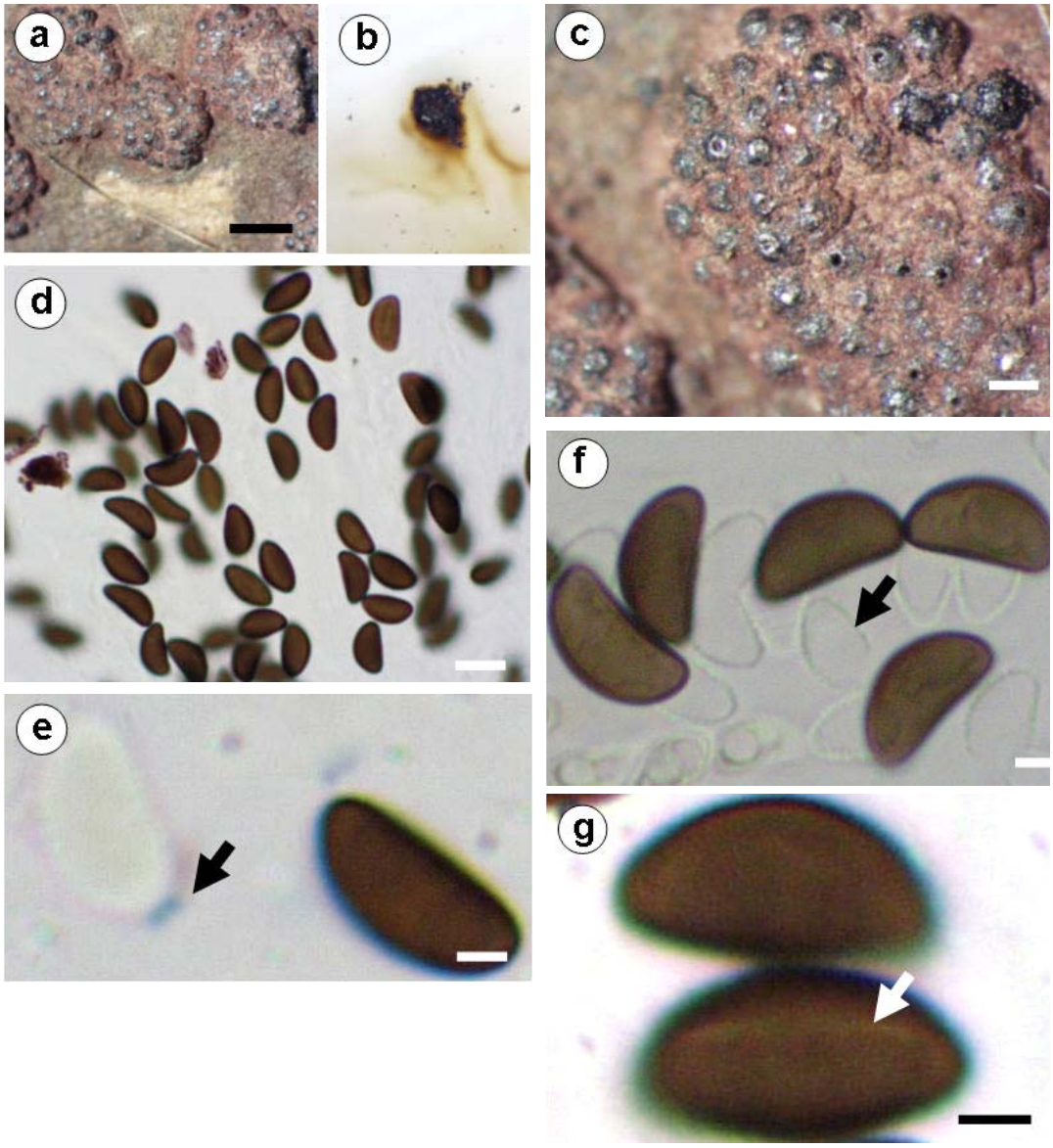

Figure 24. *Hypoxylon duranii* J.D. Rogers (SUT223); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment yellowish brown, (c) stromatal form (Bar = 0.3 mm), (d) ascospores (Bar = 10 µm), (e) apical apparatus (Bar = 2 µm), (f) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), and (g) straight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

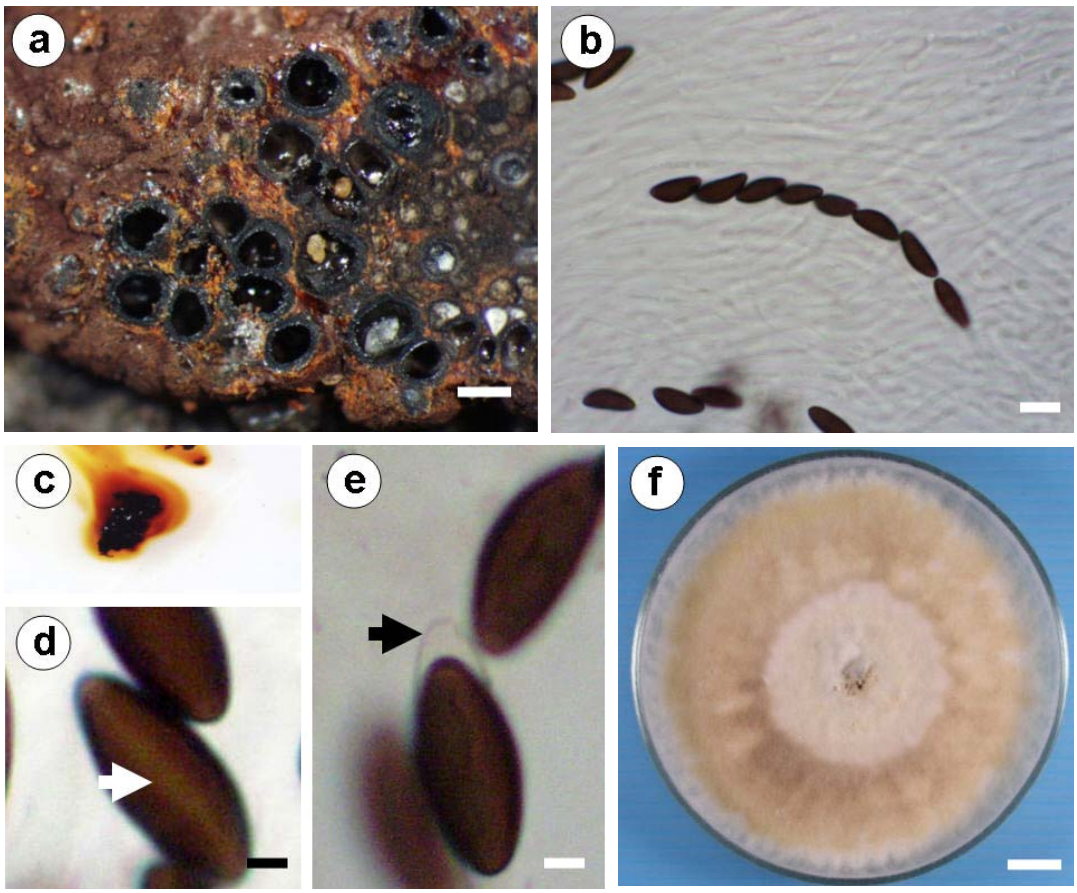

Figure 25. *Hypoxylon fendleri* Berk. *ex* Cooke (SUT162); (a) stromatal form (Bar = 0.3 mm), (b) ascospores (Bar = 10 µm), (c) KOH-extractable pigment orange, (d) slightly sigmoid germ slit spore length (arrowed) (Bar = 1 µm), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 1 µm), and (f) cultural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

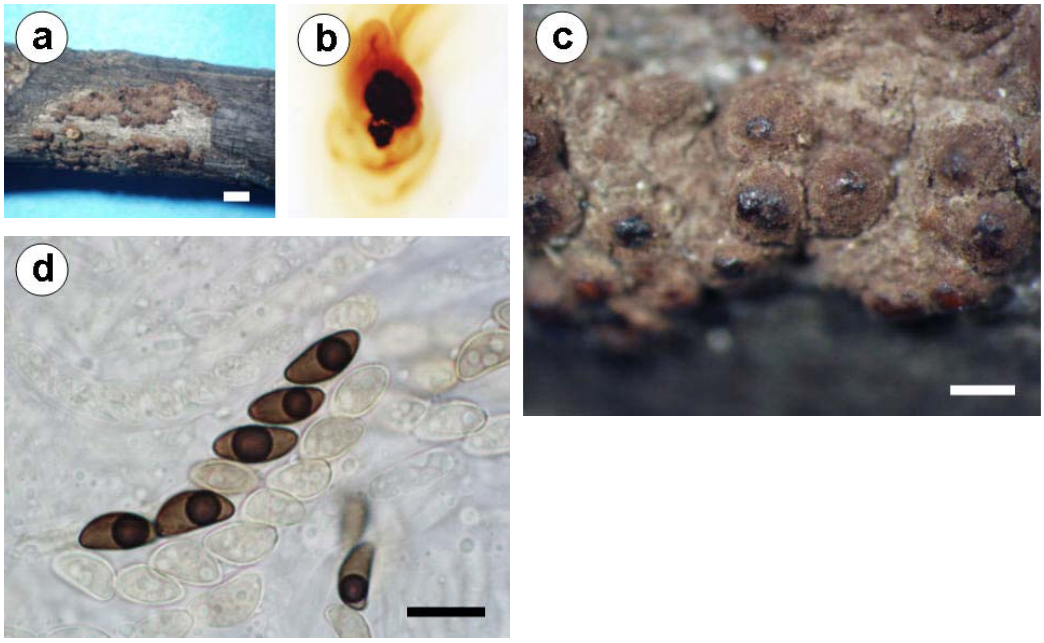

Figure 26. *Hypoxylon* cf. *ferrugineum* (SUT017); (a) and (c) stromatal form (Bars = 0.5 cm and 0.3 mm respectively), (b) KOH-extractable pigment orange, and (d) ascospores (Bar = 10 µm).

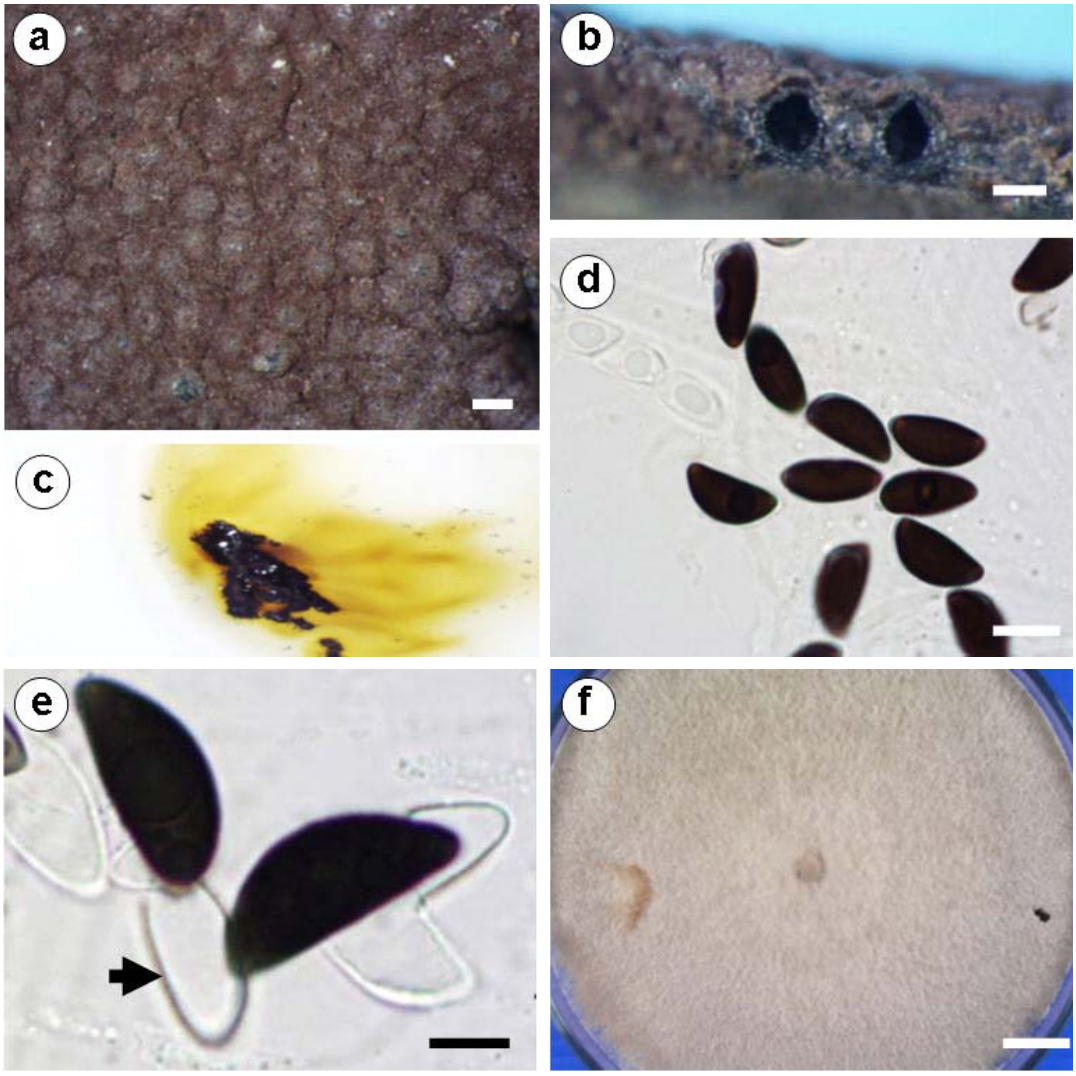

Figure 27. *Hypoxylon* cf. *ferrugineum* (SUT070); (a) stromatal form (Bar = 0.2 mm), (b) perithecia (Bar = 0.4 µm), (c) KOH-extractable pigment yellowish orange, (d) ascospores (Bar = 10 µm), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), and (f) cultural characteristics on PDA cultured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

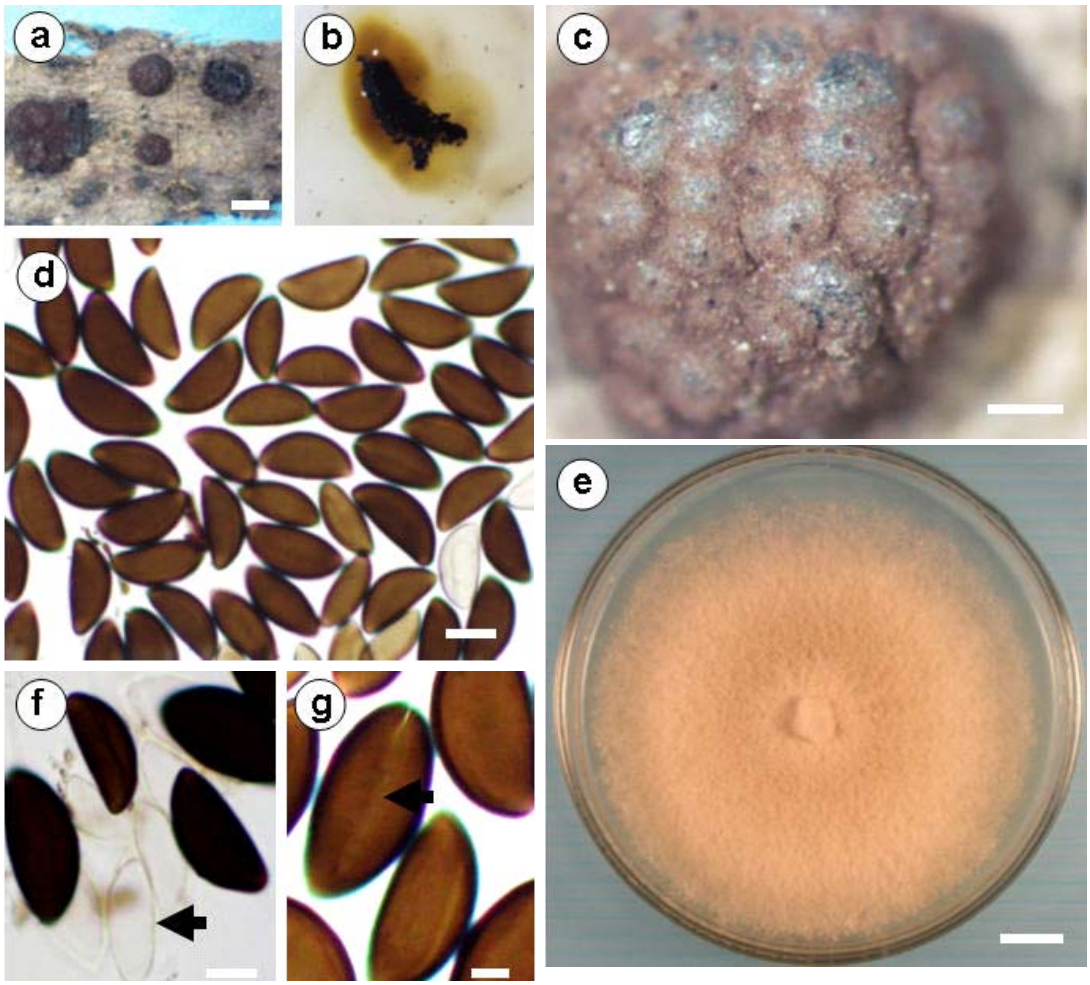

Figure 28. *Hypoxylon* cf. *ferrugineum* (SUT237); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment brownish yellow, (c) stromatal form (Bar = 0.4 µm), (d) ascospores (Bar = 10 µm), (e) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm), (f) ascospore dehiscent in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

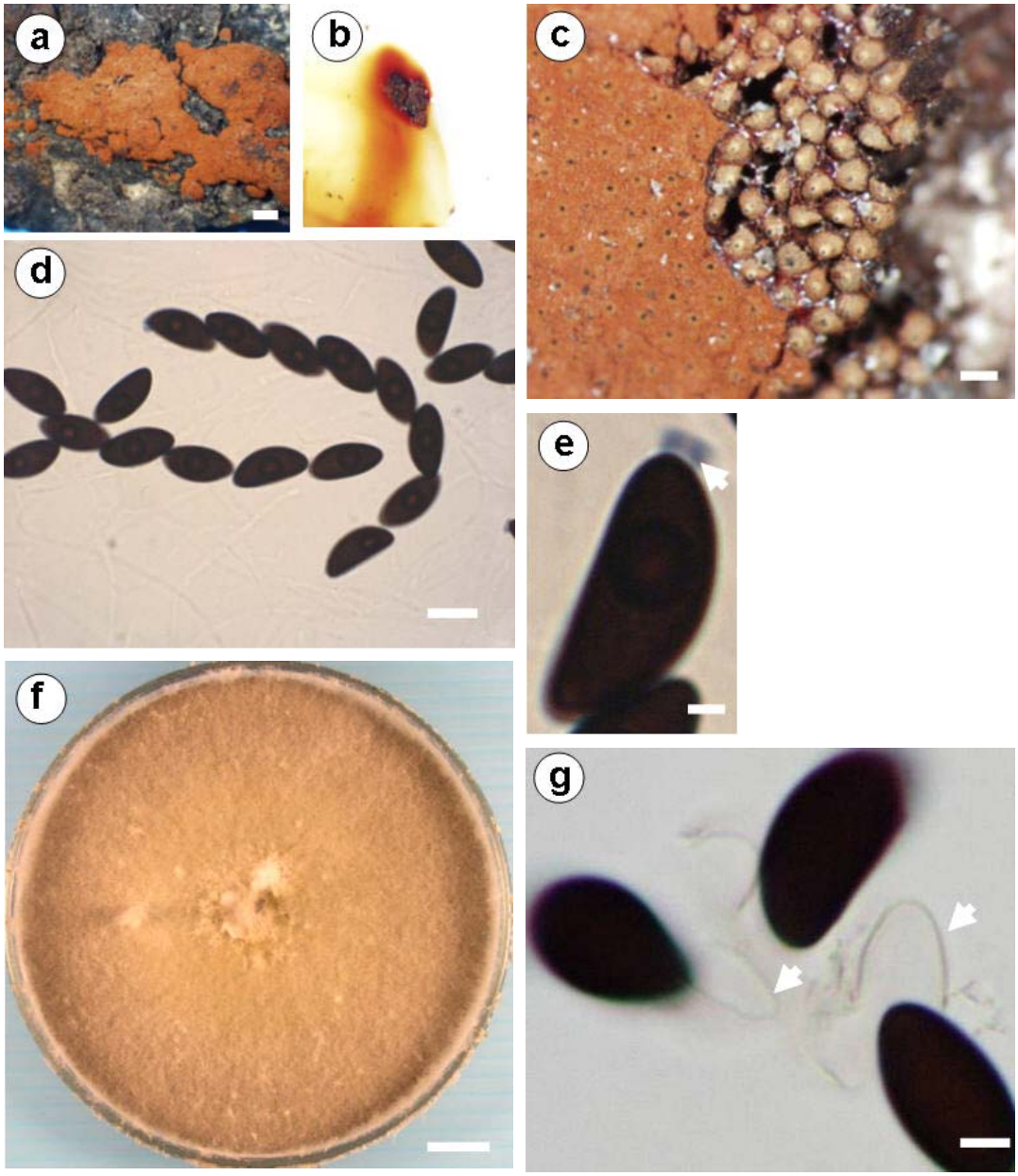

Figure 29. *Hypoxylon haematostroma* Mont. (SUT164); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment orange, (c) stromatal form (Bar = 0.3 mm), (d) ascospores (Bar = 10 µm), (e) apical apparatus (arrowed) (Bar = 3 µm), (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar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

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 µm cf. (6-)6.5-9.5(-10) x 3-4.5 µm. (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 µm and 3.8-5 x 2.5-3 µm 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 µm cf. 9-12.5(-13) x 4-5.5 µm, 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).

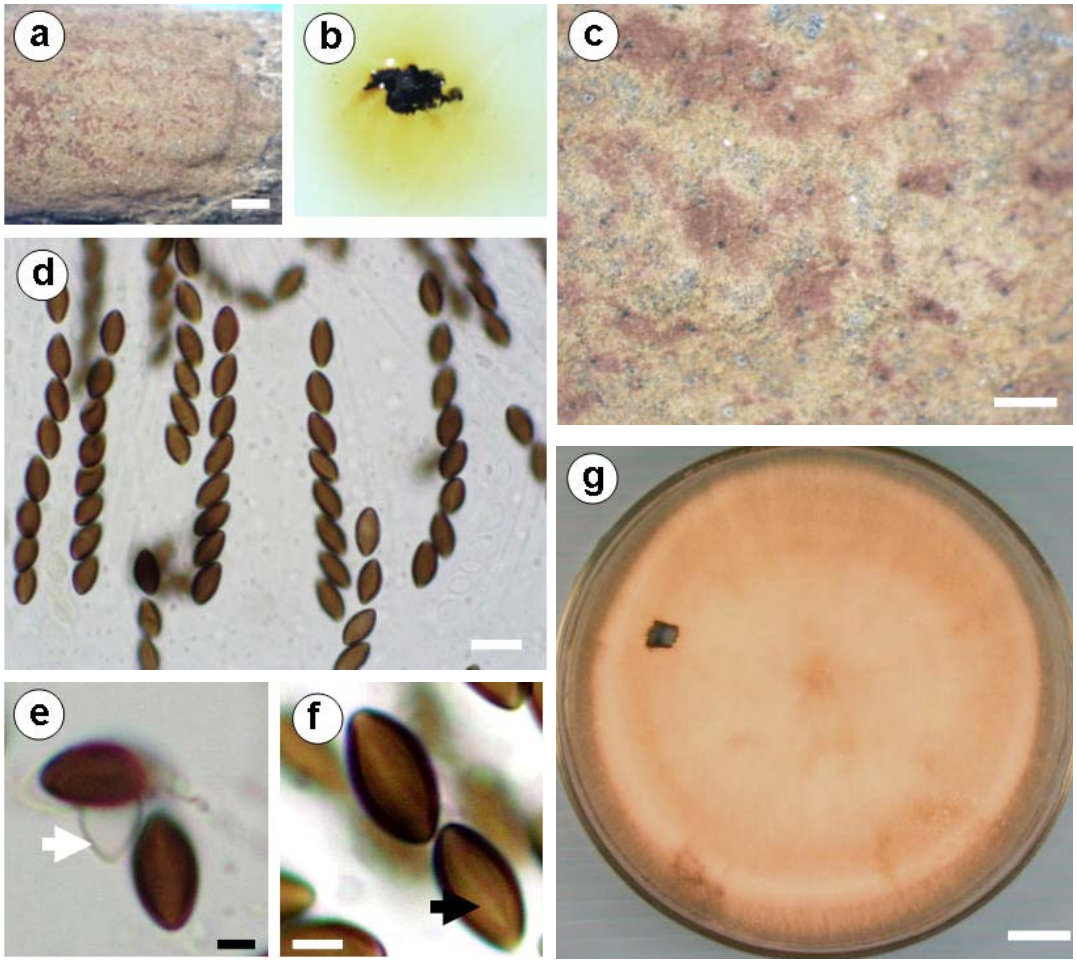

Figure 30. *Hypoxylon hypomiltum* Mont. (SUT166); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment amber, (c) stromatal form (Bar = 0.2 mm), (d) ascospore (Bar = 8 µm), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), (f) straight germ slit spore length (arrowed) (Bar = 2 µm), and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

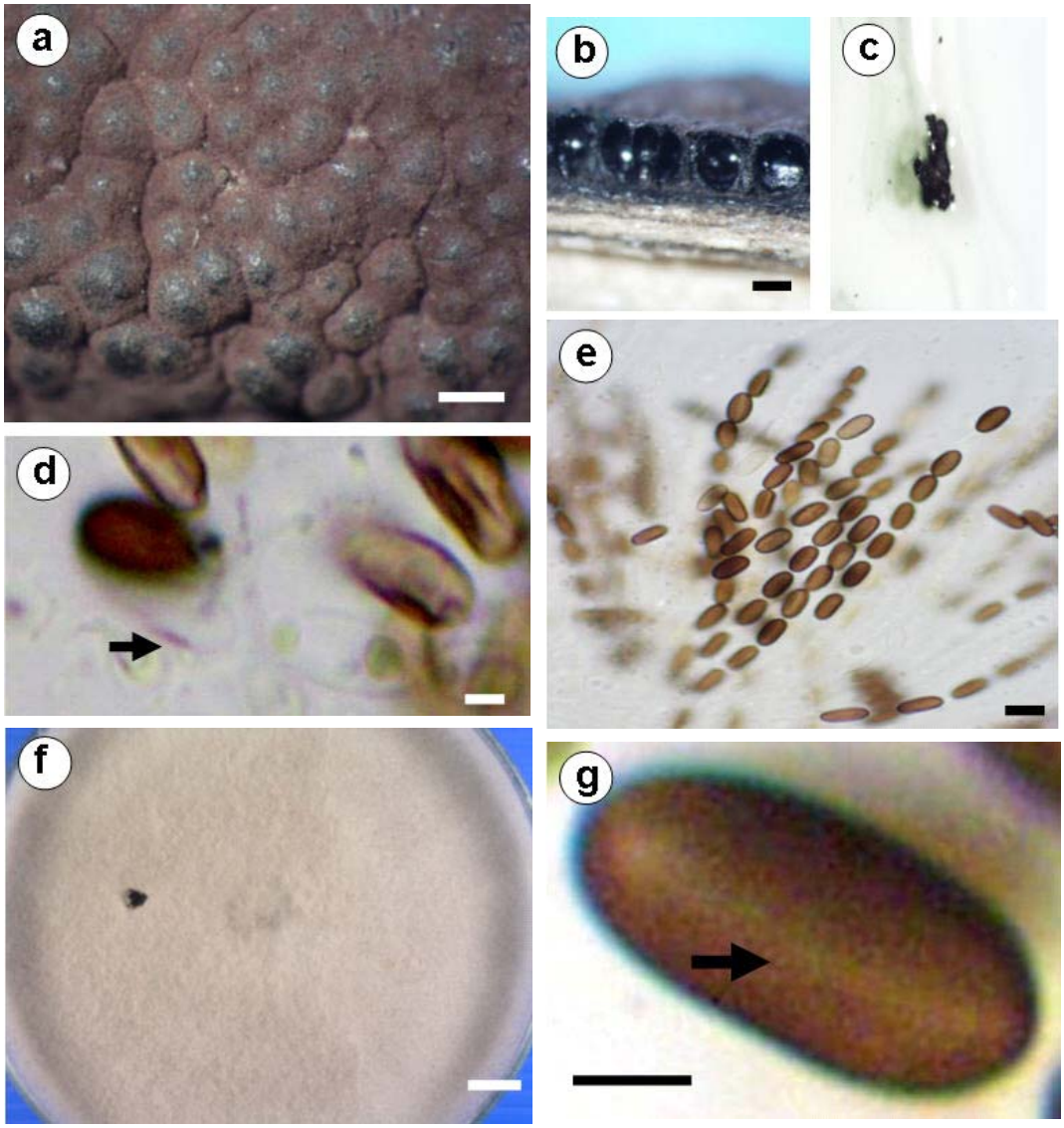

Figure 31. *Hypoxylon investiens* (Schwein.) M.A. Curtis. (SUT063); (a) stromatal form (Bar = 0.4 mm), (b) Perithecia (Bar = 0.2 mm), (c) KOH-extractable pigment dull green, (d) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), (e) ascospore (Bar = 8 µm), (f) cultural characteristics on PDA cultured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

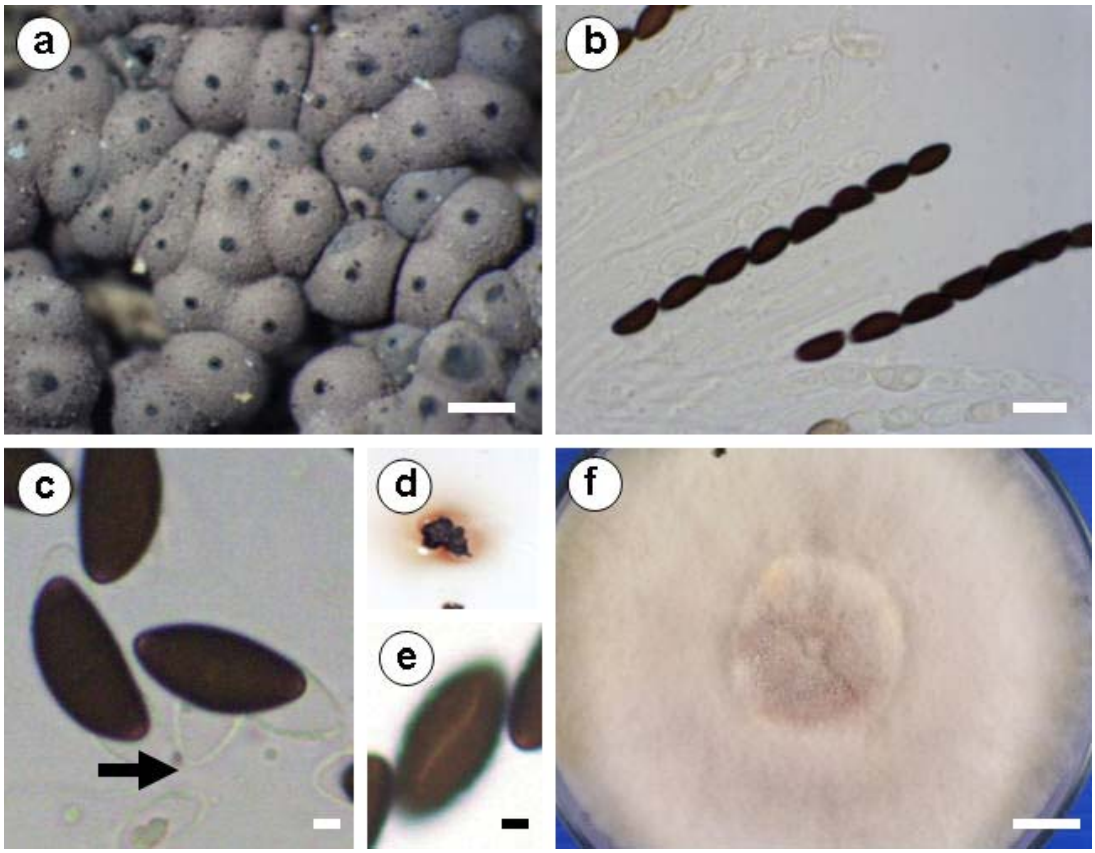

Figure 32. *Hypoxylon lenormandii* Berk. & M.A. Curtis. (SUT065); (a) stromatal form (Bar = 0.5 µm), (b) ascospores (Bar = 10 µm), (c) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), (d) KOH-extractable pigment of red, (e) slightly sigmoid germ slit spore length (arrowed) (Bar = 2 µm), and (f)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

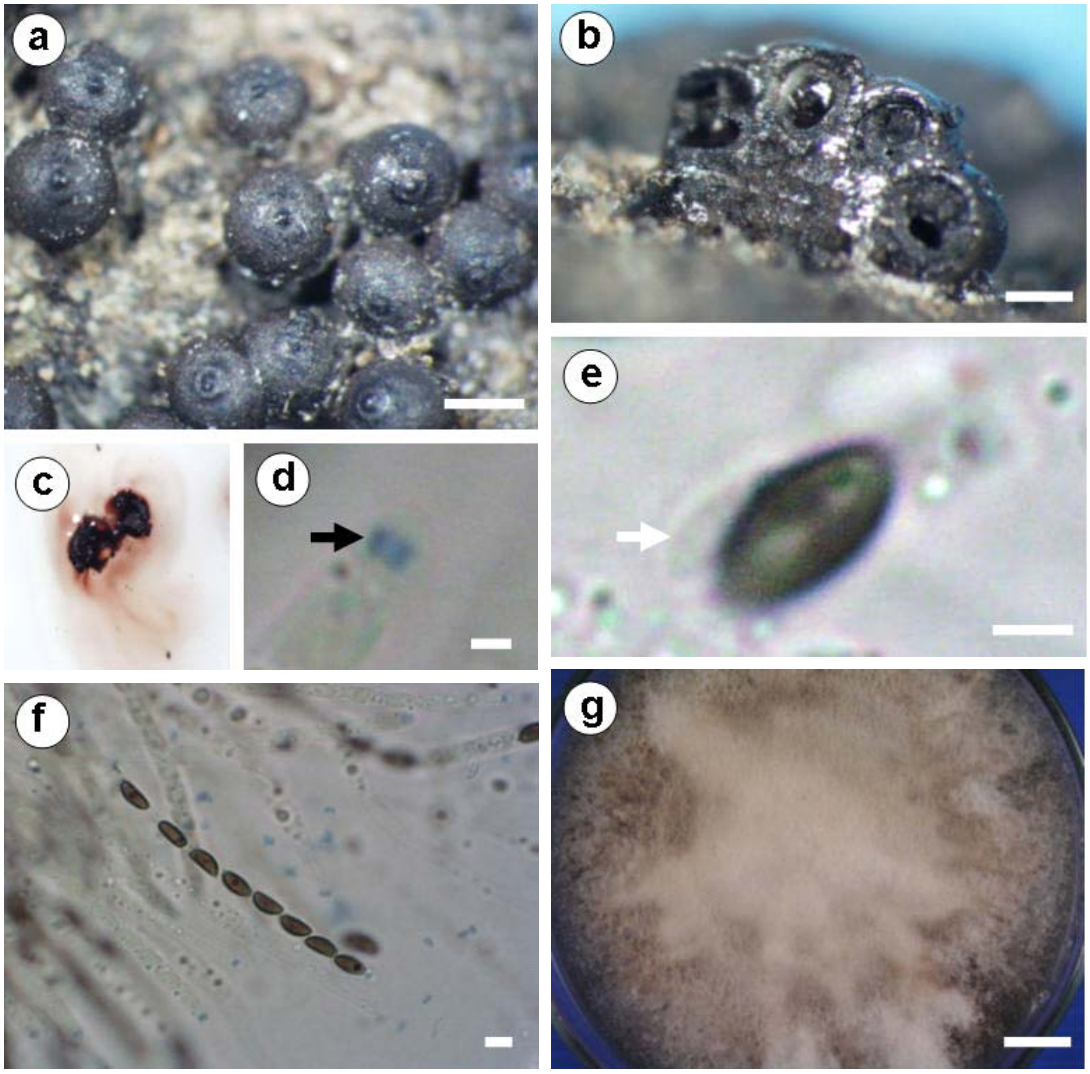

Figure 33. *Hypoxylon lenormandii* var. *microspora* (SUT022) (Thienhirun, 1997); (a) stromatal form (Bar = 0.5 µm), (b) perithecia (Bar = 0.5 µm), (c) KOH-extractable pigment of red, (d) apical apparatus (arrowed) (Bar = 1 µm), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), (f) ascospores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

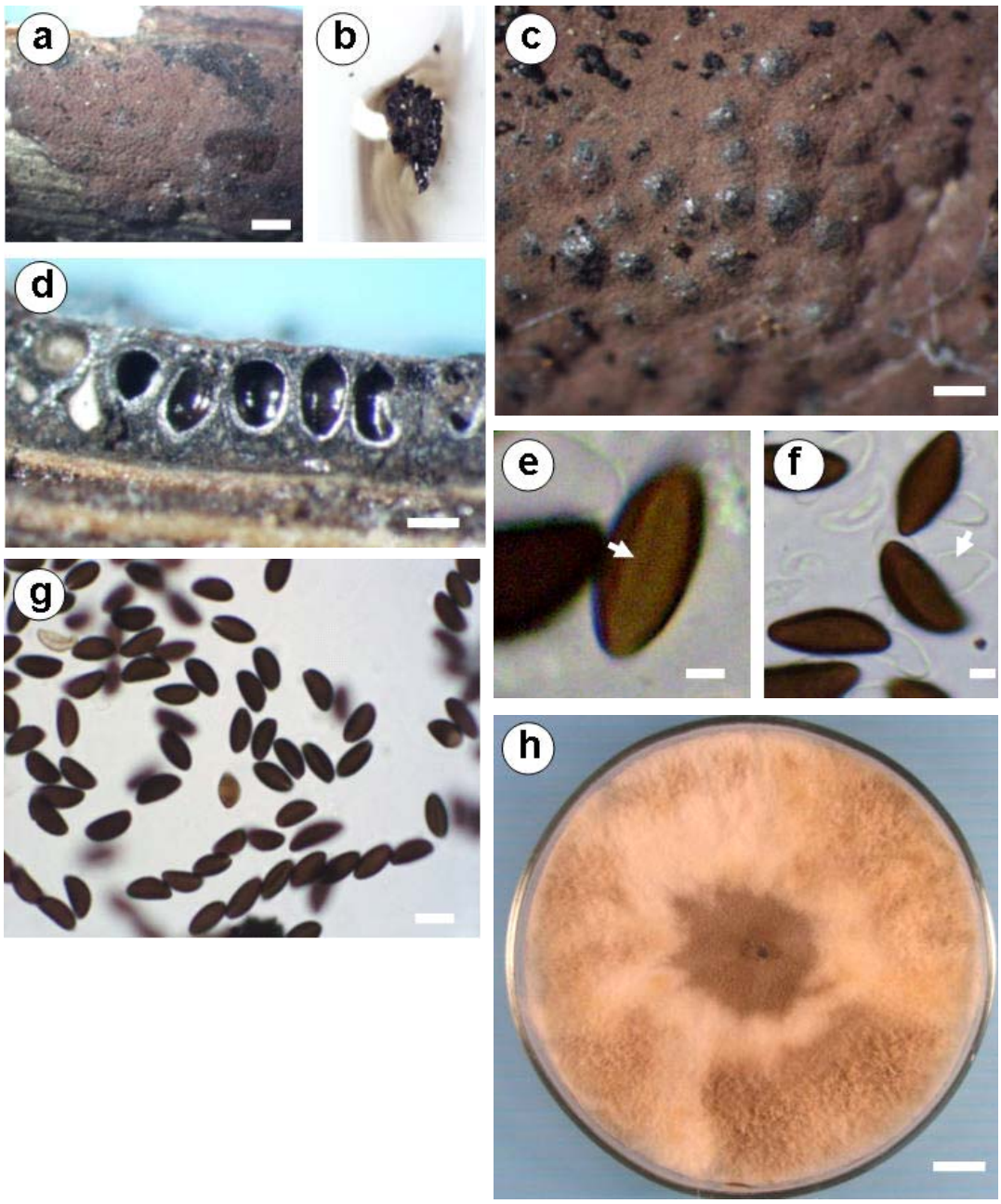

Figure 34. *Hypoxylon macrocarpum* Pouzar (SUT045); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment hazel, (c) stromatal form (Bar = 0.2 mm), (d) perithecia (Bar = 0.2 mm), (e) straight germ slit spore length (Bar = 2 µm), (f) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), (g) ascospores (Bar = 10 µm), 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 KOH-extractable 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).

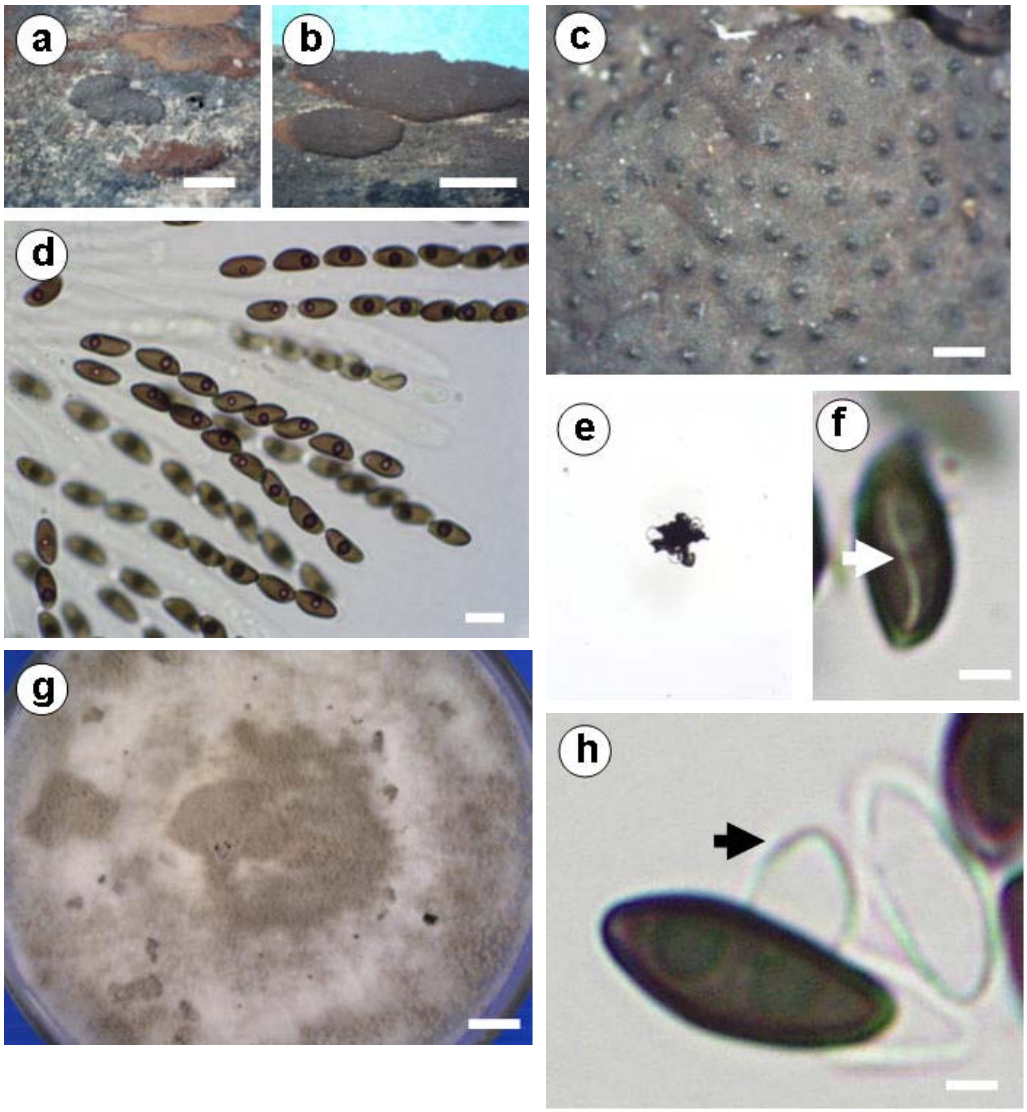

Figure 35. *Hypoxylon monticulosum* Mont. (SUT116); (a), (b), and (c) stromatal form (Bars = 1 cm, 1 cm, and 0.5 mm respectively), (d) ascospore (Bar = 10 µm), (e) KOH-extract colorless, (f) slightly sigmoid curve germ slit spore length (Bar = 2 µm), (g) cultural characteristics on PDA cultured at 25°C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

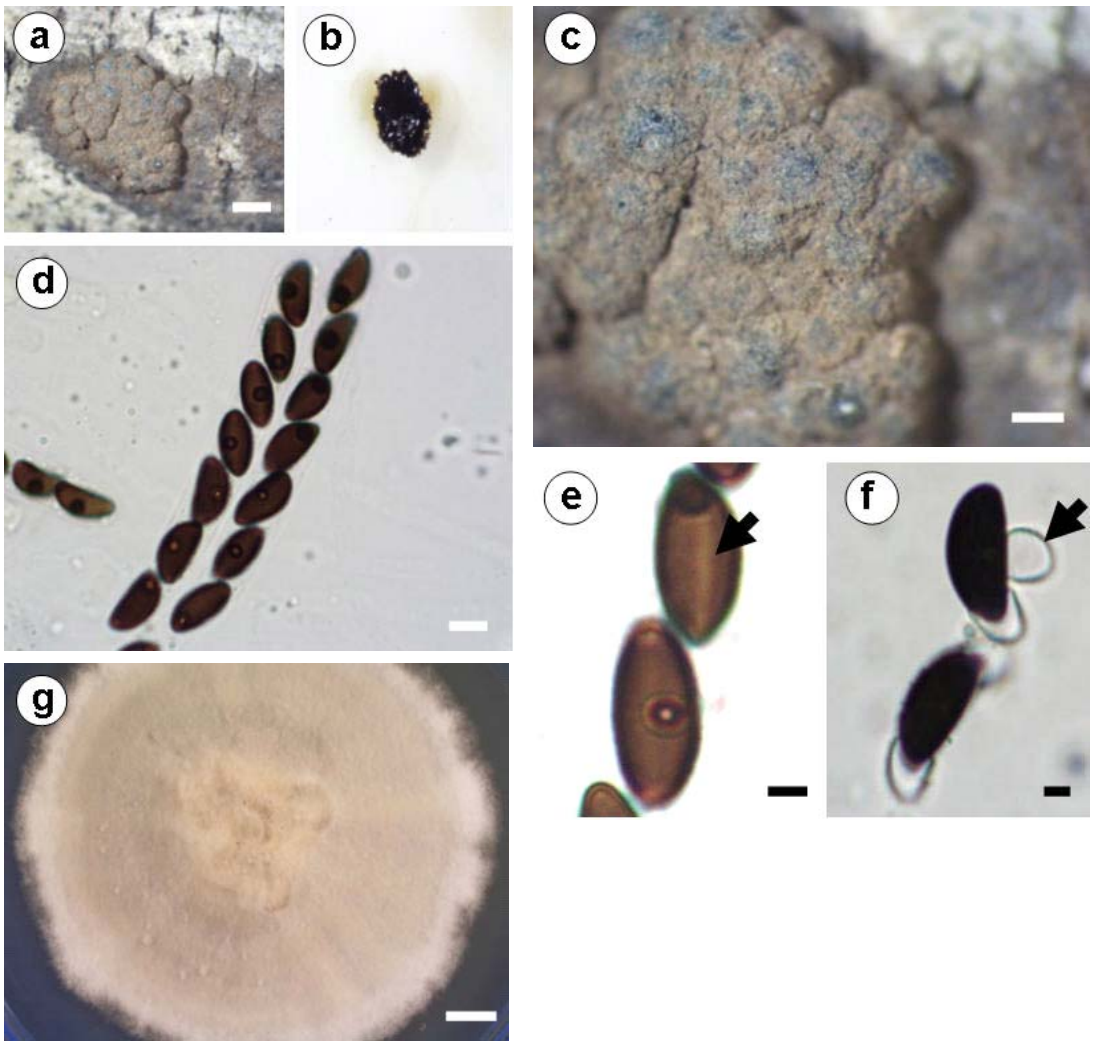

Figure 36. *Hypoxylon* cf. *perforatum* (SUT020); (a) stromatal form (Bar = 0.5 cm), (b) KOH-extractable pigment amber, (c) stromatal form (Bar = 0.3 mm), (d) ascospores (Bar = 5 µm), (e) straight germ slit spore length (Bar = 2 µm), (f) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), and (g)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

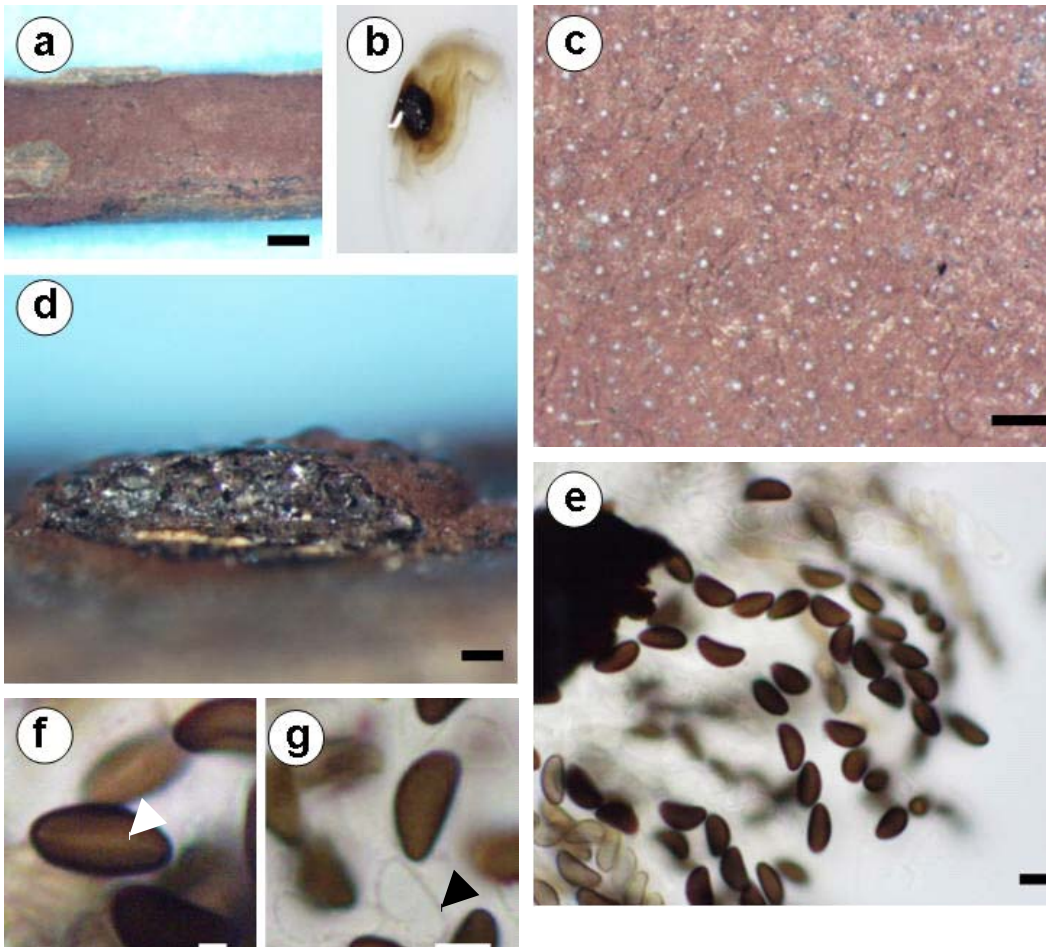

Figure 37. *Hypoxylon* cf. *perforatum* (SUT224); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment amber, (c) stromatal form (Bar = 0.4 mm), (d) perithecia (Bar = 0.2 mm), (e) ascospores (Bar = 10 µm), (f) straight germ slit spore length (Bar = 2 µm), and (g) ascospore dehiscent in 10% KOH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

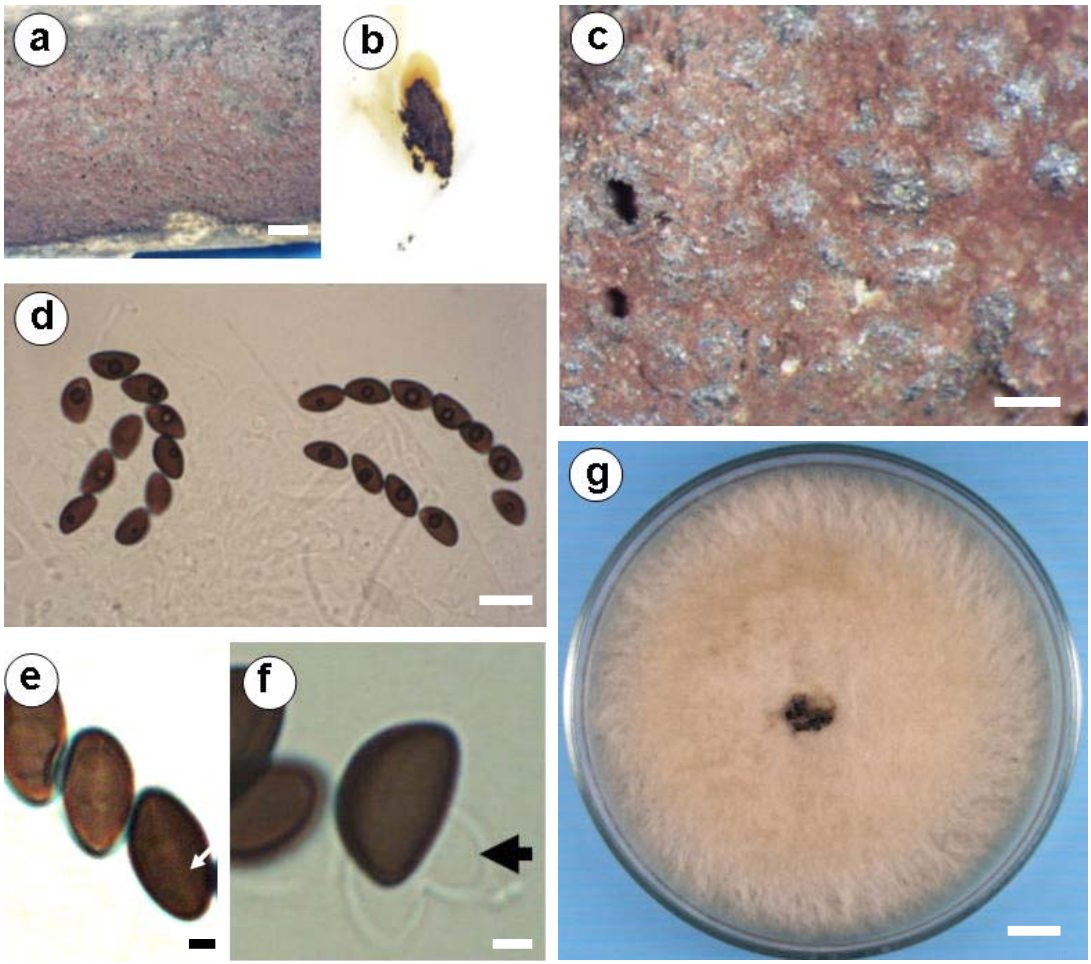

Figure 38. *Hypoxylon* cf. *perforatum* (SUT294); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment amber, (c) stromatal form (Bar = 0.3 mm), (d) ascospores (Bar = 10 µm), (e) straight germ slit spore length (Bar = 2 µm), (f) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), and (g) cultural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

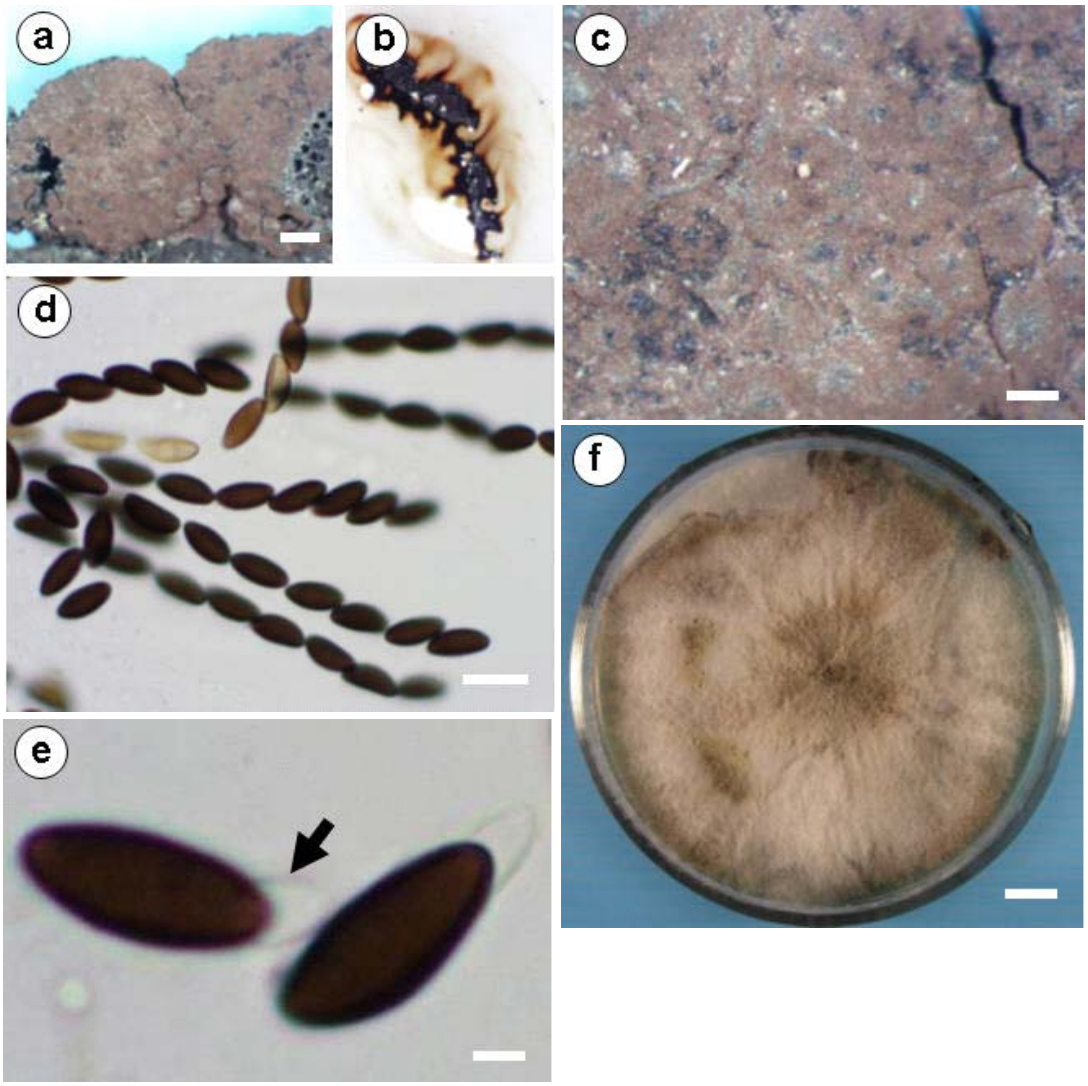

Figure 39. *Hypoxylon rubiginosum* (SUT215); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment rust, (c) stromatal form (Bar = 0.2 mm), (d) ascospores (Bar = 10 µm), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), and (f) cultural characteristics on PDA cultured at 25°C after 4 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 μm total length x 3.8-5 μm broad, the spore bearing parts 75-85 μm long with stipes 12.5-45 μm; ascospores brown, unicellular, equilateral, with narrowly rounded ends, 10-11.25(-12.5) x (0.5-)3.75-5 μm, 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) x 3.8-5 µm cf. 9.5-15(-16) x 4-6.5(-7) µm, 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 µm total length x 3.8-5 µm broad, the spore bearing parts 65-75 µm long with stipes 30-42.5 µm; ascospores brown, unicellular, equilateral, with narrowly rounded ends, 9-12 x 4-5 µm, 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 µm cf. 9.5-15(-16) x 4-6.5(-7) µm, 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 µm total length x 3.8-5 µm broad, the spore bearing parts 70-85 µm long with stipes 30-50 µm; ascospores brown to dark brown, unicellular, equilateral, with narrowly rounded ends (10-)12.5-13.8 x 5-6.3 µm, 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) x 4-6.5(-7) µm), germ slit form, and KOH pigment.

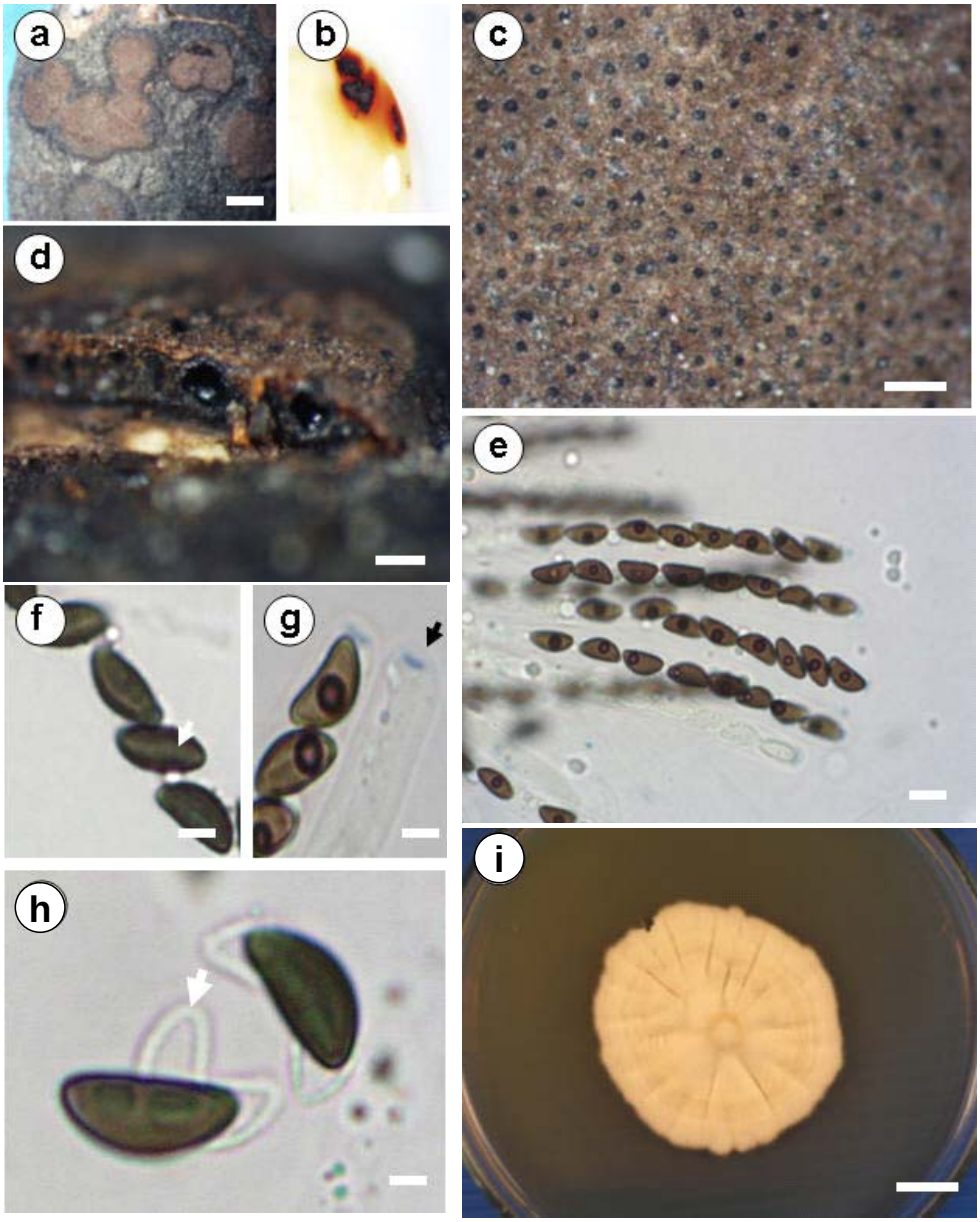

Figure 40. *Hypoxylon subgilvum* Berk. & Broome var. *microsporum* (Abe) Y.-M. Ju & J.D. Rogers. (SUT234); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment orange, (c) stromatal form (Bar = 0.5 mm), (d) perithecia (Bar = 0.5 µm), (e) ascospores (Bar = 7 µm), (f) straight to slightly sigmoid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

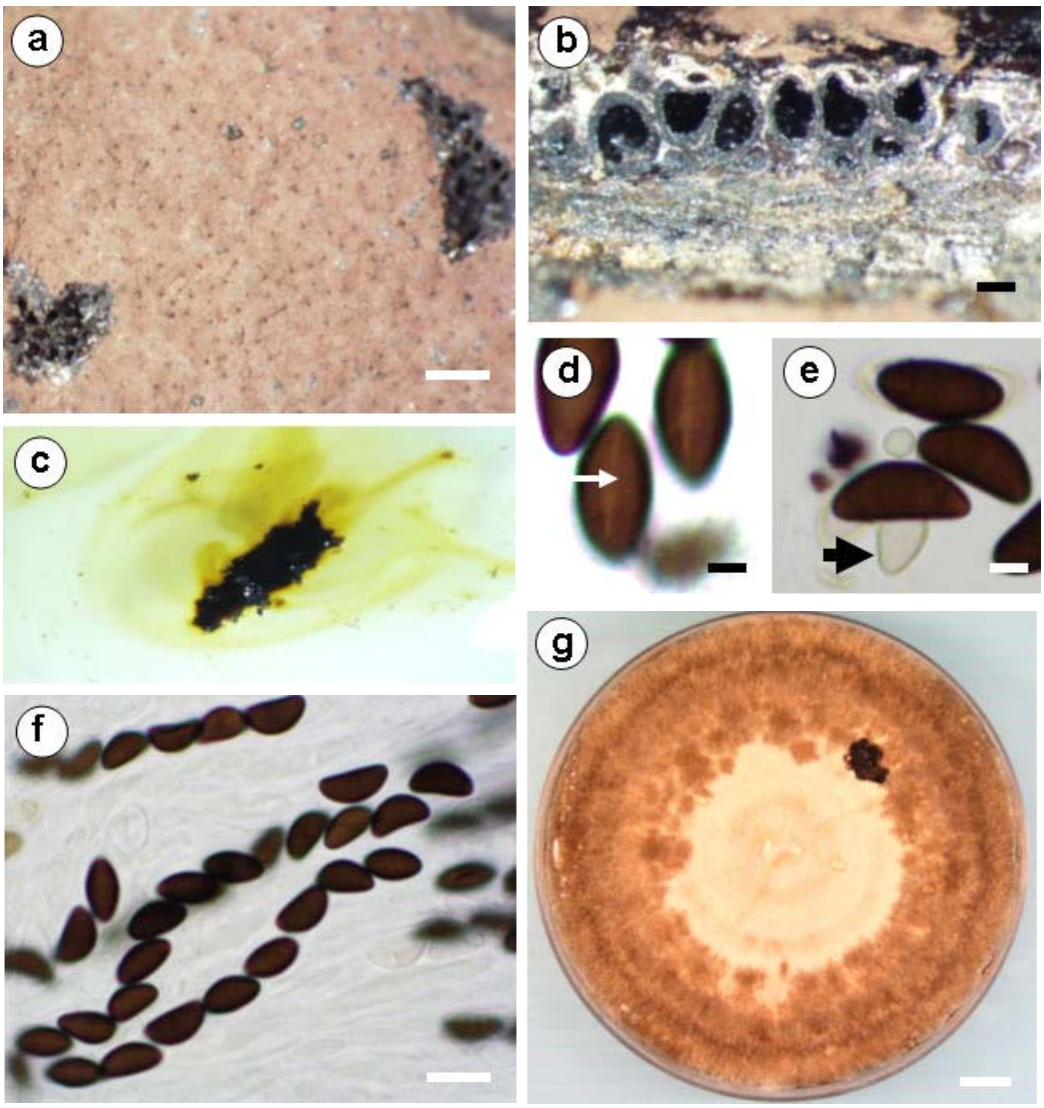

Figure 41. *Hypoxylon trogodes* Berk. & Broome (SUT187); (a) stromatal form (Bar = 1 cm), (b) perithecia (Bar = 0.2 µm), (c) KOH-extractable pigment yellow, (d) straight germ slit spore length (arrowed) (Bar = 2 µm), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), (f) ascospores (Bar = 10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

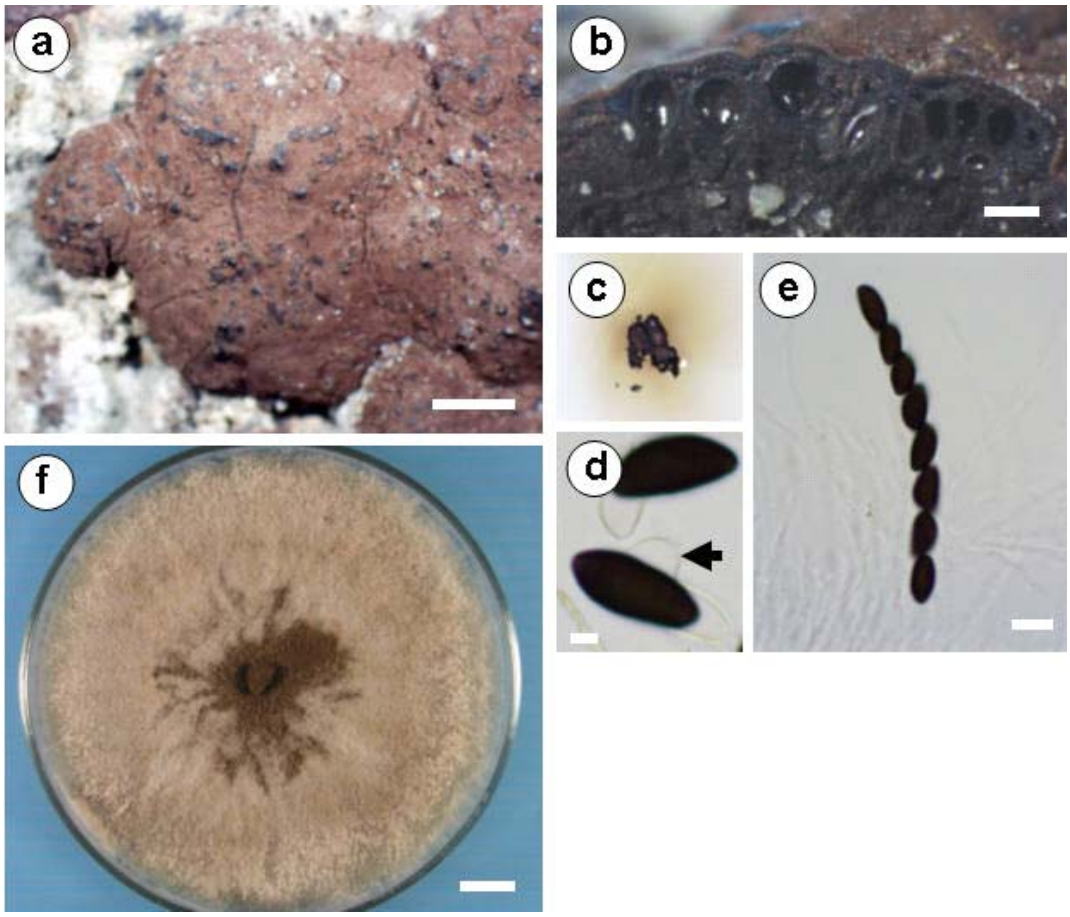

Figure 42. *Hypoxylon trugodes* Berk. & Broome (SUT154); (a) stromatal form (Bar = 1 cm), (b) perithecia (Bar = 0.4 µm), (c) KOH-extractable pigment yellowish brown, (d) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), (e) ascospores (Bar = 10 µm), and (f) cultural characteristics on PDA cultured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

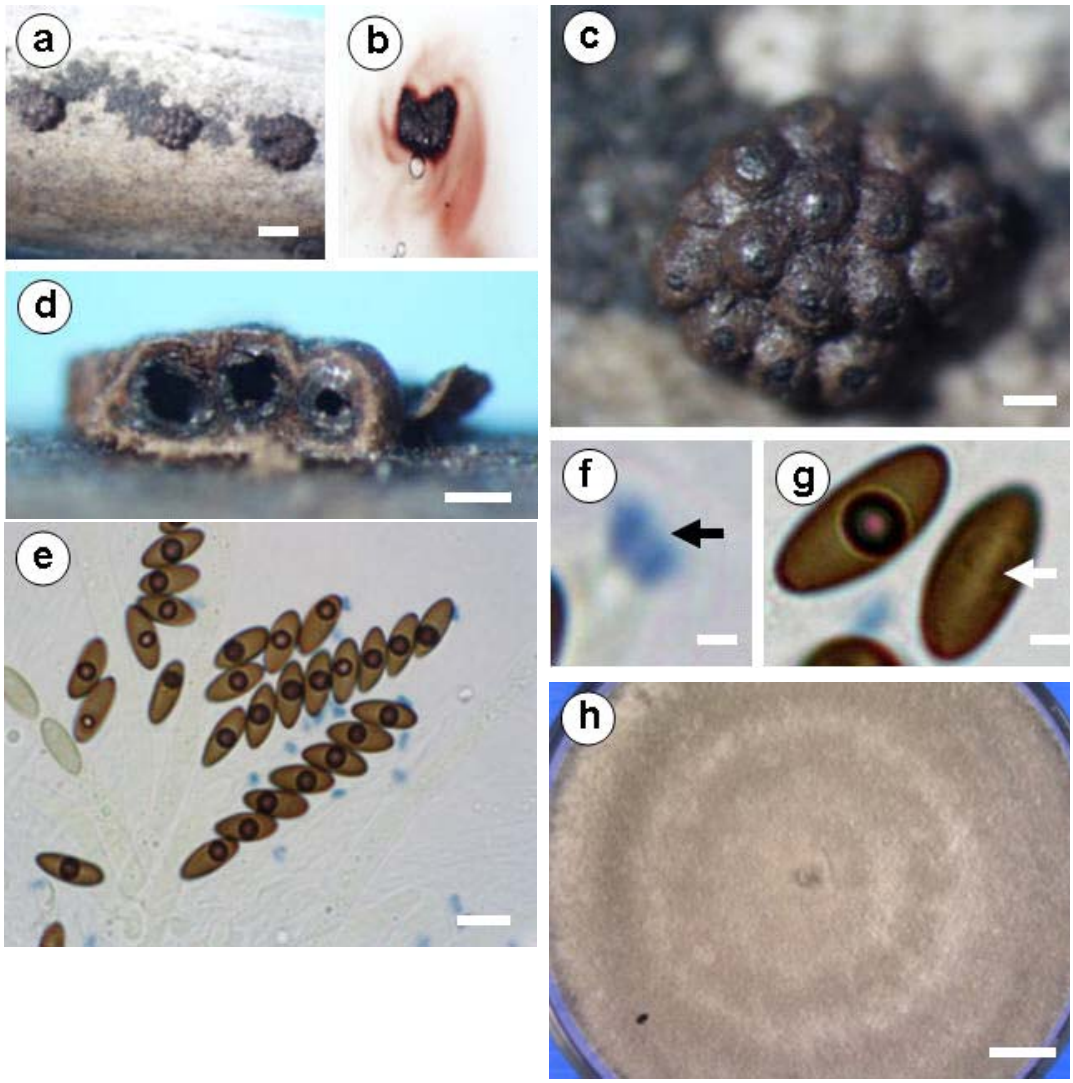

Figure 43. *Hypoxylon kanchanapisekii* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley, sp. nov. (SUT069); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment reddish brown, (c) stromatal form (Bar = 0.2 mm), (d) perithecia (Bar = 0.2 µm), (e) ascospores (Bar = 10 µm), (f) apical apparatus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

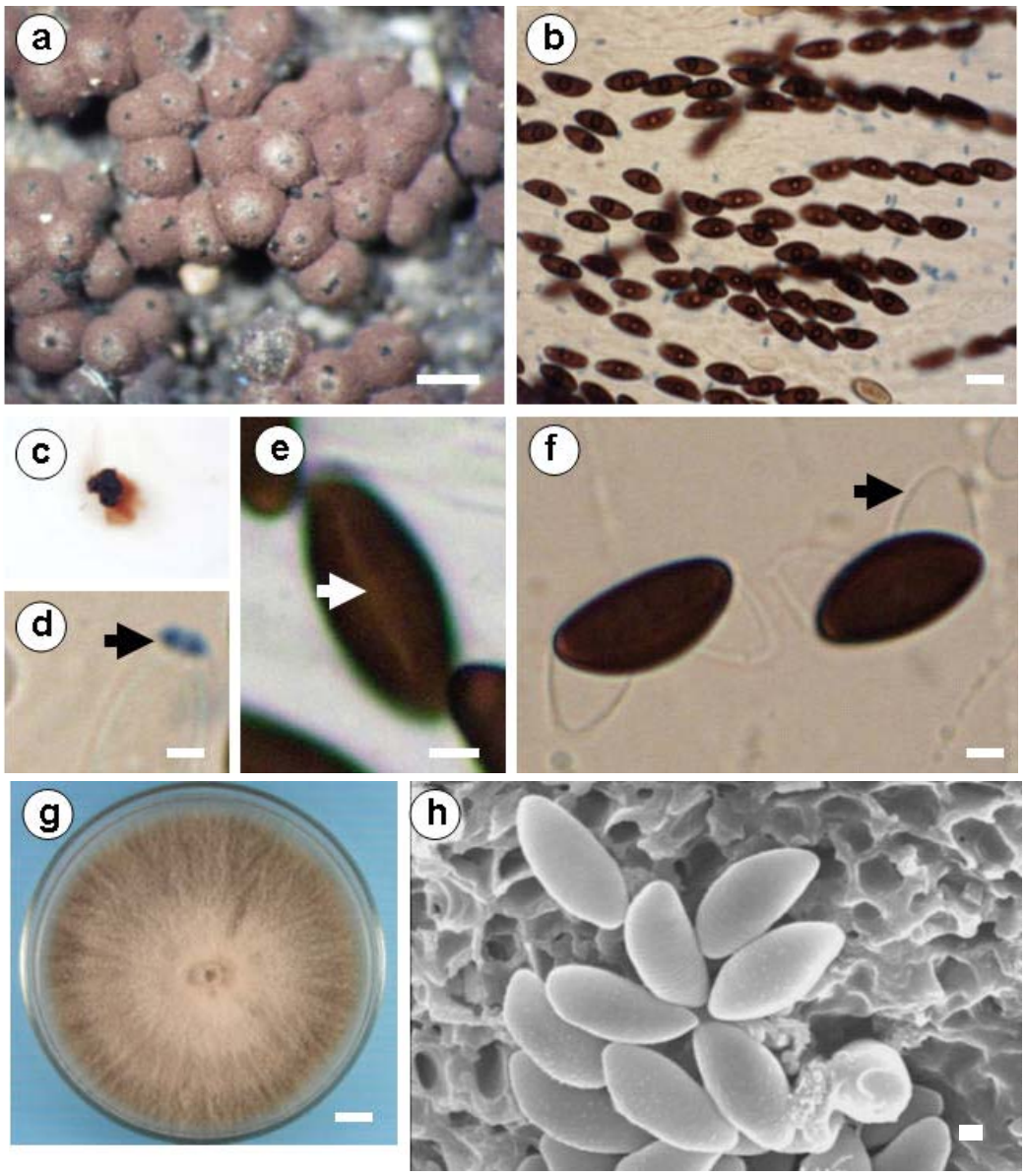

Figure 44. *Hypoxylon sublenormandii* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley, sp. nov. (SUT282); (a) stromatal form (Bar = 0.5 cm), (b) ascospores (Bar = 10 µm), (c) KOH-extractable pigment of reddish brown, (d) apical apparatus (arrowed) (Bar = 1 µm), (e) straight germ slit spore length (arrowed) (Bar = 2 µm), (f) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), (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 µm).

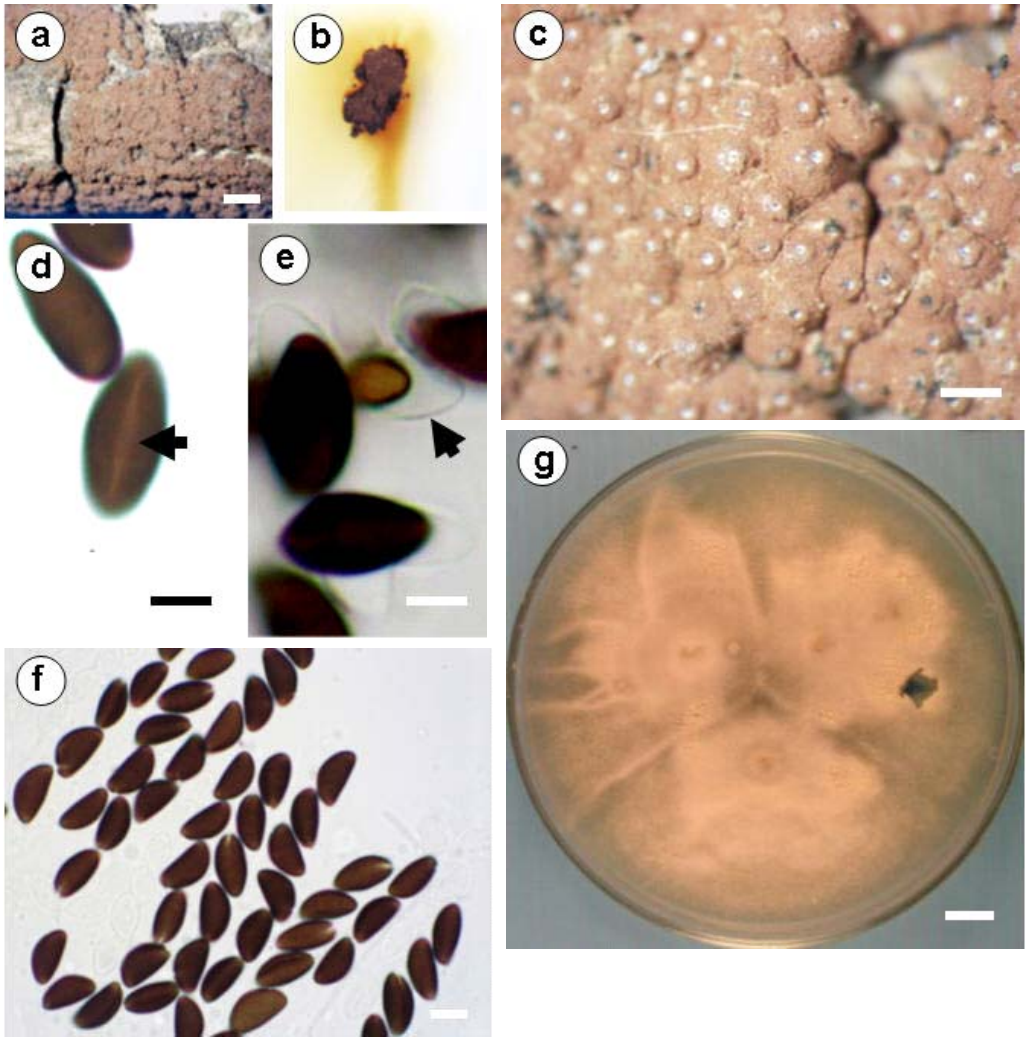

Figure 45. *Hypoxylon suranareei* N. Suwannasai, S. Rodtong, S. Thienhirun & A.J.S. Whalley, sp. nov. (SUT182); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment yellowish orange, (c) stromatal form (Bar = 0.5 µm), (d) straight germ slit spore length (arrowed) (Bar = 5 µm), (e) ascospore dehiscent 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 µm) than *Hypoxylon rubiginosum* (Pers.: Fr.) Fr. ((8-)9-12 x 4-4.5 µm). 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 µm and 10-11.3 x 3.8-5 µm respectively.

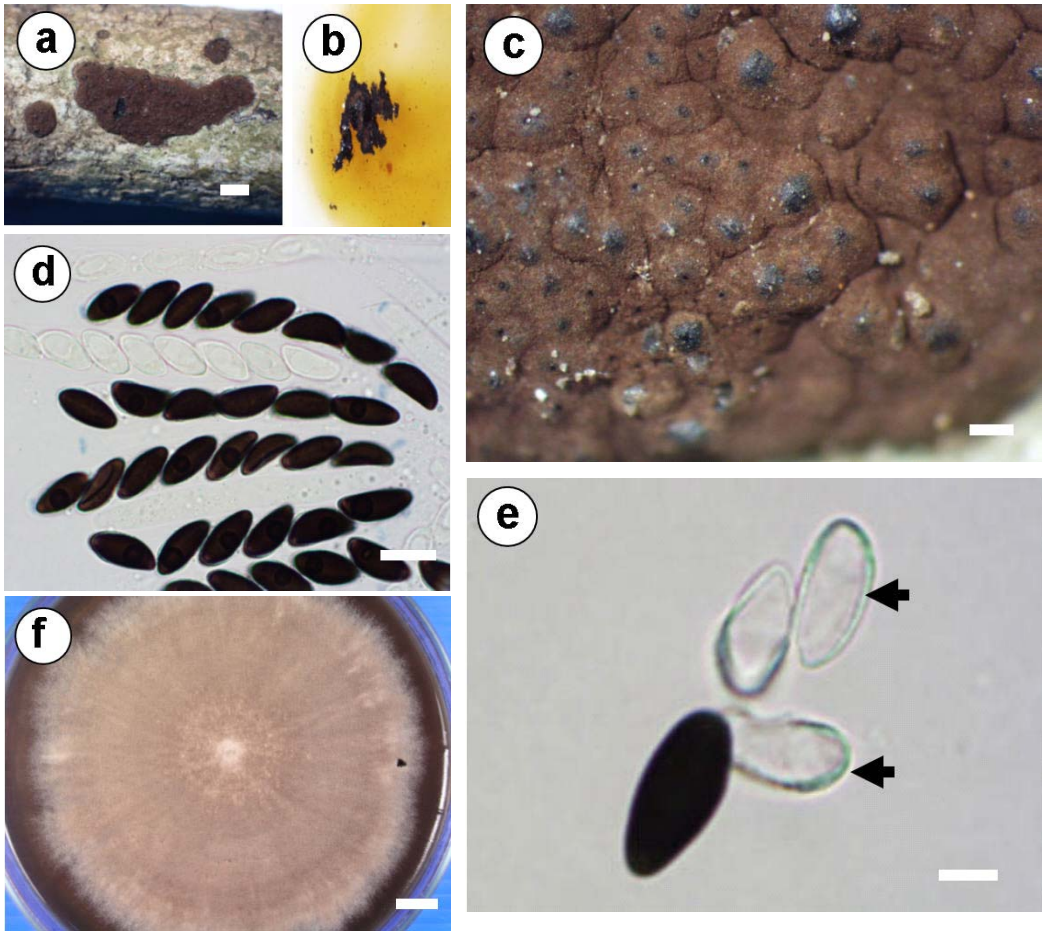

Figure 46. *Hypoxylon* taxonomic species 2 (SUT082); (a) stromatal form (Bar = 1 cm), (b) KOH-extractable pigment rust, (c) stromatal form (Bar = 0.2 mm), (d) ascospores (Bar = 10 µm), (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), and (f) cultural characteristics on PDA cultured at 25°C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

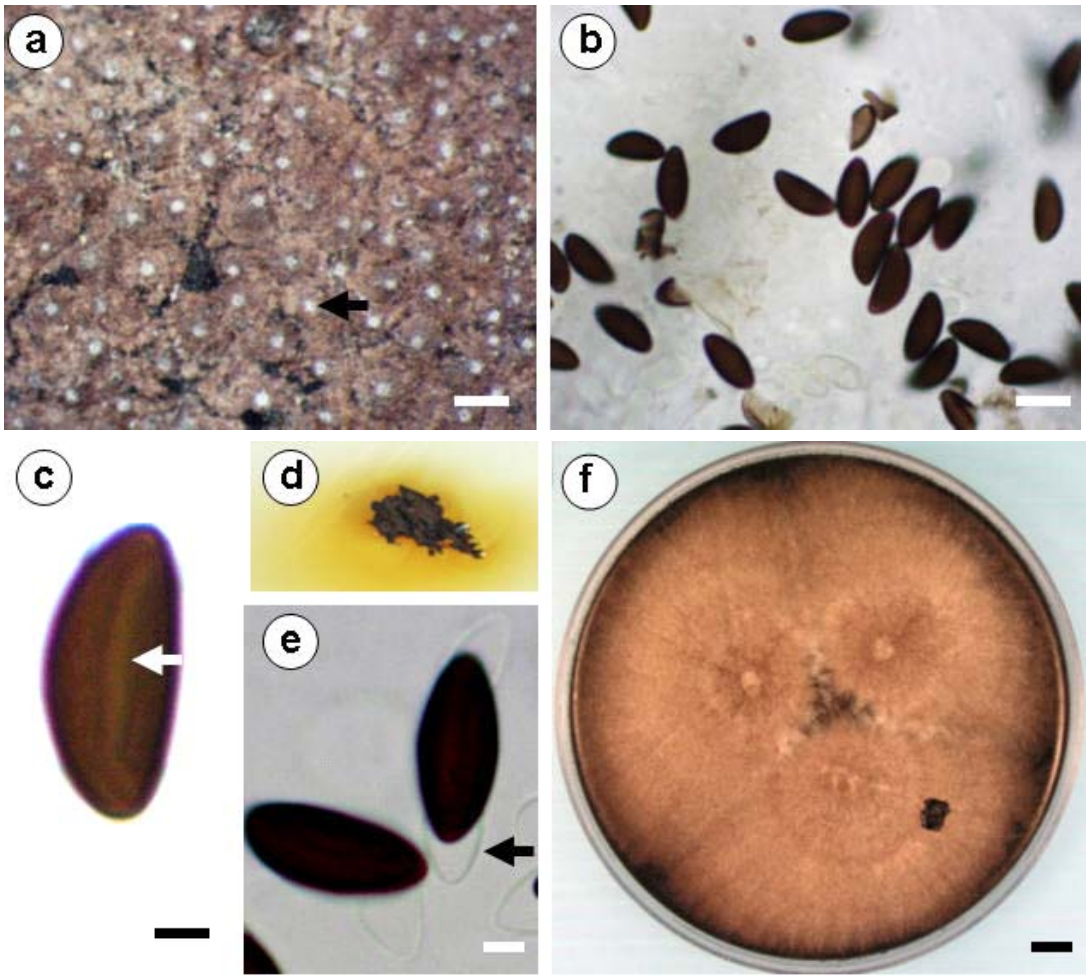

Figure 47. *Hypoxylon* taxonomic species 3 (SUT158); (a) stromatal form (Bar = 0.5 cm), (b) ascospores (Bar = 10 µm), (c) straight germ slit spore length (arrowed) (Bar = 2 µm), (d) KOH-extractable pigment amber, (e) ascospore dehiscent in 10% KOH (arrowed) (Bar = 2 µm), and (f) cultural characteristics 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
Xylaria		
X. anisopleura (Mont.) Fr.	3	According to Rogers and Samuels (1986), Rogers (1988), González and Rogers (1989), and Thienhirun (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 Thienhirun (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 *et al*. (1988), 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), Rogers *et al*. (1987 and 1988), Callan and Rogers (1990), and Thienhirun (1997)
Xylaria species 2	17	Thienhirun, 1997
Xylaria sp. nov.	27	Rough stromatal surface, finely reticulately cracked into small angular closely spaced scales so as to outline the individual perithecia
Xylaria taxonomic species 1 (SUT075)	1	Smooth stromatal surface except for peeling layer, externally blackish with dark brown outer peeling layer, internally creamy white
Xylaria 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
Xylaria taxonomic species 3 (SUT204)	1	Rugose and usually roughened stromatal surface by warts, externally black and internally white
Xylaria taxonomic species 4 (SUT207)	1	Smooth stromatal surface except for 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 fertile head with short stalk, not branched, externally blackish, internally white
Nemania		
Nemania species (SUT258)	2	Erumpent to superficial stromata, smooth with slightly papillate surface

* Number of collections

Table 16. (Continued).

Species	No.*	Remark
Biscogniauxia		
Biscogniauxia capnodes (Berk.) Y.-M. Ju & J.D. Rogers	4	Ju and Rogers (1998)
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) µm cf. 17.5-22.5 x 10-11.3 µm (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.

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

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

* More details on collections are given in Appendix B.

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

* More details on collections are given in Appendix B.

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

* More details on collections are given in Appendix B.

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 μm high x 2 μm broad
Ascospores			
Color	Brown	Brown	Brown to dark brown
Shape	Ellipsoid-inequilateral, with rounded ends	Ellipsoid-inequilateral with rounded to acute ends	Ellipsoid-inequilateral with broadly rounded 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

* More details on collections are given in Appendix B.

Table 17. (Continued).

Character	***X. mellisii* (Berk.) Cooke ***	***X. multiplex* (Kunze) Fr. ***	***X. muscula* Lloyd. ***
Stromata			
Shape	Fertile part cylindrical to cylindric-conical, with acute sterile apices, on narrow hirsute stipes	Cylindrical, with acute sterile apices, smooth stipe, arising from enlarged tomentose base	Cylindrical with sterile apices, on short stipes
Color	Externally blackish with grey to brown outer peeling layer, internally white to creamy white	Externally blackish with light brown outer peeling layer, which splited longitudinally into narrow strips, internally white	Externally white with black ostioles, 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 μm high x 1.5 μm 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 rounded 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

* More details on collections are given in Appendix B.

Table 17. (Continued).

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 μm high x 3.8-5 μm 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

* More details on collections are given in Appendix B.

Table 17. (Continued).

Character	***Xylaria* species 2 ***	***X. telfairii* (Berk.) Fr.***	***Xylaria* sp. nov.***
Stromata			
Shape	Cylindrical, with rounded fertile apices, smooth stipe	Cylindrical to fusiform, not branched or occasionally branched near the base, with rounded fertile apices, smooth stipes, concolorous to the fertile part	Cylindrical, gregarious, with narrowly rounded fertile apices, smooth stipe, which was 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 angular dark brownish scales	Cracked into minute scales	Rough, finely reticulately cracked into small angular closely spaced scales so as to outline the individual perithecia
Perithecia			
Shape	Immersed, subglobose	Partially immersed, subglobose	Immersed, sometimes vaguely evident in outline, 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 punctiform	Slightly papillate
Apical apparatus	Rectangular, 1.5-2 μm high x 1.5 μm broad	Rectangular, constricted subapically, 4.5-5 μm high x 3.8-5 μm broad	Quadrate to rectangular, 1.5 μm high x 1.5-2 μm broad
Ascospores			
Color	Brown	Dark brown	Light brown to brown to dark brown
Shape	Ellipsoid-equilateral with narrowly rounded ends	Ellipsoid-inequilateral with narrowly rounded to pinched ends	Ellipsoid-equilateral with narrowly rounded ends
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 oriented to the long axis of the spore, less than spore length	Straight full 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, SUT134, SUT171, SUT195, SUT271, and SUT274	SUT206	SUT006, SUT012, SUT027, SUT031, SUT033, SUT034, SUT087, SUT093, SUT131, SUT133, SUT136, SUT141, SUT143, SUT155, SUT172, SUT197, SUT198, SUT272, SUT273, and SUT275

* More details on collections are given in Appendix B.

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 short 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 broad
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

* More details on collections are given in Appendix B.

Table 17. (Continued).

Character	***Xylaria* taxonomic species4 ***	***Biscogniauxia capnodes* (Berk.) Y.-M. 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	Not observed	Not observed	Discoid, 1.5 µm high x 2.5-3 µm
Ascospores			
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 length	Straight germ slit spore-length	Straight germ slit spore-length
Habitat	On wood	On wood	On wood
Location	Trad	Trad	Songkhla
Specimen examined	SUT207	SUT212	SUT290

* More details on collections are given in Appendix B.

Table 17. (Continued).

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		
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

* More details on collections are given in Appendix B.

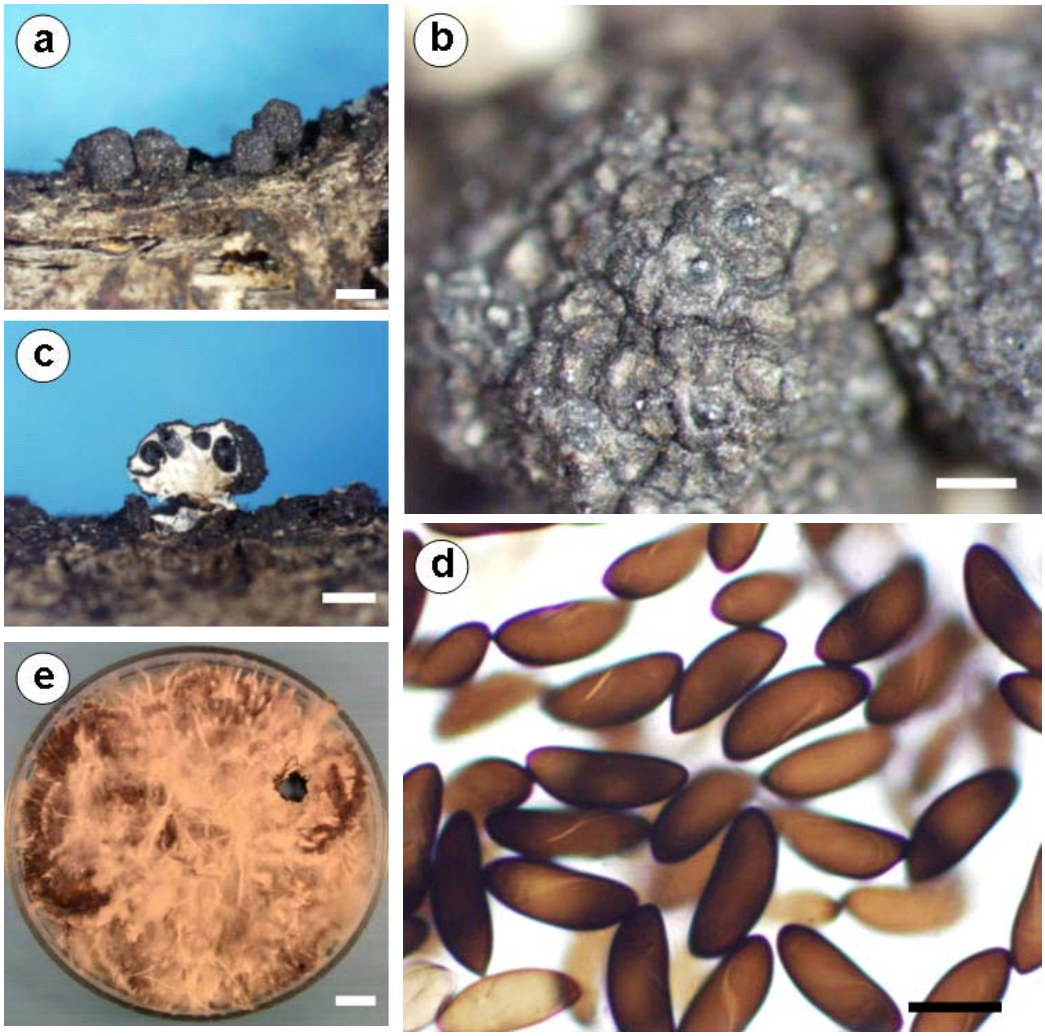

Figure 48. *Xylaria anisopleura* (Mont.) Fr. (SUT205); (a) and (b) stromatal form (Bars = 1 cm and 1 mm respectively), (c) perithecia (Bar = 2 mm), (d) ascospores with germ slits (arrowed) (Bar = 10 µm), and (e) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).

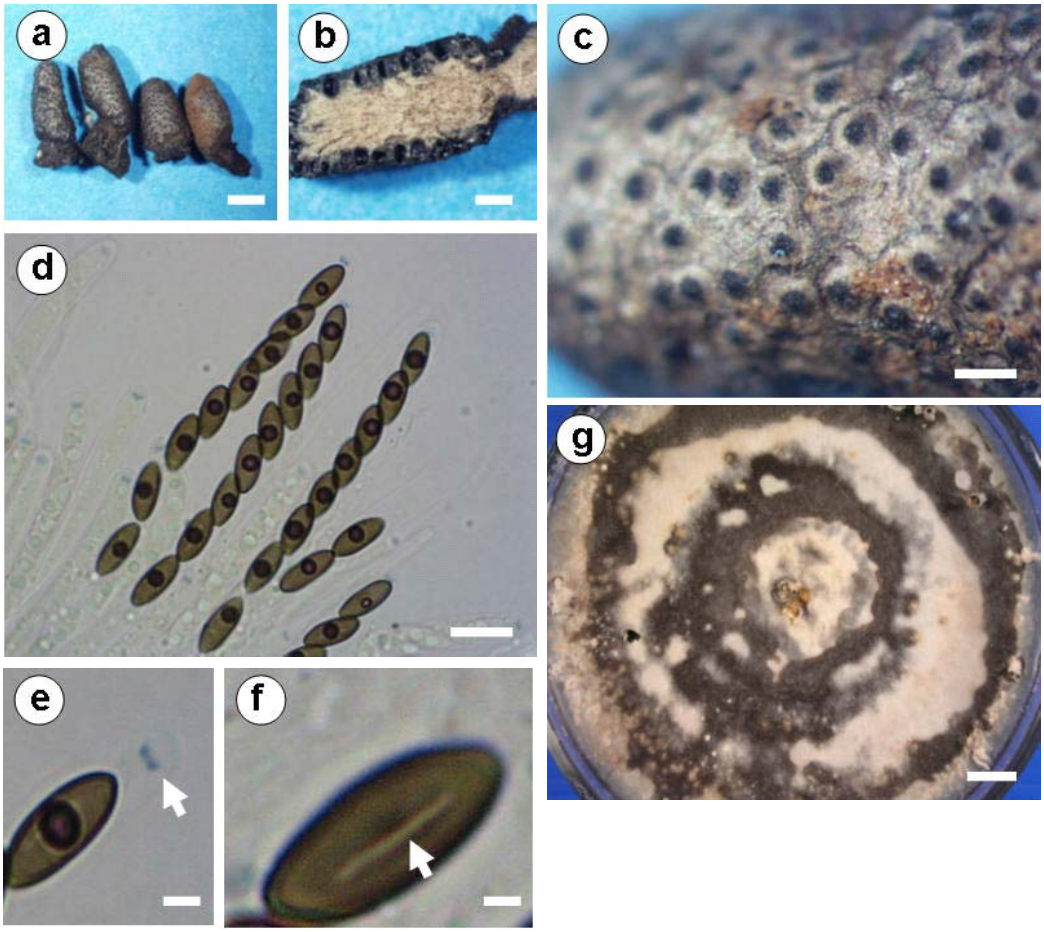

Figure 49. *Xylaria badia* Pat. (SUT076); (a) stromatal form (Bar = 1 cm), (b) perithecia (Bar = 1 mm), (c) stromatal form (Bar = 0.2 mm), (d) ascospores (Bar = 10 µm), (e) apical apparatus (arrowed) (Bar = 1 µm), (f) straight germ slit less than spore length (Bar = 1 µm), and (g) cultural characteristics 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 µm and (11-14(-16) x 5-6 µm) 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 µm) compared to those given by Rogers and Samuels (1986), Rogers *et al*. (1988), and González and Rogers (1989) (8-10.5 x 4-5 µm). 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 µm and 10-11.3 x 5-5.6 µm 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 µm). *Xylaria inthino-velutina* usually occurs on legume fruits.

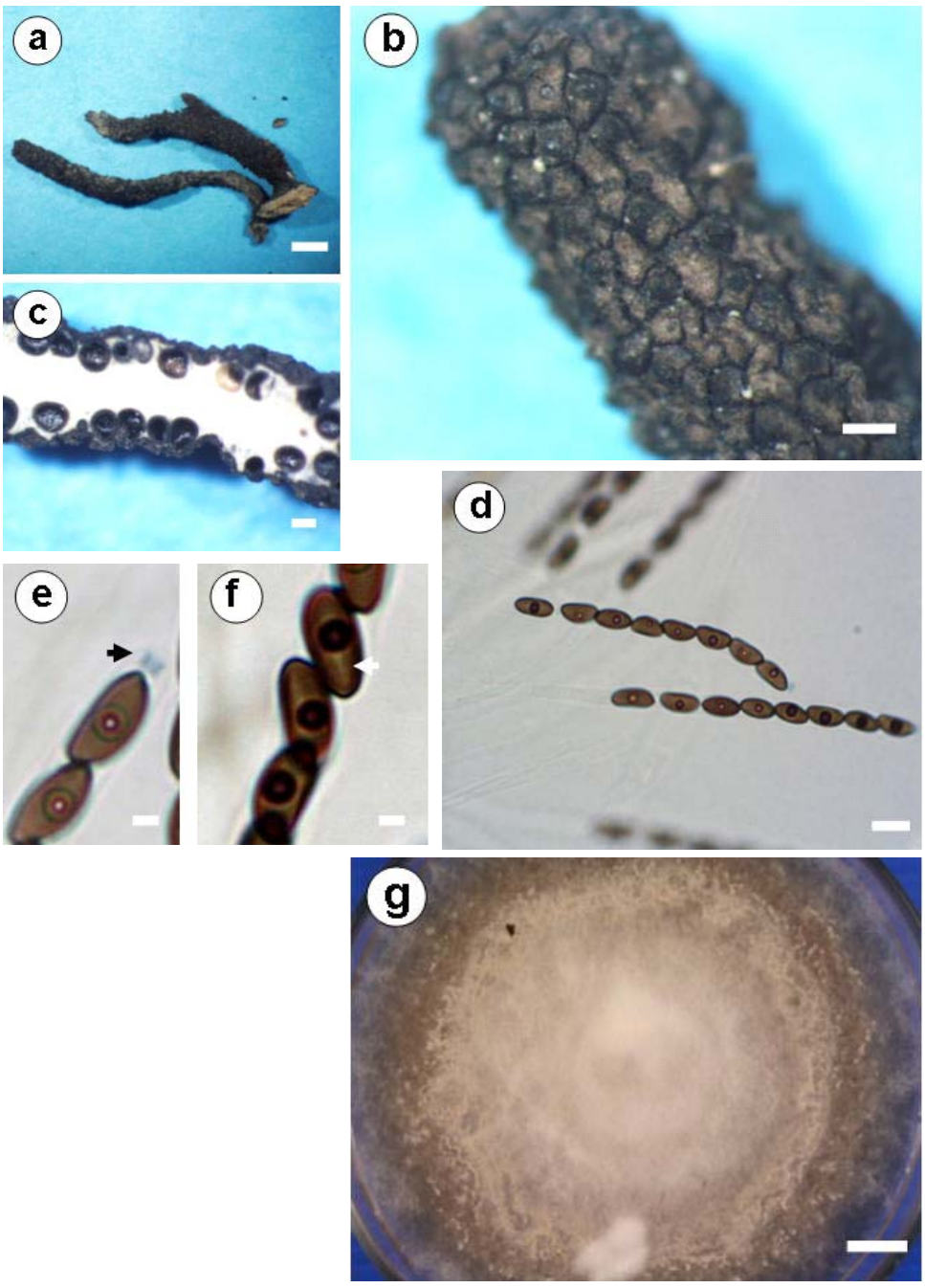

Figure 50. *Xylaria beccari* Lloyd. (SUT092); (a) and (b) stromatal forms (Bars = 1 and 2 mm respectively), (c) perithecia (Bar = 1 mm), (d) ascospores (Bar = 5 µm), (e) apical apparatus (arrowed) (Bar = 1 µm), (f) straight germ slit spore length (Bar = 1 µm), and (g) cultural characteristics on PDA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

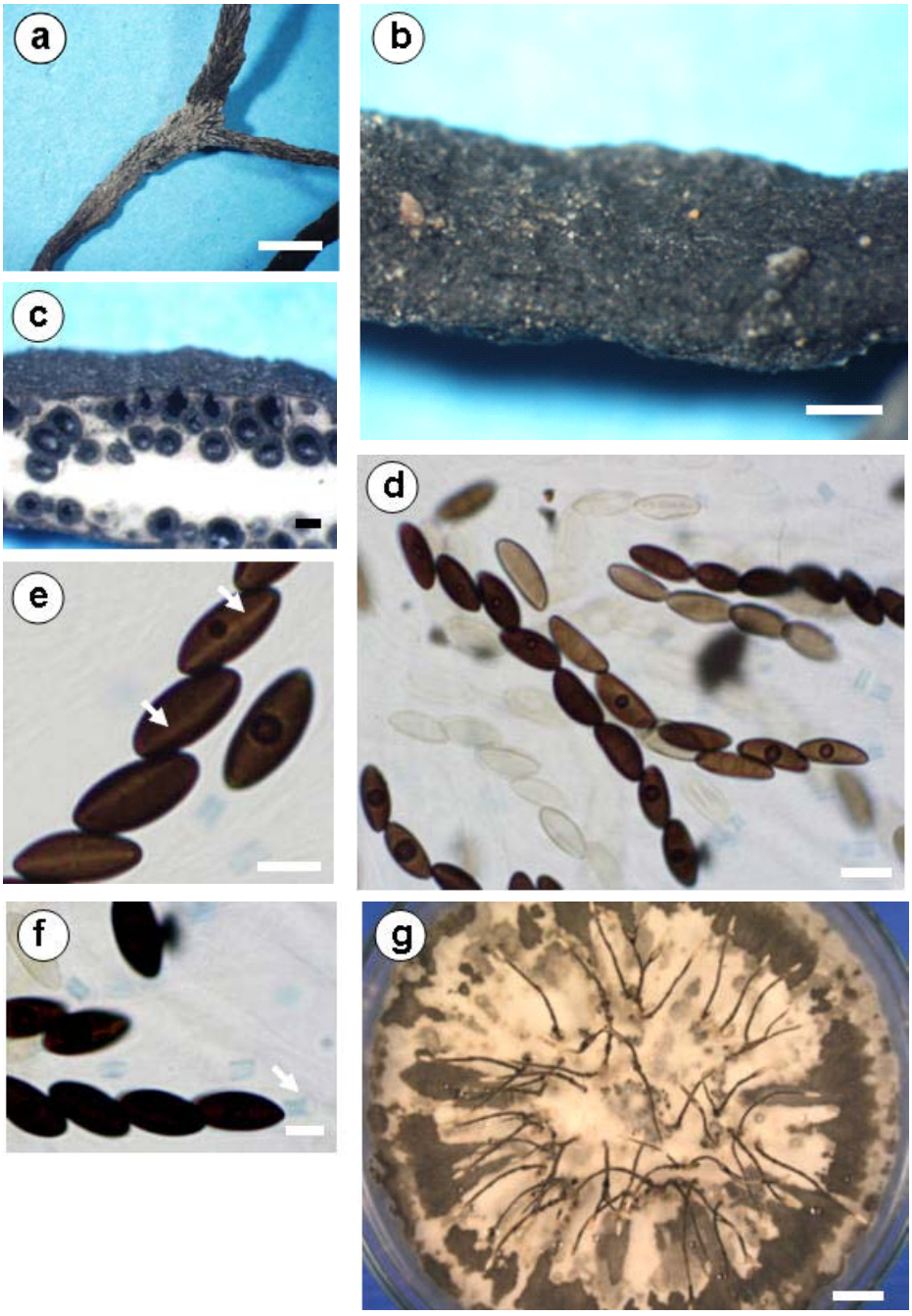

Figure 51. *Xylaria brachiata* Sacc. (SUT078); (a) and (b) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (c) perithecia (Bar = 0.5 mm), (d) ascospores (Bar = 10 µm), (e) straight germ slit spore length (arrowed) (Bar = 5 µm), (f) apical apparatus (arrowed) (Bar = 5 µm), and (g) cultural characteristics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

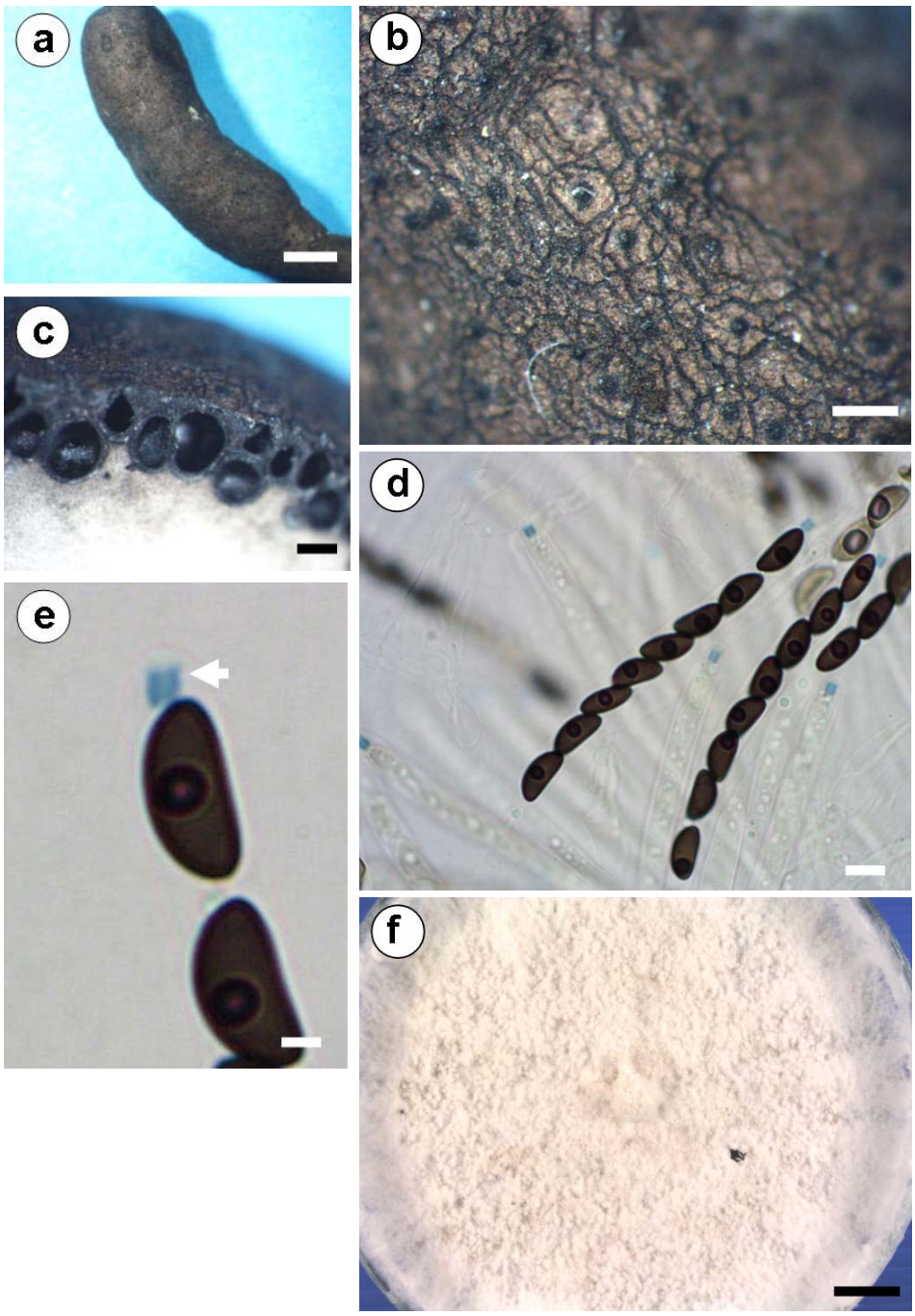

Figure 52. *Xylaria cubensis* (Mont.) Fr. (SUT089); (a) and (b) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (c) perithecia (Bar = 0.5 mm), (d) ascospores (Bar = 5 µm), (e) apical apparatus (arrowed) (Bar = 2 µm), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar =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

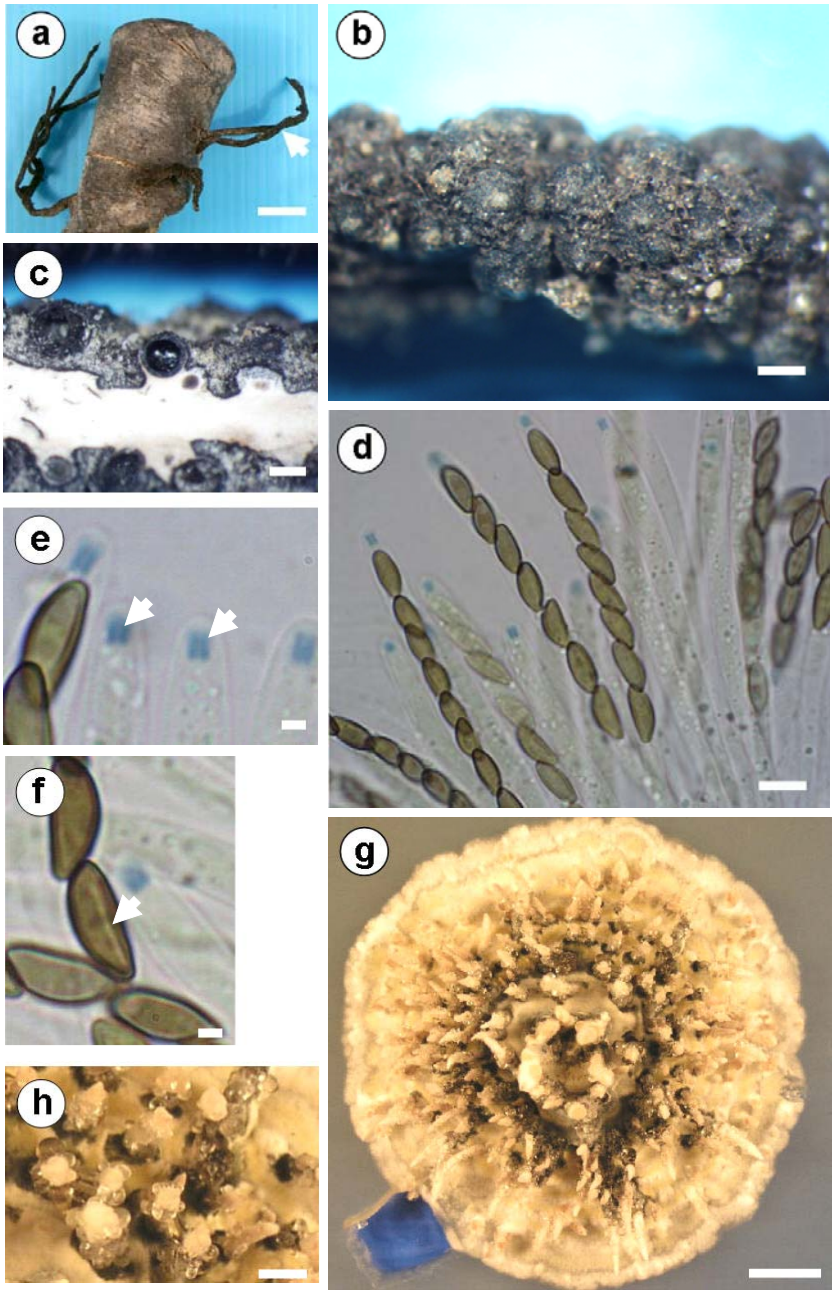

Figure 53. *Xylaria inthino-velutina* (Mont.) Fr. (SUT123); (a) and (b) stromatal forms (Bars = 1 cm and 0.2 mm respectively), (c) perithecia (Bar = 0.2 mm), (d) ascospores (Bar = 10 µm), (e) apical apparatus (arrowed) (Bar = 1 µm), (f) straight germ slit spore length (Bar = 2 µm), (g) cultural characteristics 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 µm), and *X. multiplex* (14.5-17(-18) x 5-5.5(-6.5) µm).

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) x 3.8-5 µm cf. 9-10.5(-11) x 3.5-4 µm (Dennis, 1957; 1961; Thienhirun, 1997; González and Rogers, 1989).

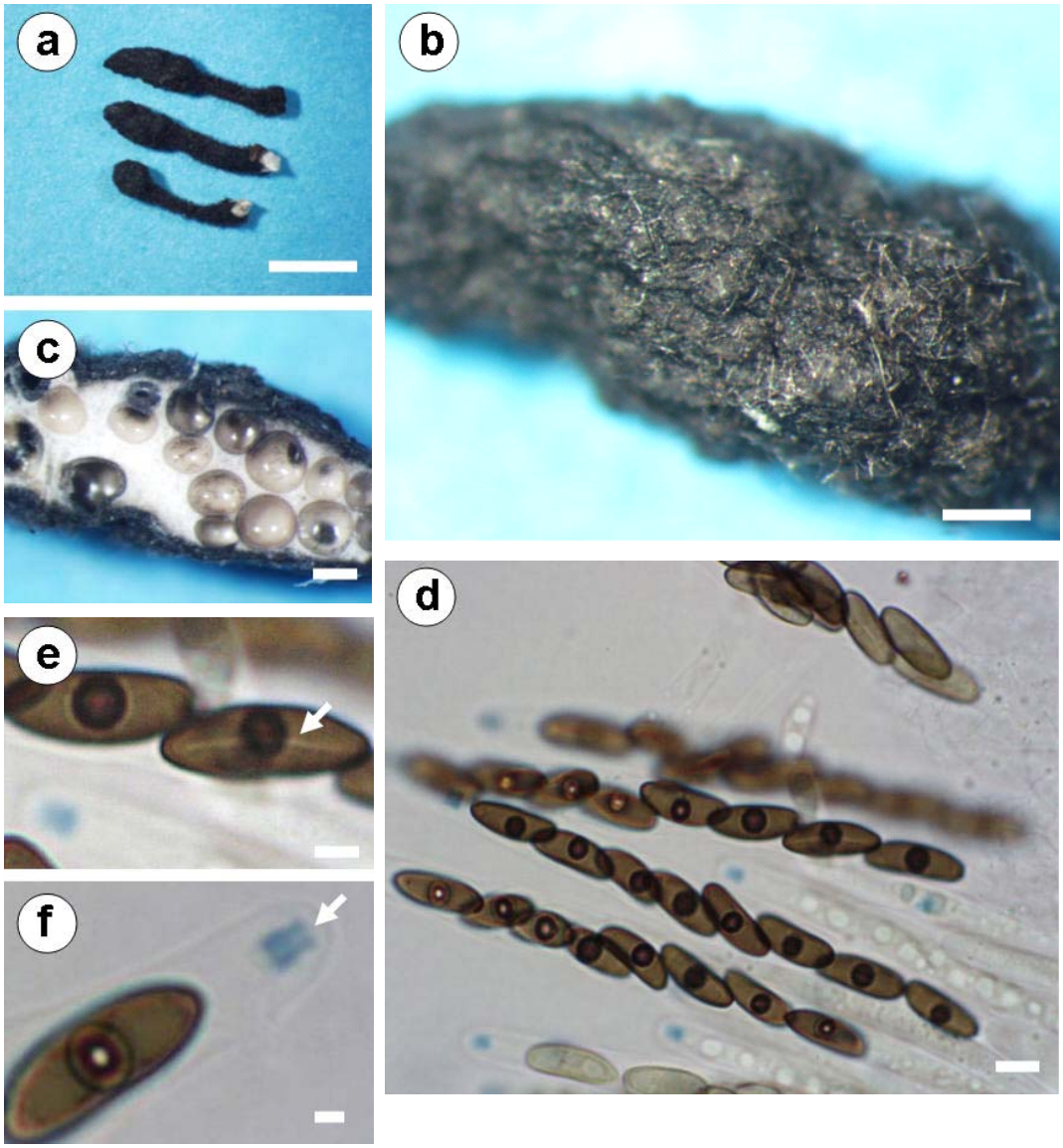

Figure 54. *Xylaria* cf. *juruensis* (SUT035); (a) and (b) stromatal forms (Bars = 0.5 cm and 0.5 mm respectively), (c) perithecia (Bar = 0.5 mm), (d) ascospores (Bar = 5 µm), (e) straight germ slit spore length (Bar = 2 µm), and (f) apical apparatus (arrowed) (Bar = 2 µm).

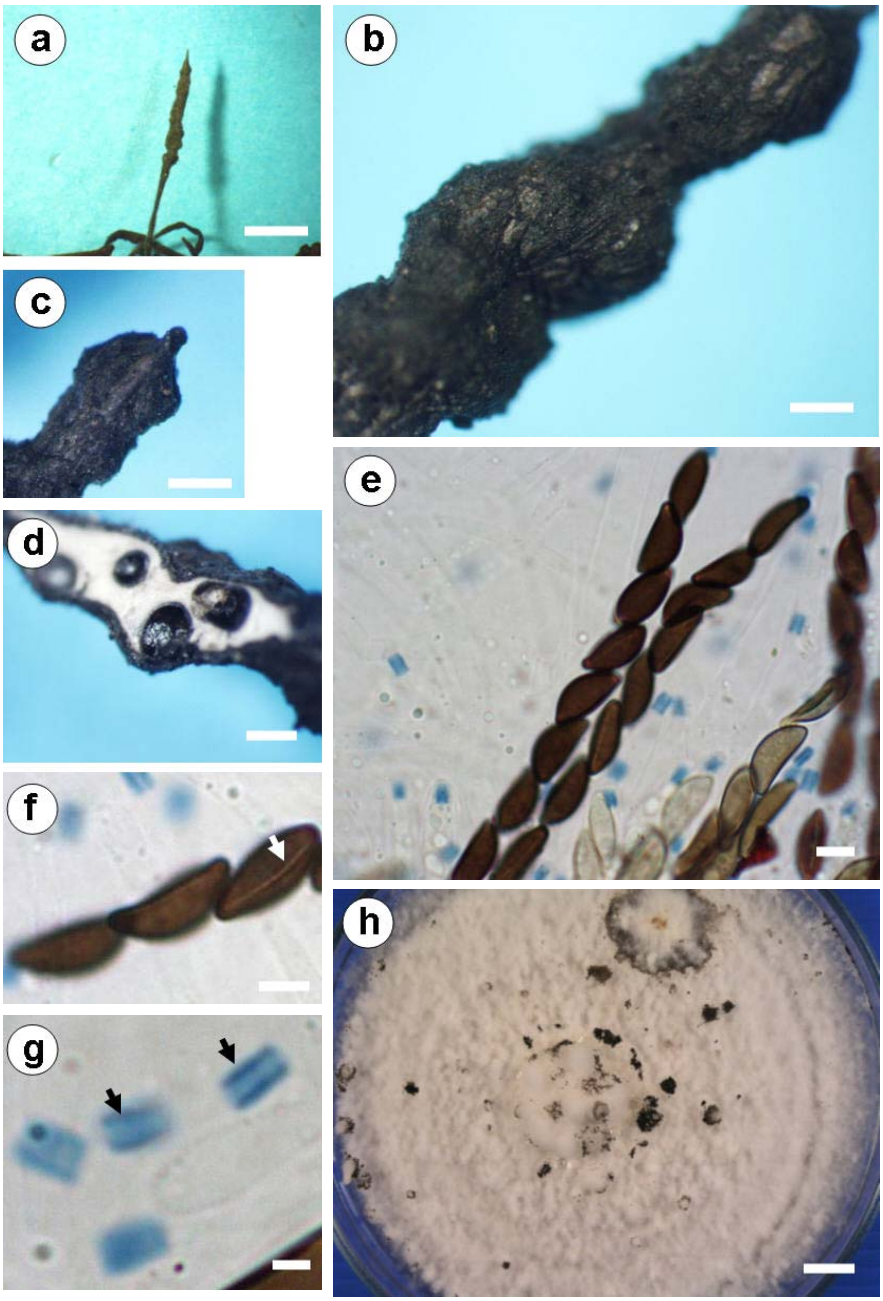

Figure 55. *Xylaria* cf. *juruensis* (SUT088); (a) and (b) stromatal forms (Bars = 5 and 1 mm respectively), (c) acute apex (Bar = 1 mm), (d) perithecia (Bar = 0.5 mm), (e) ascospores (Bar = 5 µm), (f) straight germ slit spore length (Bar = 5 µm), (g) apical apparatus (arrowed) (Bar = 1 µm), and (h)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

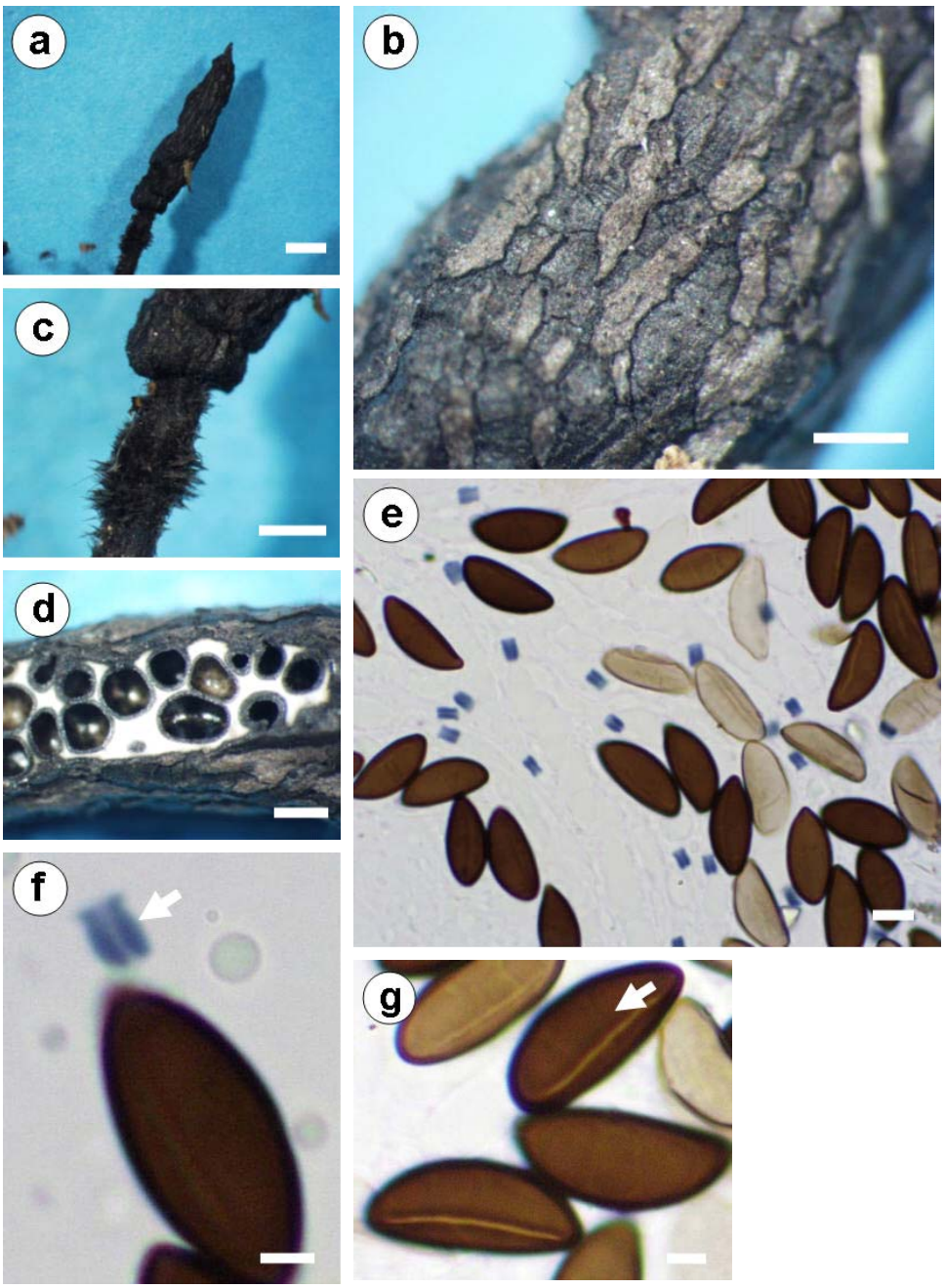

Figure 56. *Xylaria* cf. *juruensis* (SUT140); (a) and (b) stromatal forms (Bars = 1 and 0.1 cm respectively), (c) stalk (Bar = 1 mm), (d) perithecia (Bar = 0.5 mm), (e) ascospores (Bar = 5 µm), (f) apical apparatus (arrowed) (Bar = 1 µm), and (g) straight germ slit spore length (Bar = 5 µm).

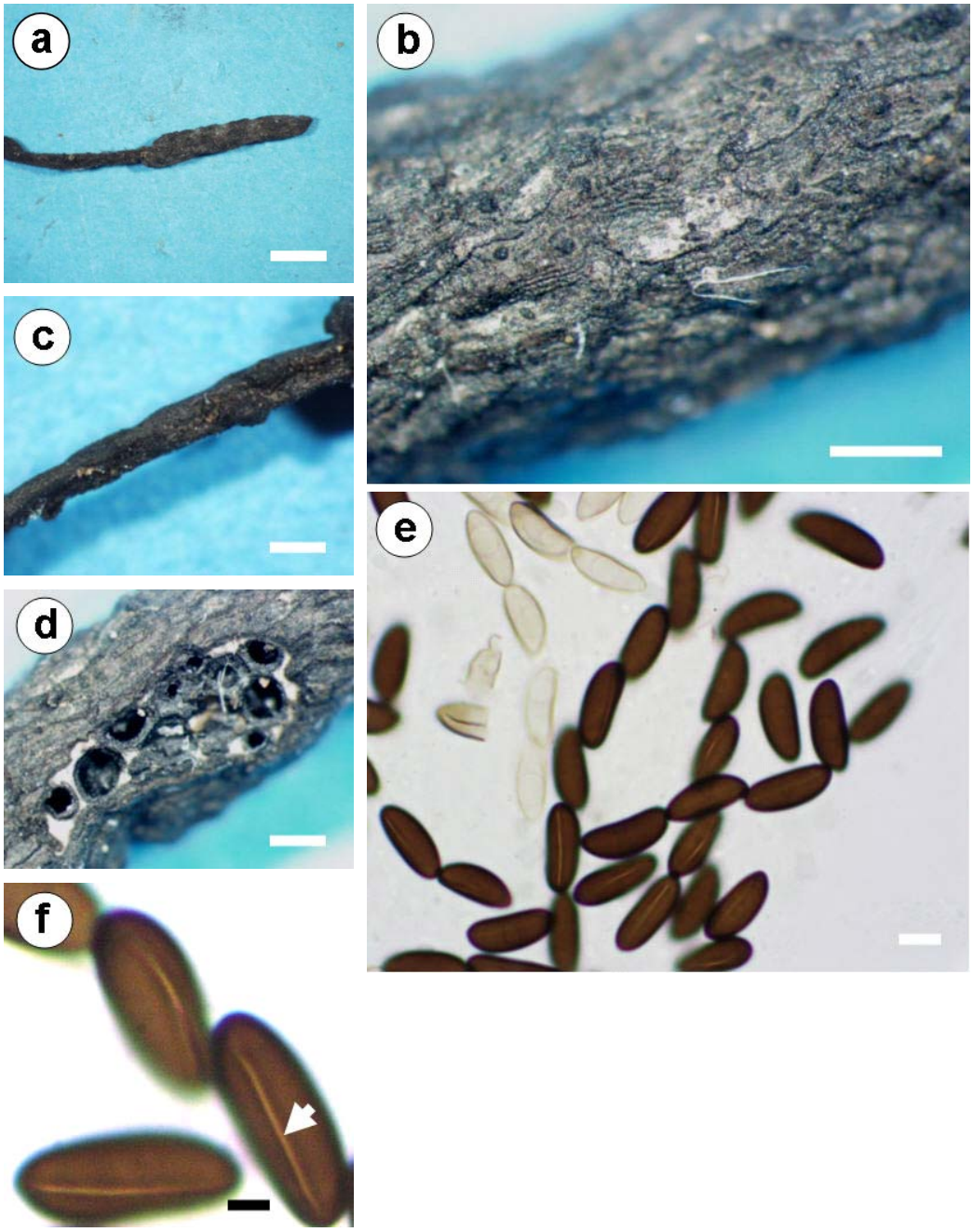

Figure 57. *Xylaria* cf. *juruensis* (SUT170); (a) and (b) stromatal forms (Bars = 1 cm and 1 mm respectively), (c) stalk (Bar = 1 mm), (d) perithecia (Bar = 0.5 mm), (e) ascospores (Bar = 5 µm), and (f) apical apparatus (arrowed) (Bar = 1 µm).

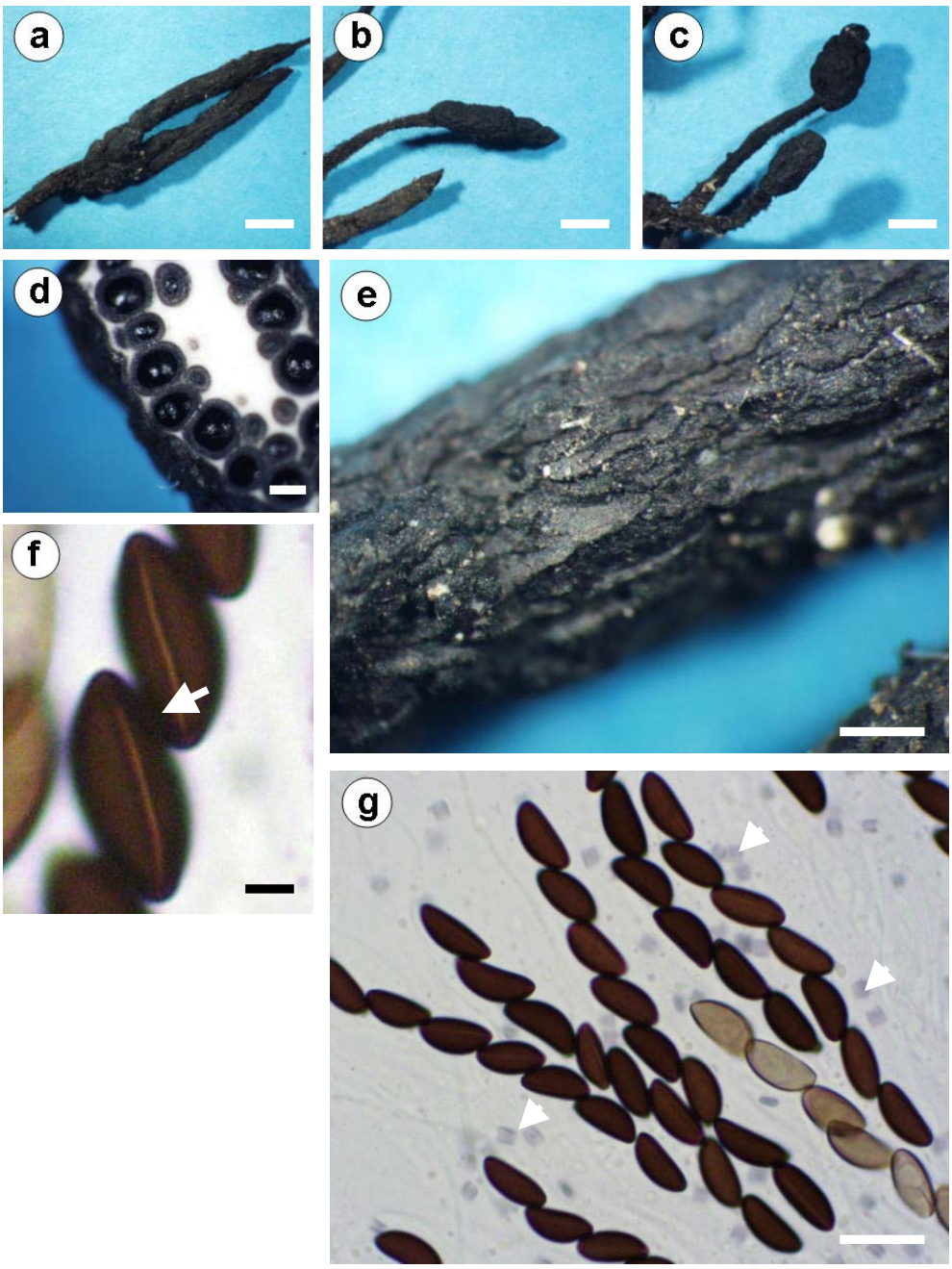

Figure 58. *Xylaria juruensis* var. *microspora* (Thienhirun, 1997); (a), (b), (c), and (e) stromatal forms of SUT129, SUT138, SUT139, and SUT129 respectively (Bars = 1, 1, 1, and 0.01 cm), d) perithecia (Bar = 0.5 mm), (f) straight germ slit spore length (Bar = 2 µm), and (g) ascospores with apical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

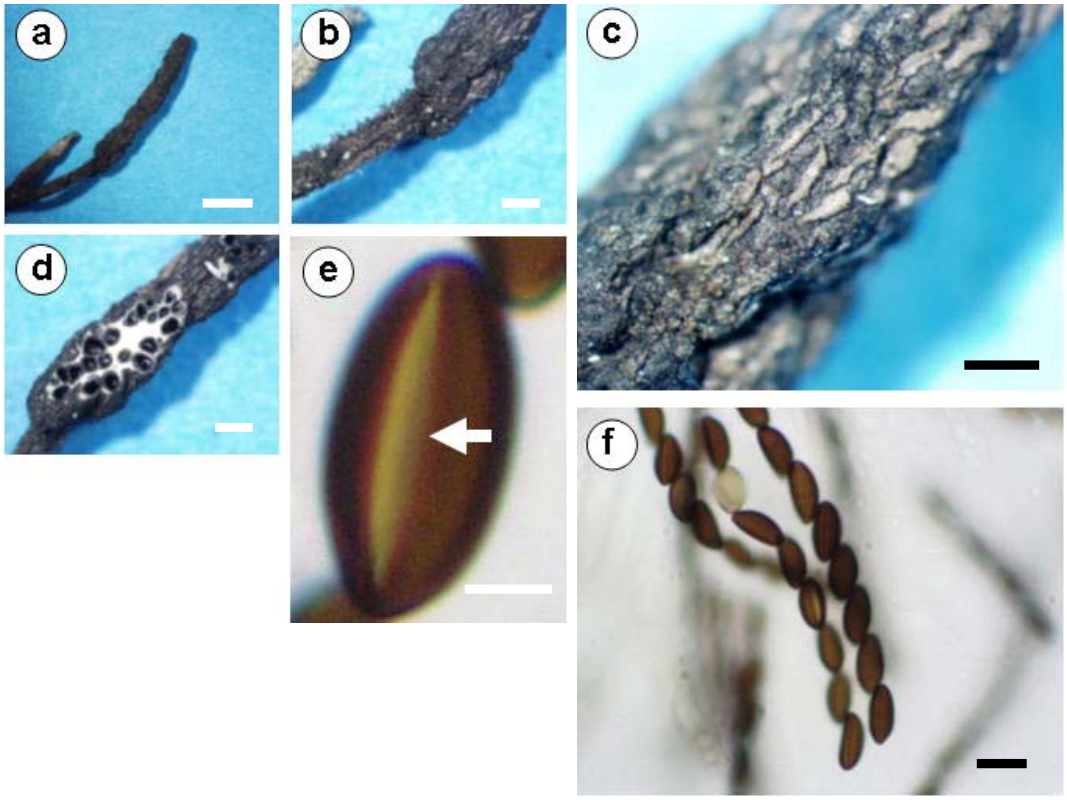

Figure 59. *Xylaria maitlandii* (Dennis) D. Hawksw (SUT177); (a), (b) and (c) stromatal forms (Bars = 0.5 cm, 2 mm, and 0.2 mm respectively), (d) perithecia (Bar = 0.5 mm), (e) straight germ slit spore length (Bar = 2 µm), and (f) ascospores (Bar = 10 µm).

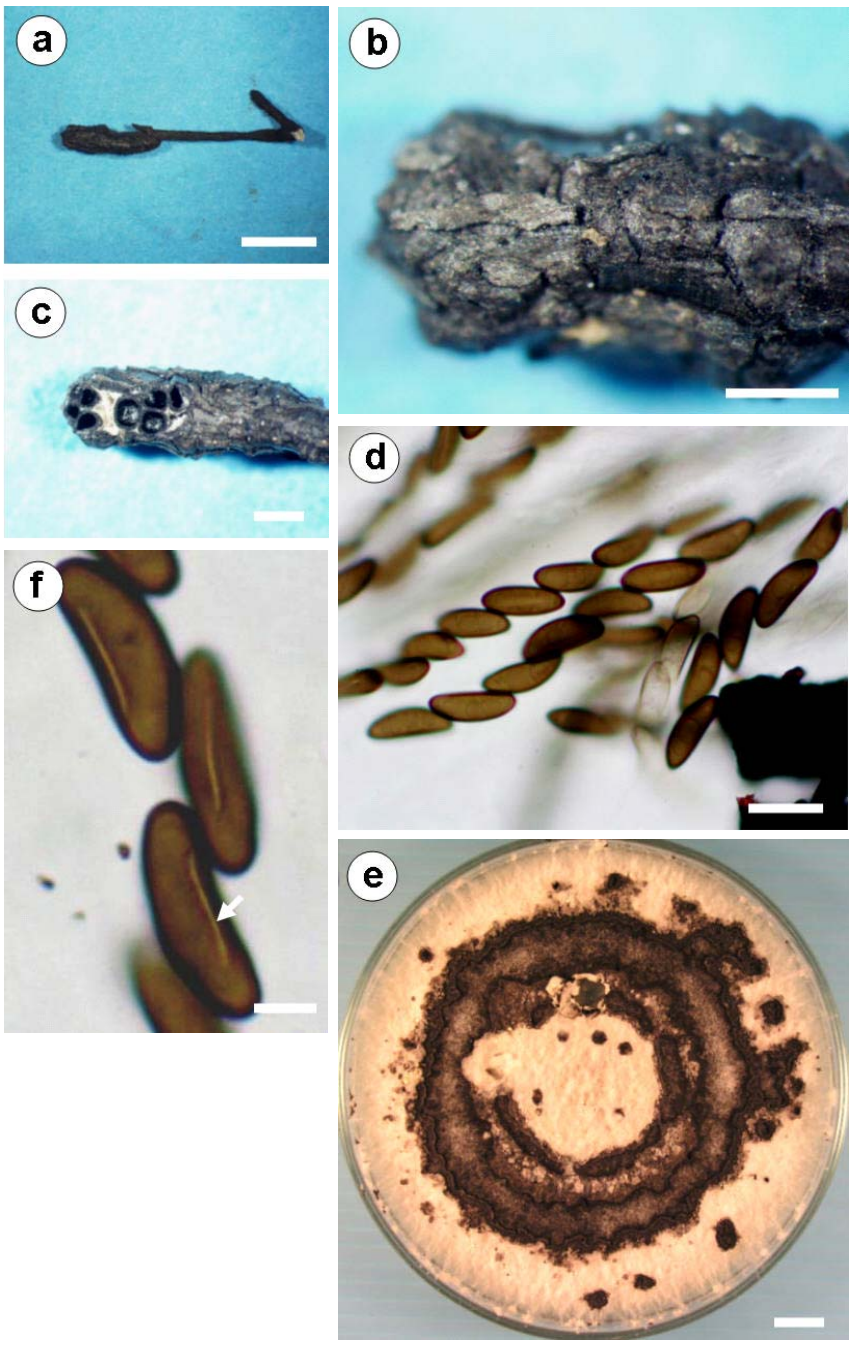

Figure 60. *Xylaria mellisii* (Berk.) Cooke. (SUT192); (a) and (c) stromatal forms (Bars = 0.2 cm and 0.4 mm respectively), (b) perithecia (Bar = 0.5 mm), (d) ascospores (Bar = 15 µm), (e) straight germ slit spore length (Bar = 5 µm), and (f) cultural characteristics on PDA cultured at 25°C after 4 weeks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

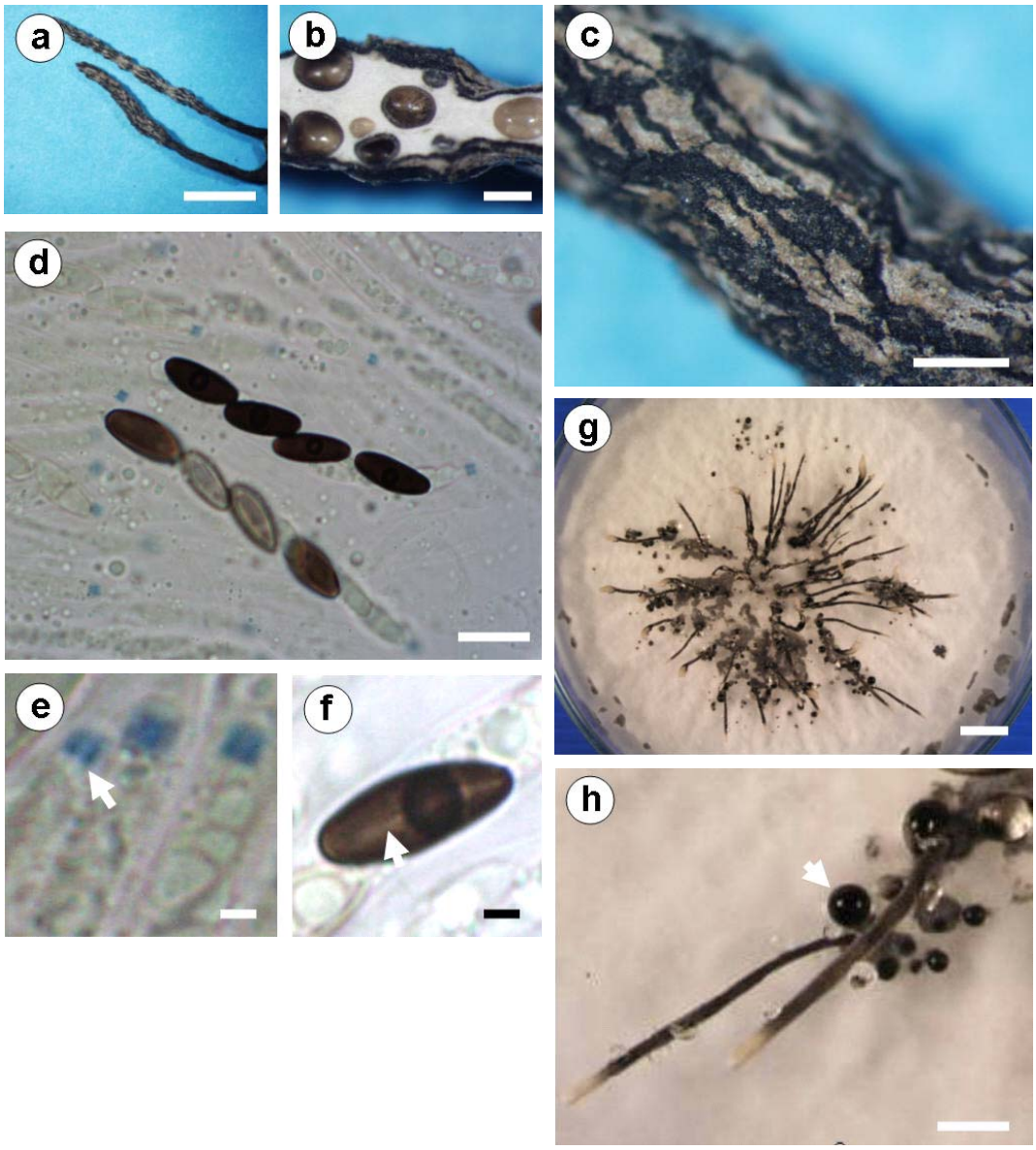

Figure 61. *Xylaria* cf. *multiplex* (SUT028); (a) and (c) stromatal forms (Bars = 0.5 cm and 0.5 mm respectively), (b) perithecia (Bar = 0.5 mm), (d) ascospores (Bar = 10 µm), (e) apical apparatus (arrowed) (Bar = 1 µm), (f) straight germ slit spore length (arrowed) (Bar = 2 µm), (g) cultural characteristics 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 μm cf. 18.8-21.3 x 6.3-7.5 μm, 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 μm high x 1.5-2 μm 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 μm; 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).

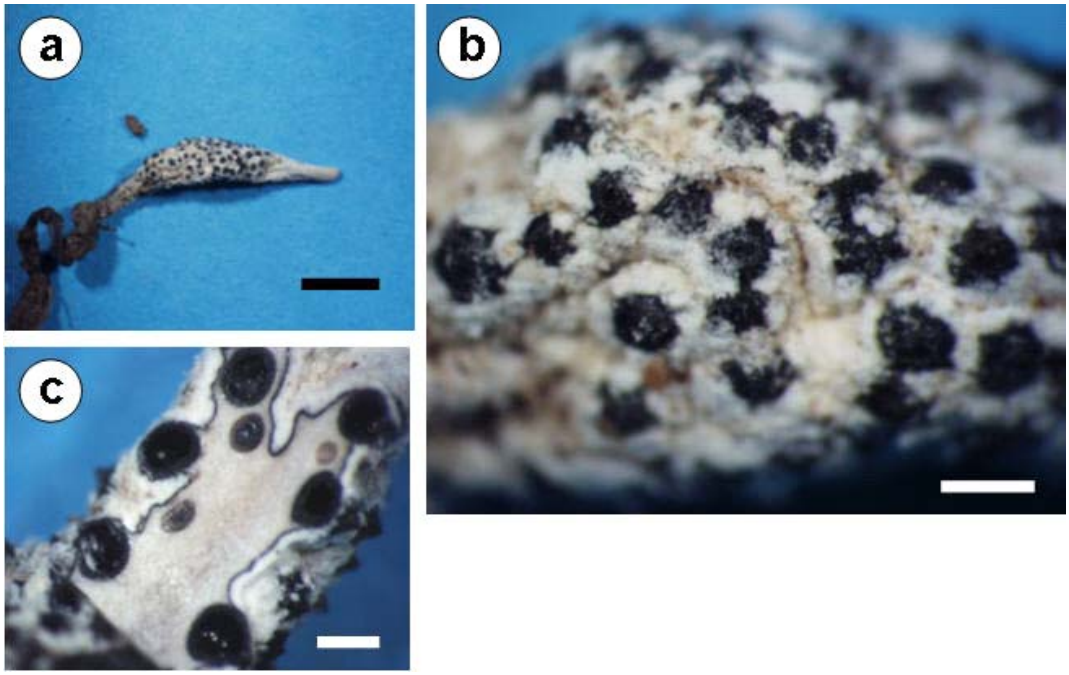

Figure 62. *Xylaria muscula* Lloyd. (SUT029); (a) and (b) stromatal forms (Bars = 0.5 cm and 0.2 mm respectively), and (c) perithecia (Bar = 0.2 mm).

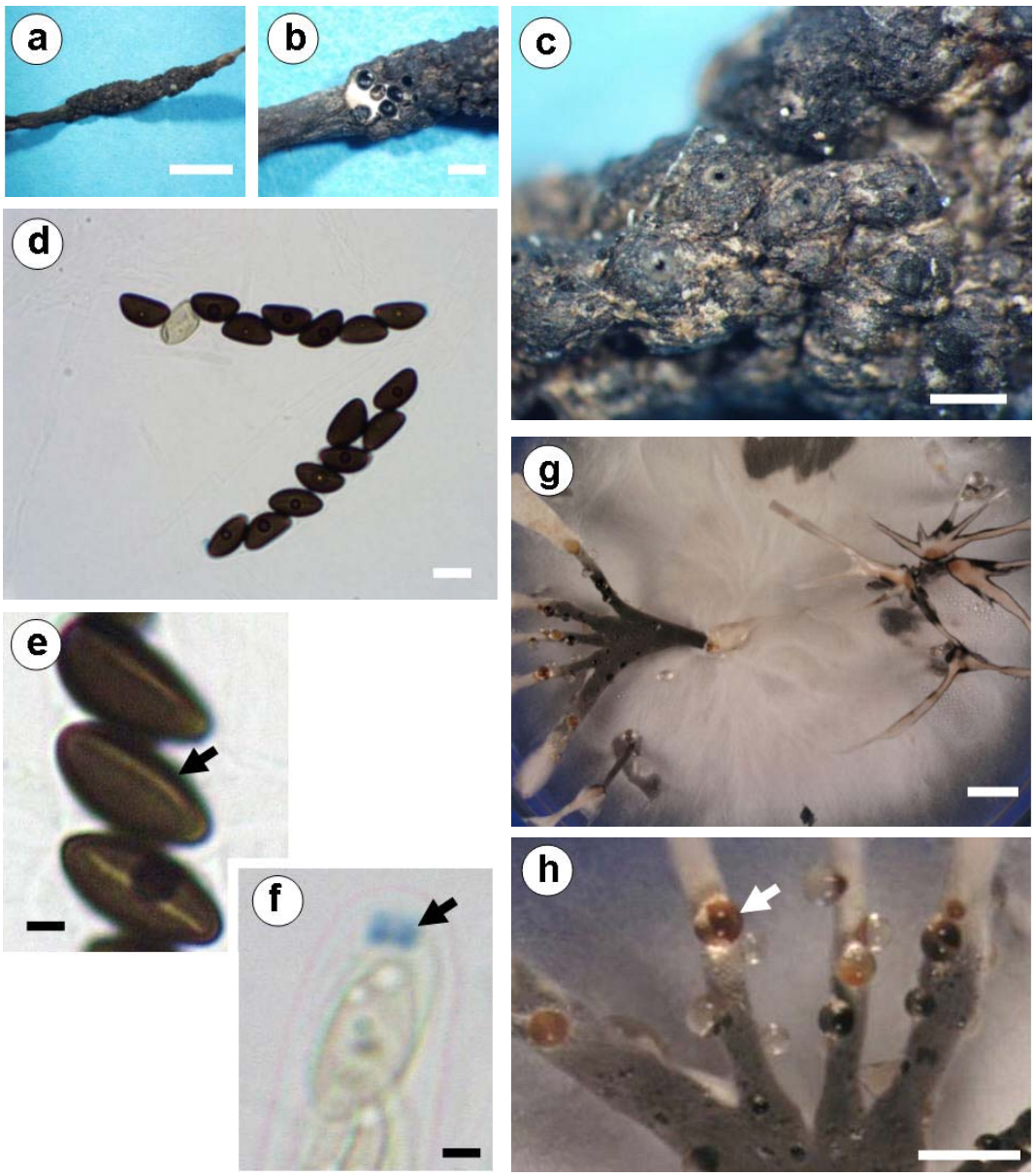

Figure 63. *Xylaria psidii* J.D. Rogers & Hemmes. (SUT125); (a), (b) and (c) stromatal forms (Bars = 0.5, 0.1, and 0.03 cm respectively), (d) ascospores (Bar = 5 µm), (e) straight germ slit spore length (arrowed) (Bar = 2 µm), (f) apical apparatus (arrowed) (Bar = 2µm), (g) cultural characteristics on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

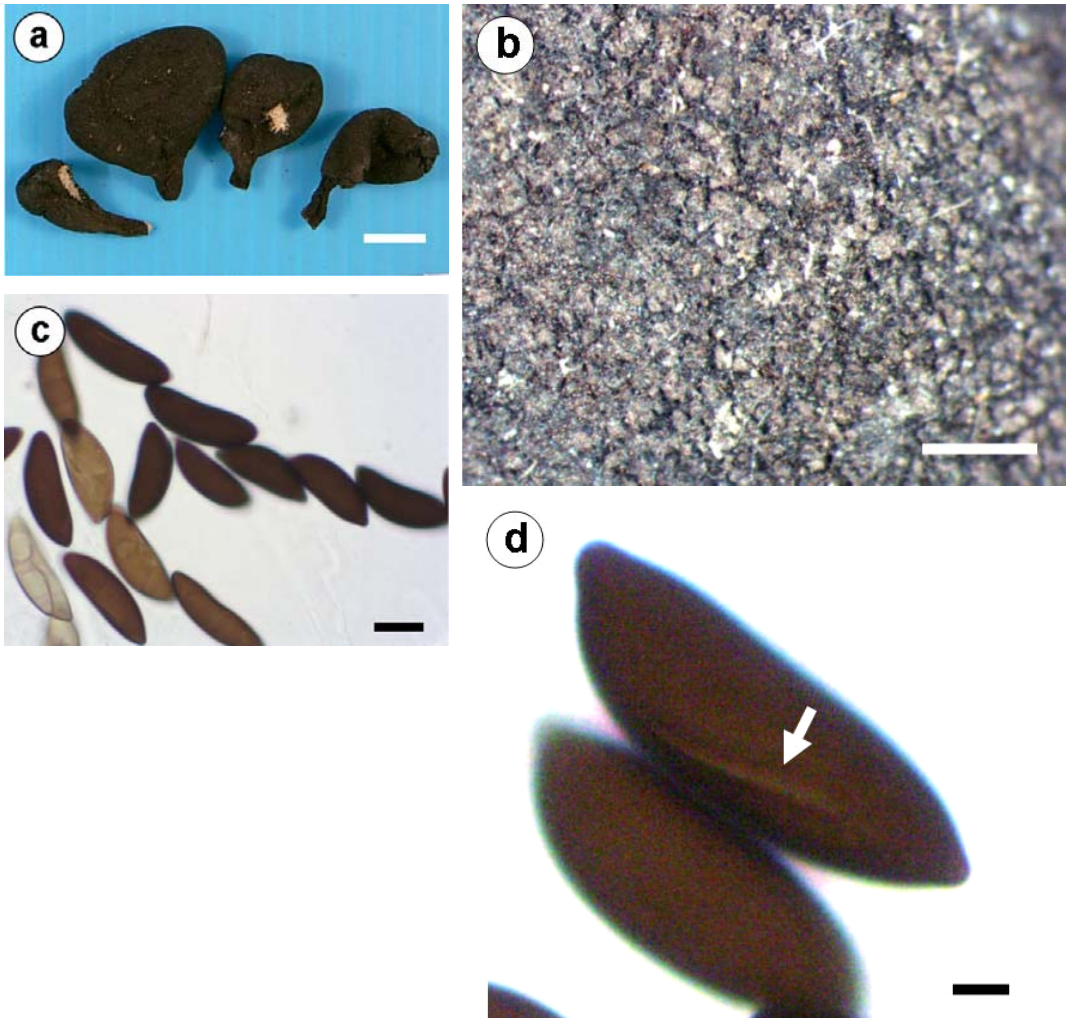

Figure 64. *Xylaria schweinitzii* Berk. & M.A. Curtis. (SUT201); (a) and (b) stromatal forms (Bars = 1 cm and 3 mm respectively), (c) ascospores (Bar = 10 µm), and (d) straight to slightly spiraling germ slit (arrowed) (Bar = 2 µm).

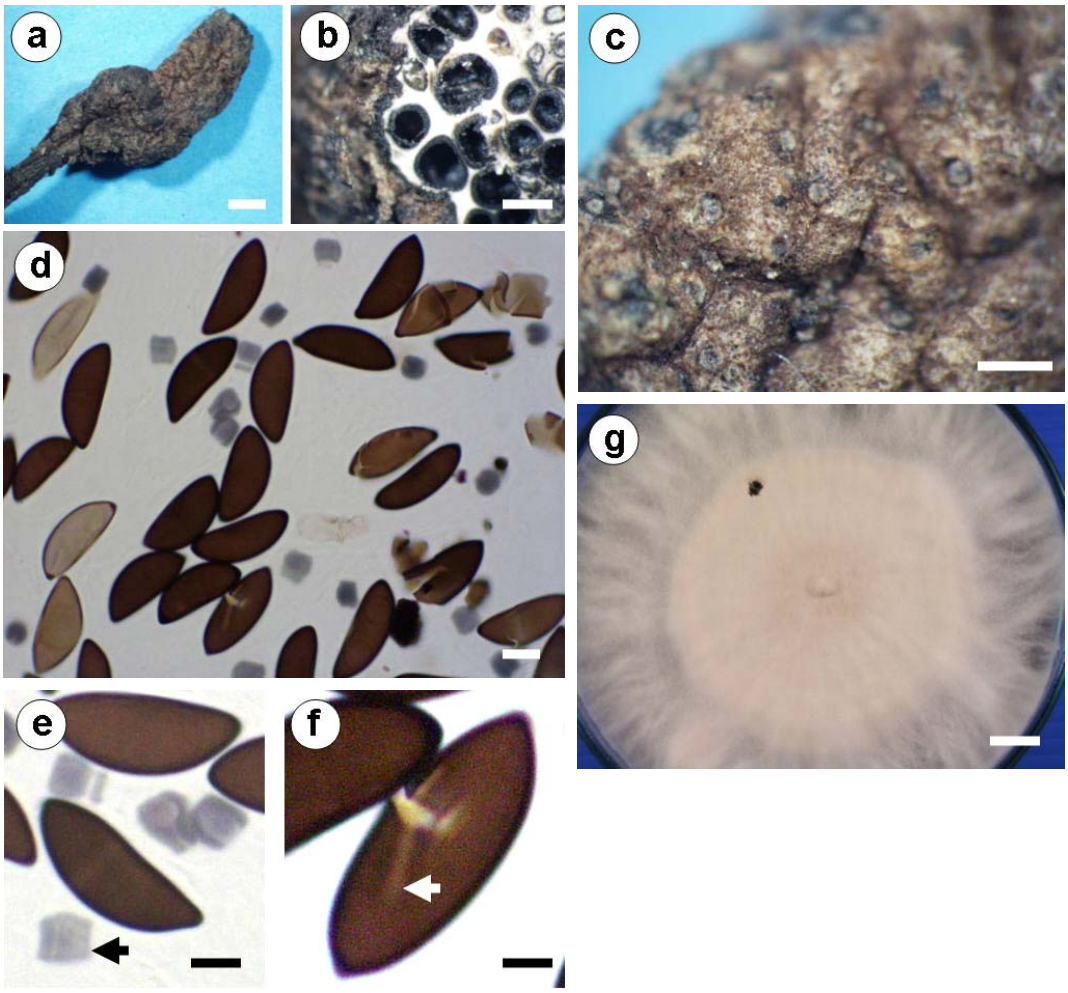

Figure 65. *Xylaria scruposa* (Fr.) Fr. (SUT117); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (b) perithecia (Bar = 0.5 µm), (d) ascospores (Bar = 5 µm), (e) apical apparatus (arrowed) (Bar = 2µm), (f) straight to slightly sigmoid germ slit slightly obliquely oriented to the long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

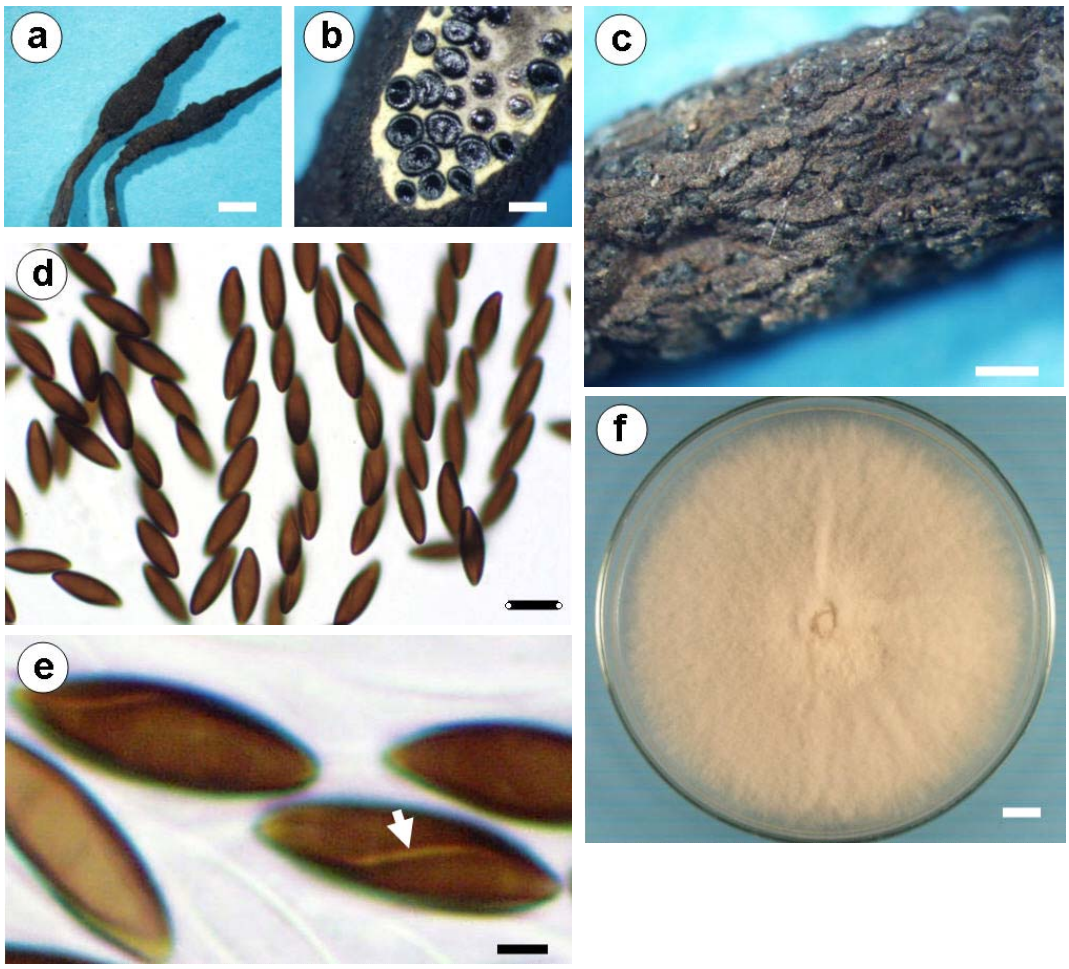

Figure 66. *Xylaria* species 2 (SUT155); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (b) perithecia (Bar = 0.5 µm), (d) ascospores (Bar = 10 µm), (e) slightly sigmoid germ slit (arrowed) (Bar = 1 µm), and (f) cultural characteristics on PDA cultured at 25°C for 2 weeks (Bar = 1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

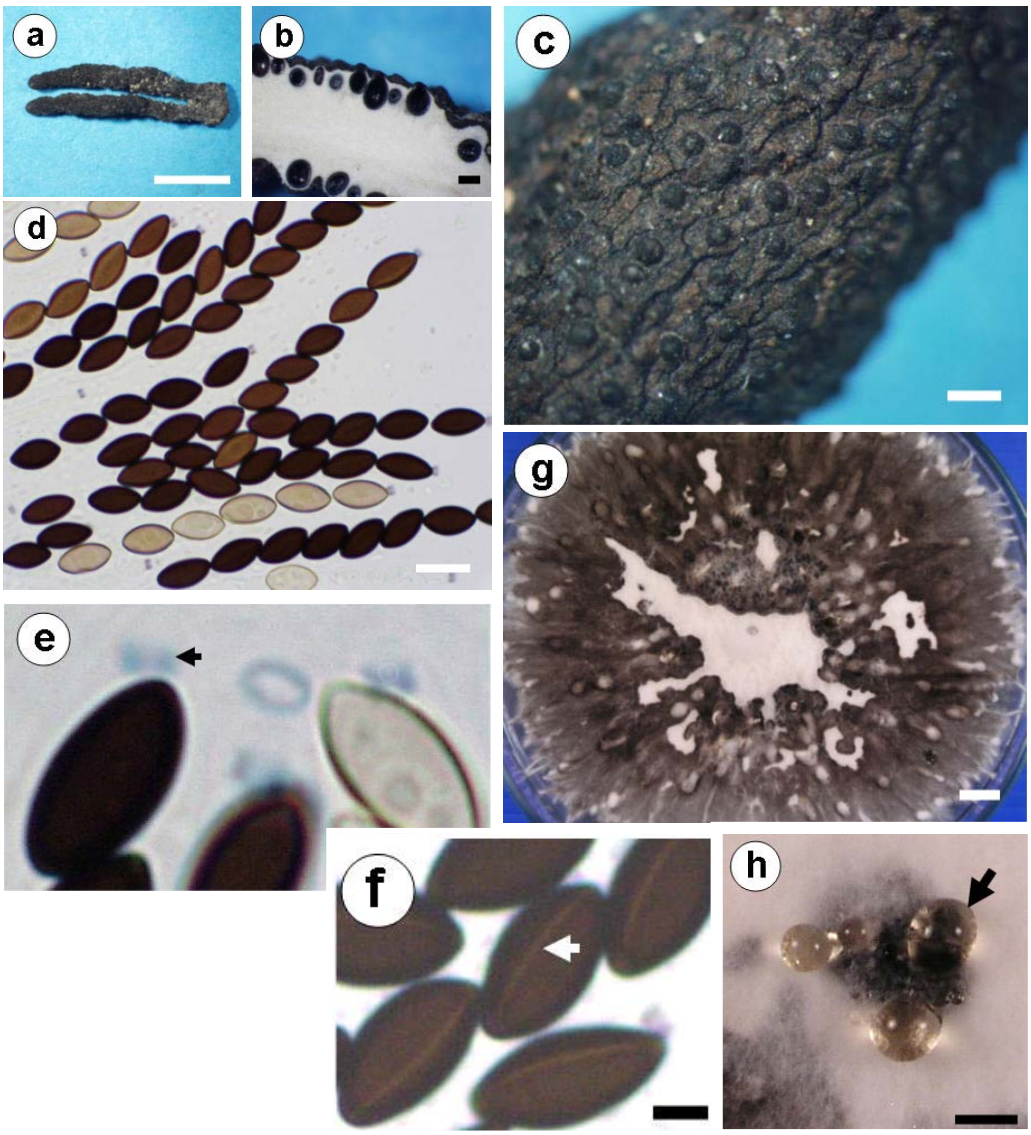

Figure 67. *Xylaria* sp. nov.(SUT195); (a) and (c) stromatal forms (Bars = 0.5 cm and 0.3 mm respectively), (b) perithecia (Bar = 0.5 µm), (d) ascospores (Bar = 10 µm), (e) apical apparatus (Bar = 1 µm), (f) straight germ slit spore length (arrowed) (Bar = 2 µm), (g) cultural characteristics on PDA cultured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

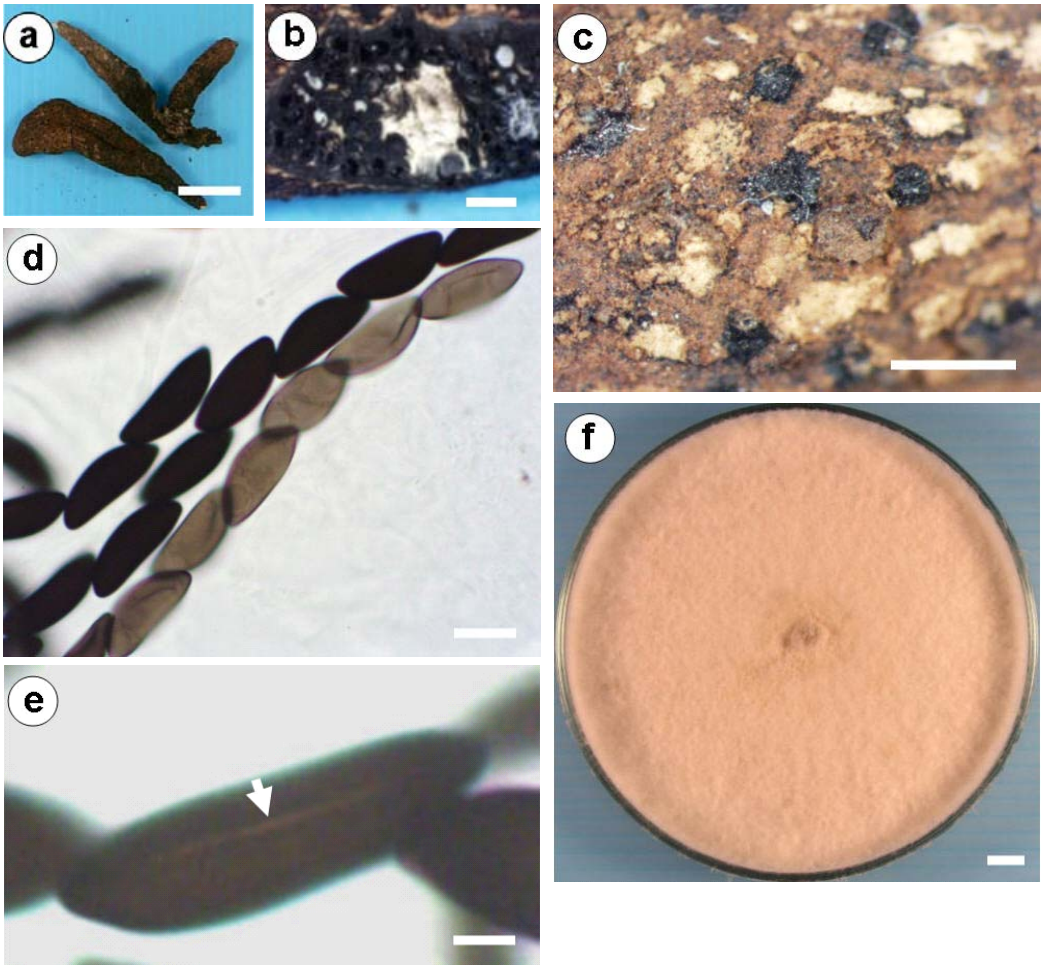

Figure 68. *Xylaria telfairii* (Berk.) Fr. (SUT206); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (b) perithecia (Bar = 0.5 µm), (d) ascospores (Bar = 10 µm), (e) straight germ slit (arrowed) (Bar = 1 µm), and (f) cultural characteristics on PDA cultured at 25°C after 2 weeks (Bar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

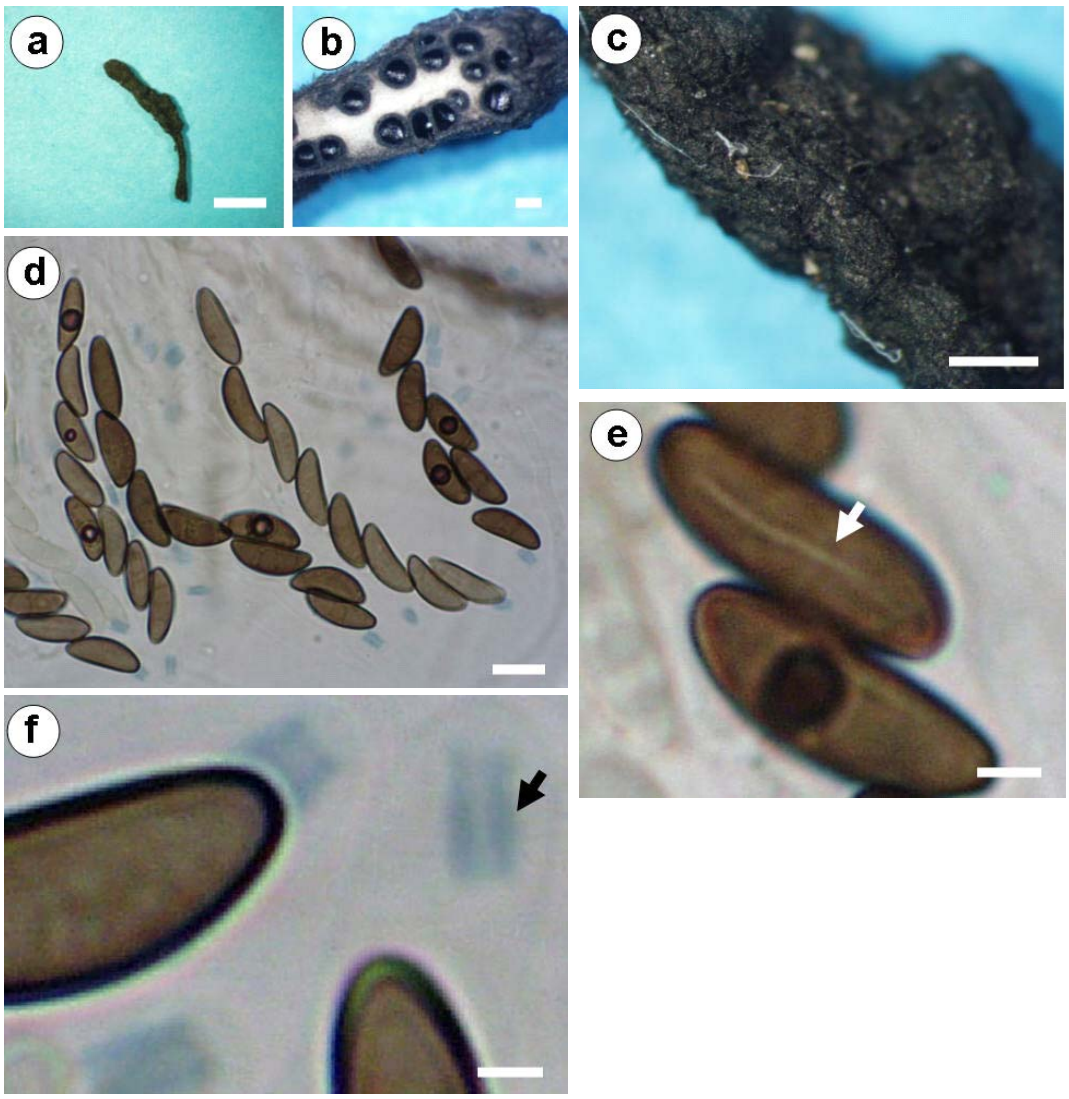

Figure 69. *Xylaria* taxonomic species 1 (SUT075); (a) and (c) stromatal forms (Bars = 0.5 cm and 0.5 mm respectively), (b) perithecia (Bar = 0.3 µm), (d) ascospores (Bar = 12 µm), (e) straight to slightly sigmoid germ slit less than spore length (arrowed) (Bar = 2 µm), and (f) apical apparatus (Bar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

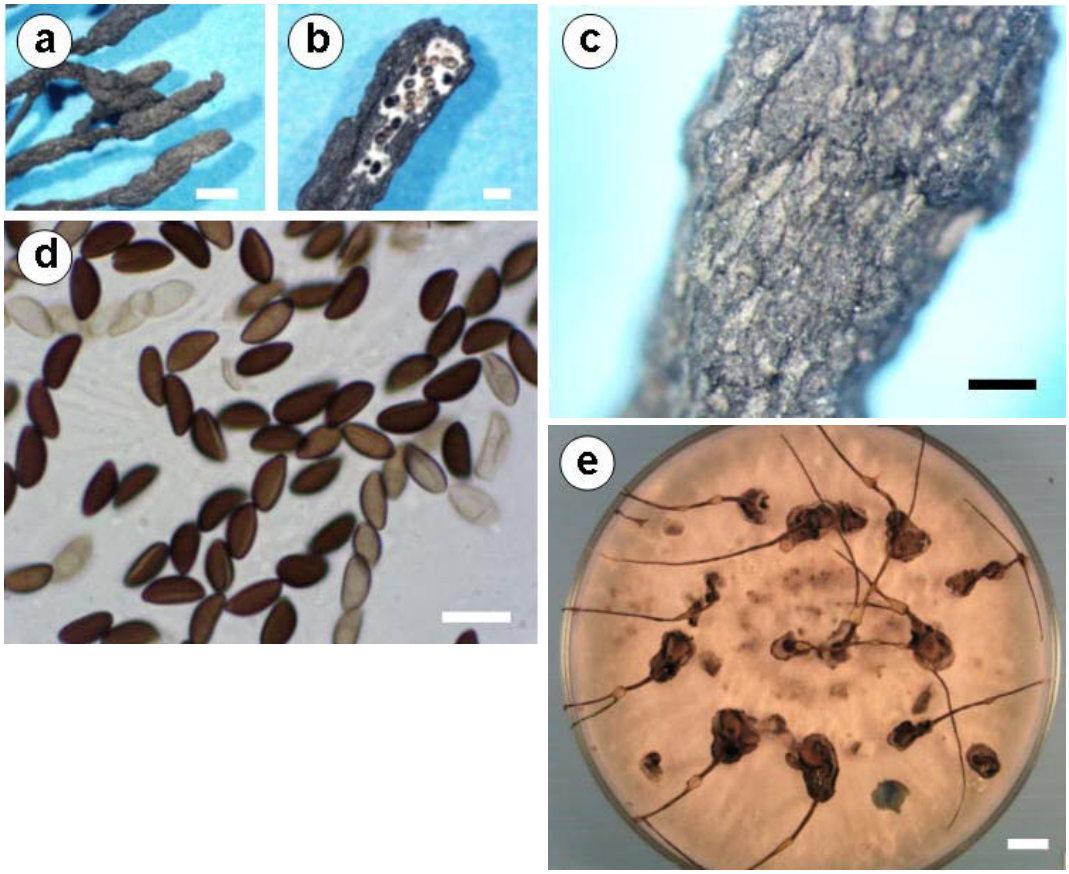

Figure 70. *Xylaria* taxonomic species 2 (SUT203); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (b) perithecia (Bar = 0.5 µm), (d) ascospores (Bar = 10 µm), and (e) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).

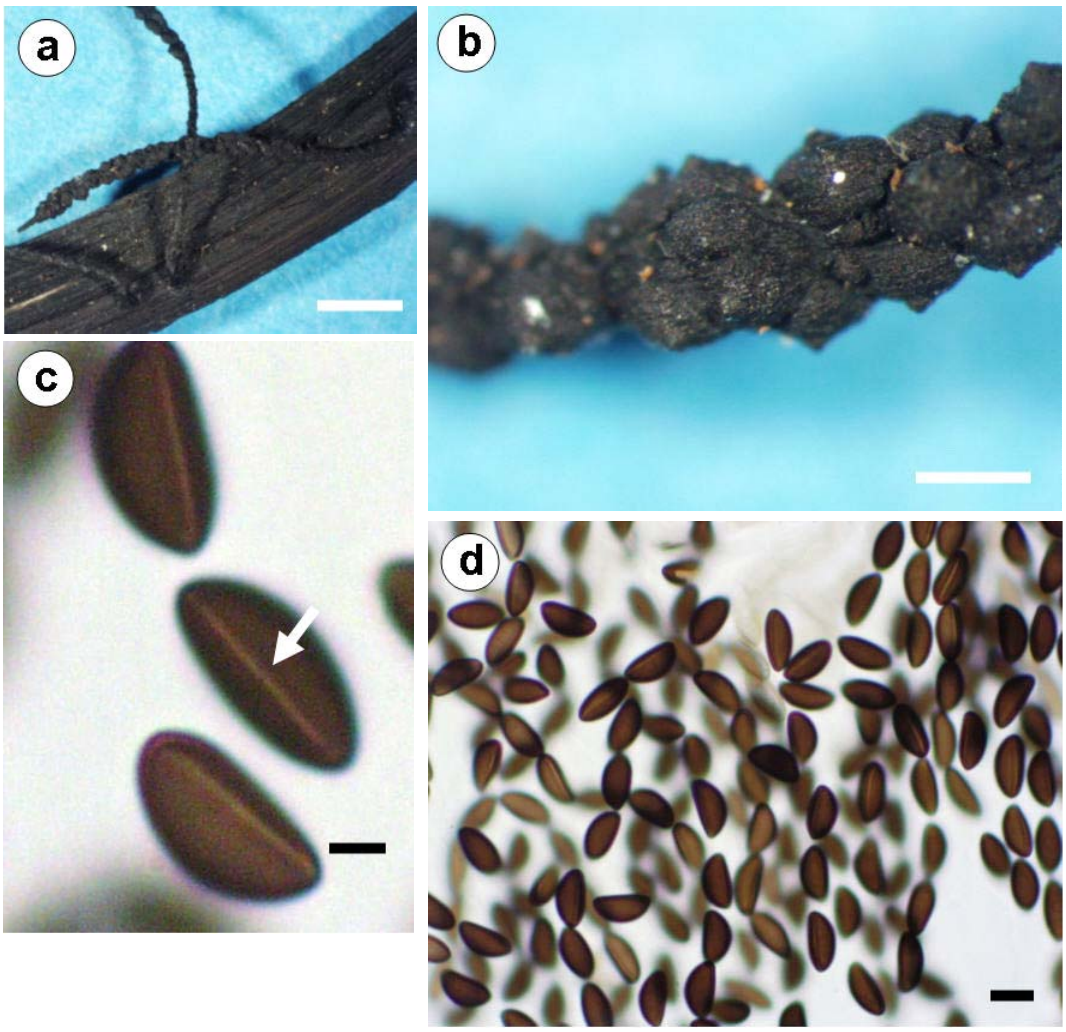

Figure 71. *Xylaria* taxonomic species 3 (SUT204); (a) and (b) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (c) straight germ slit spore length (arrowed) (Bar = 2 µm), and (d) ascospores (Bar = 8 µm).

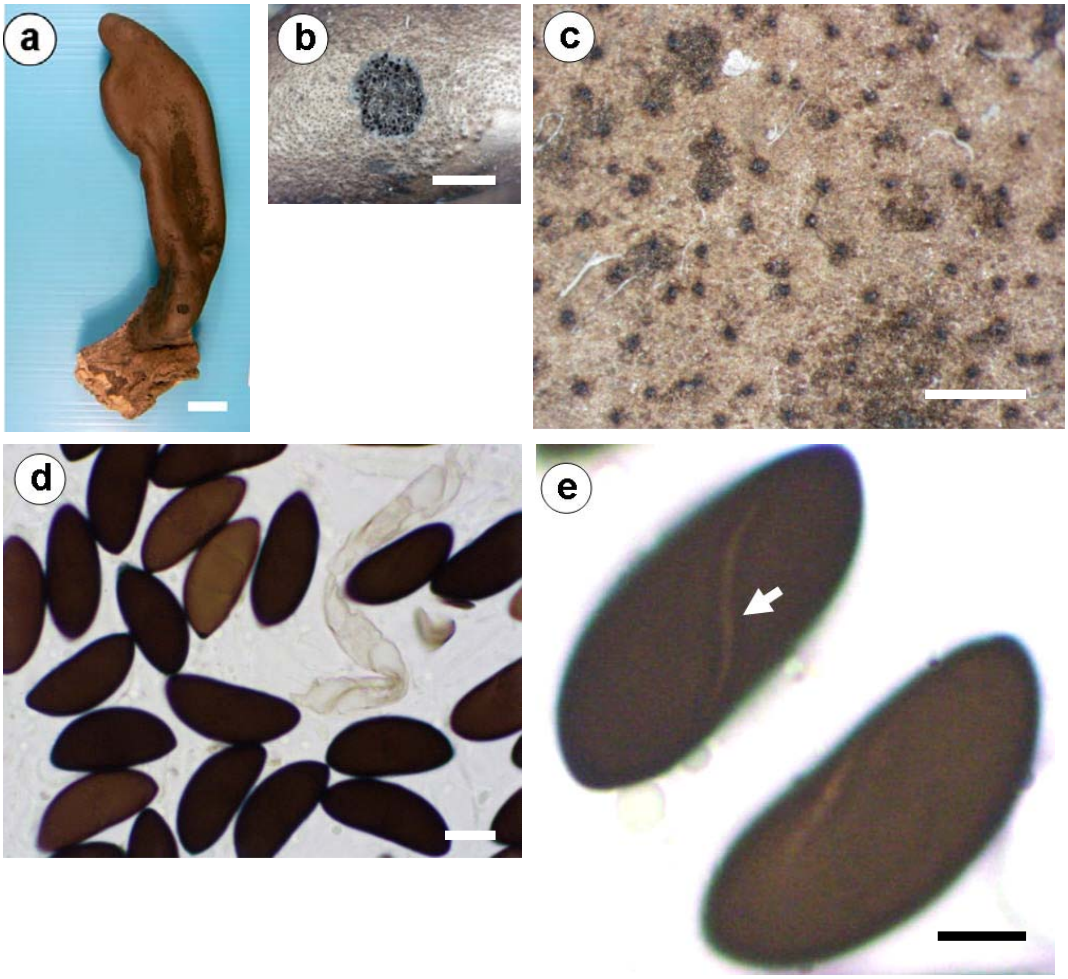

Figure 72. *Xylaria* taxonomic species 4 (SUT207); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm respectively), (b) perithecia (Bar = 0.5 µm), (d) ascospores (Bar = 10 µm), and (e) sigmoid germ slit (arrowed) (Bar = 5 µm).

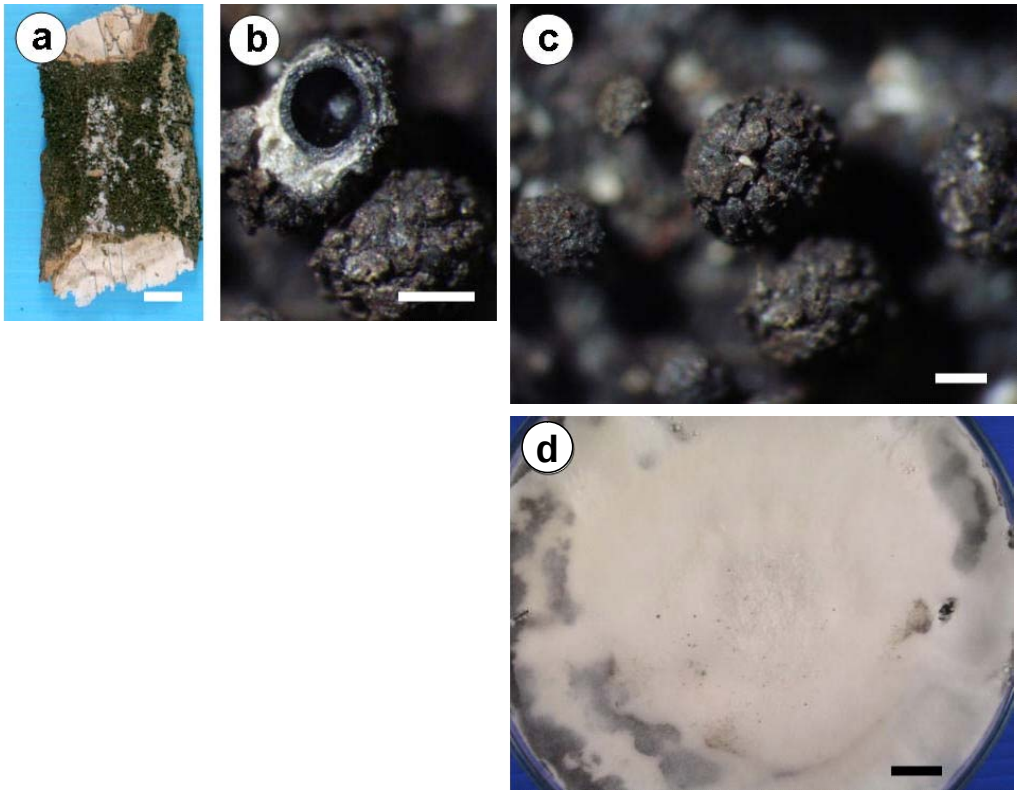

Figure 73. *Kretzschmaria* species (SUT101); (a) and (c) stromatal forms (Bars = 1 cm and 0.2 mm respectively), (b) perithecia (Bar = 0.3 µm), and (d) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm).

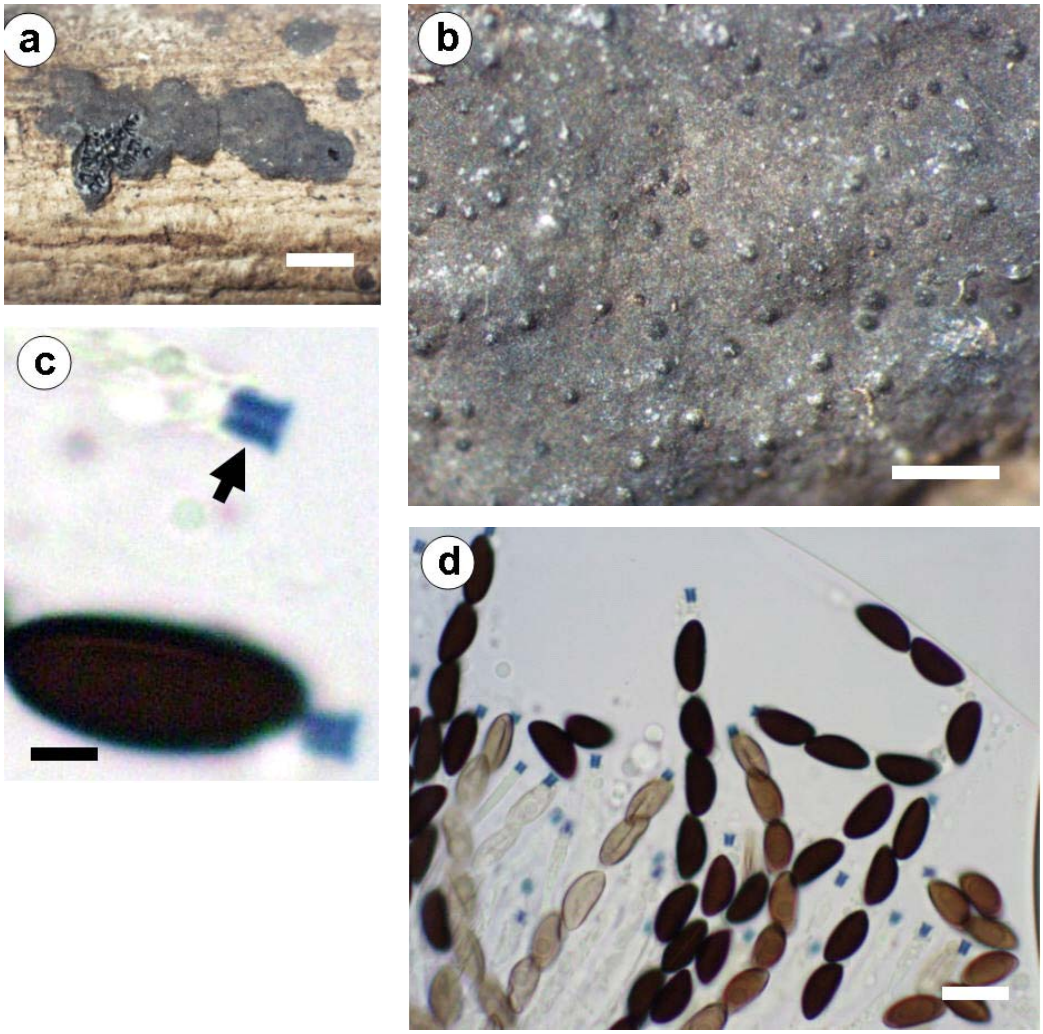

Figure 74. *Nemania* species (SUT258); (a) and (b) stromatal forms (Bars = 1 cm and 0.5 cm respectively), (c) apical apparatus (Bar = 2 µm), and (d) ascospores (Bar = 12 µm).

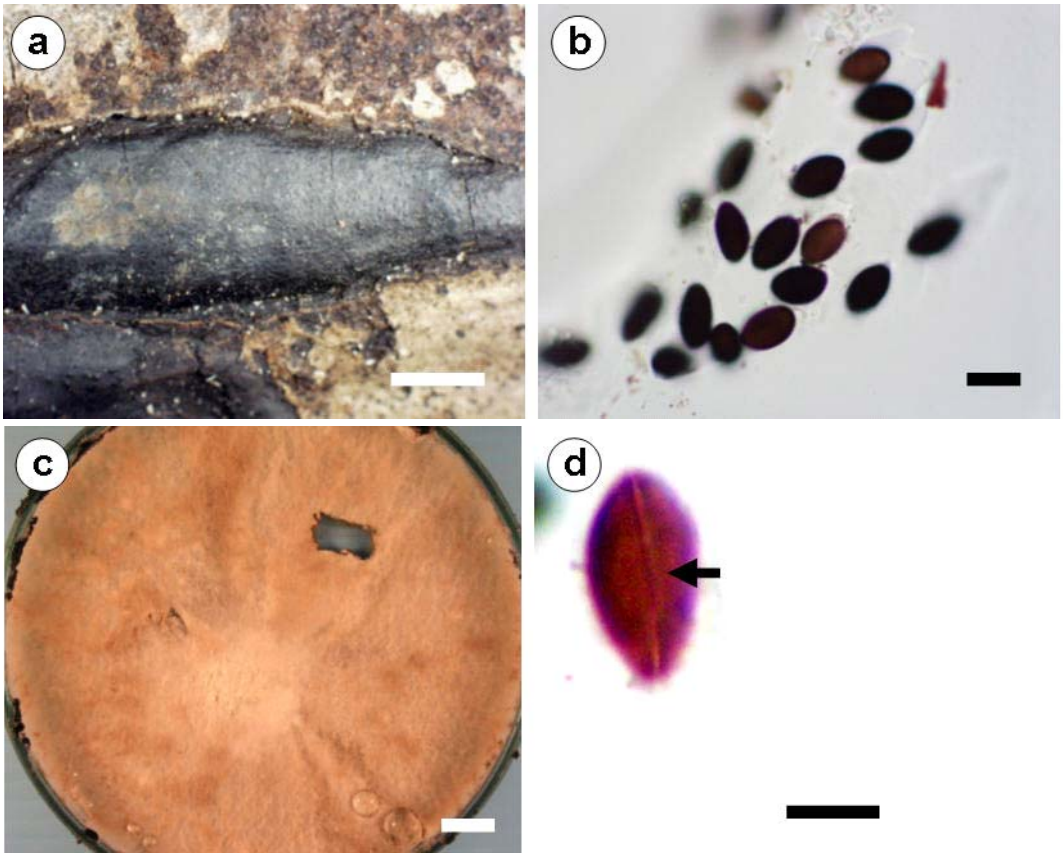

Figure 75. *Biscogniauxia capnodes* (Berk.) Y.-M. Ju & J.D. Rogers (SUT212); (a) stromatal forms (Bar = 1 cm), (b) ascospores (Bar = 0.5 µm), (c) cultural characteristics on PDA cultured at 25°C after 4 weeks (Bar = 1 cm), and (d) straight germ slit spore length (arrowed) (Bar = 2 µm).

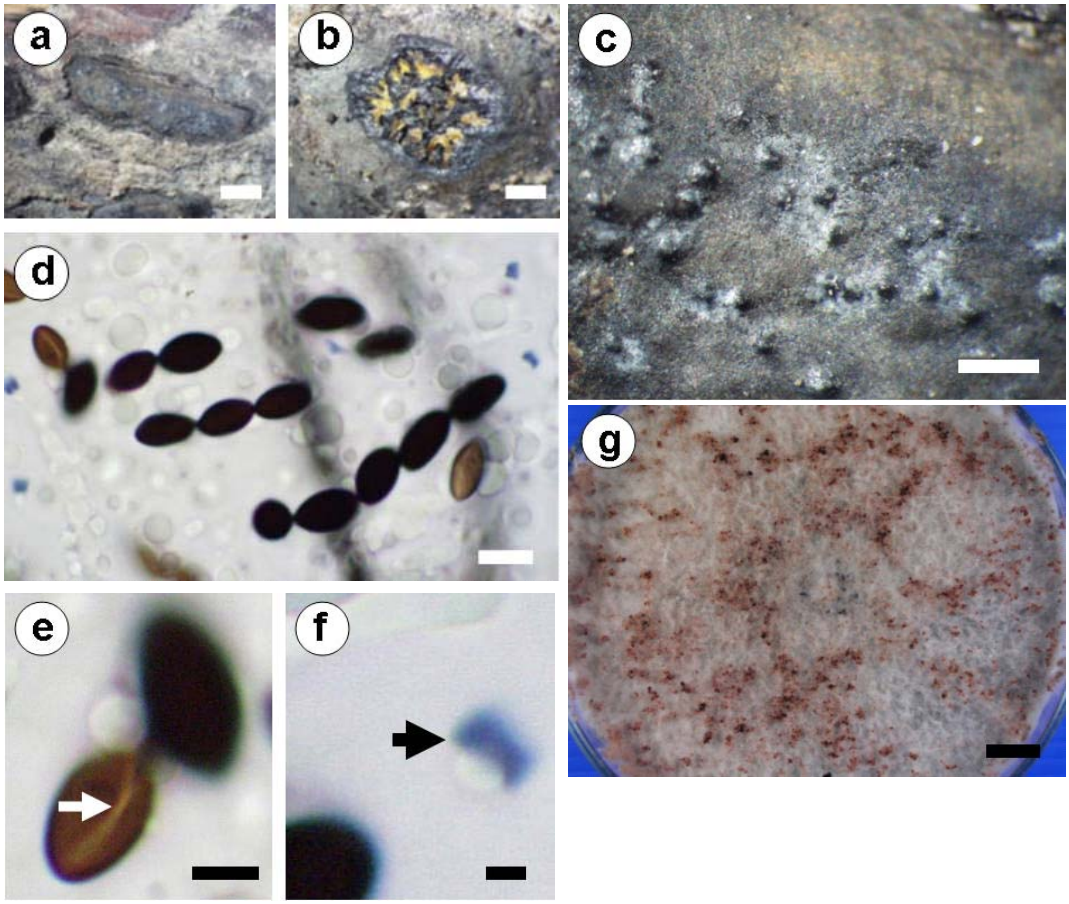

Figure 76. *Biscogniauxia* sp. nov. (SUT290); (a) and (c) stromatal forms (Bars = 1 cm and 0.5 mm, respectively), (d) ascospores (Bar = 0.5 µm), (e) straight germ slit spore length (arrowed) (Bar = 2 µm), apical apparatus (arrowed) (Bar = 2 µm), and (g) cultural characteristics on PDA cultured at 25°C 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.

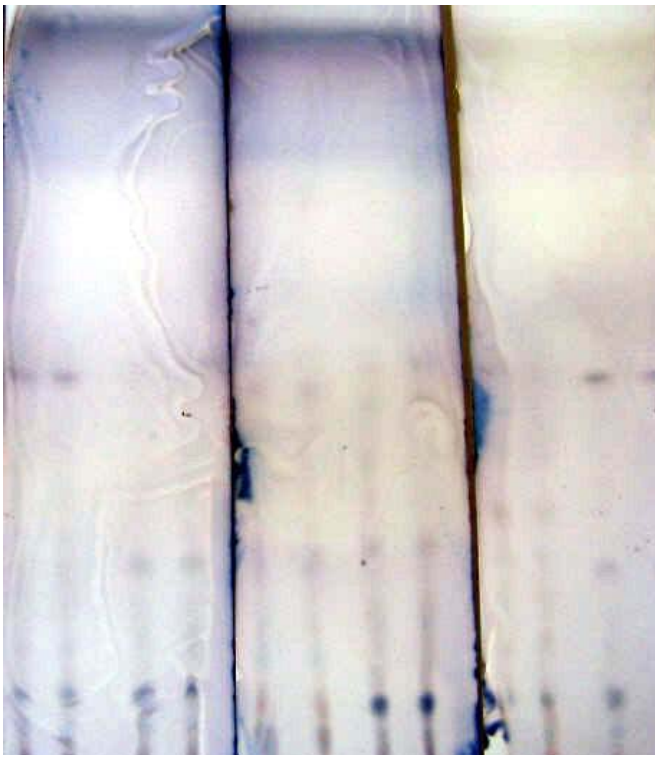

Figure 77. Secondary metabolite profiles of *Xylaria* isolates compared to those of *Xylaria* endophytes extracted from 100-mL cultural broth and analyzed by TLC method under visible light. UX5 = *Xylaria hypoxylon* endophyte, UX3 = *Xylaria apiculata* endophyte, X1 = *X. cubensis* ST2326, X2 = *X. arbuscula* 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.

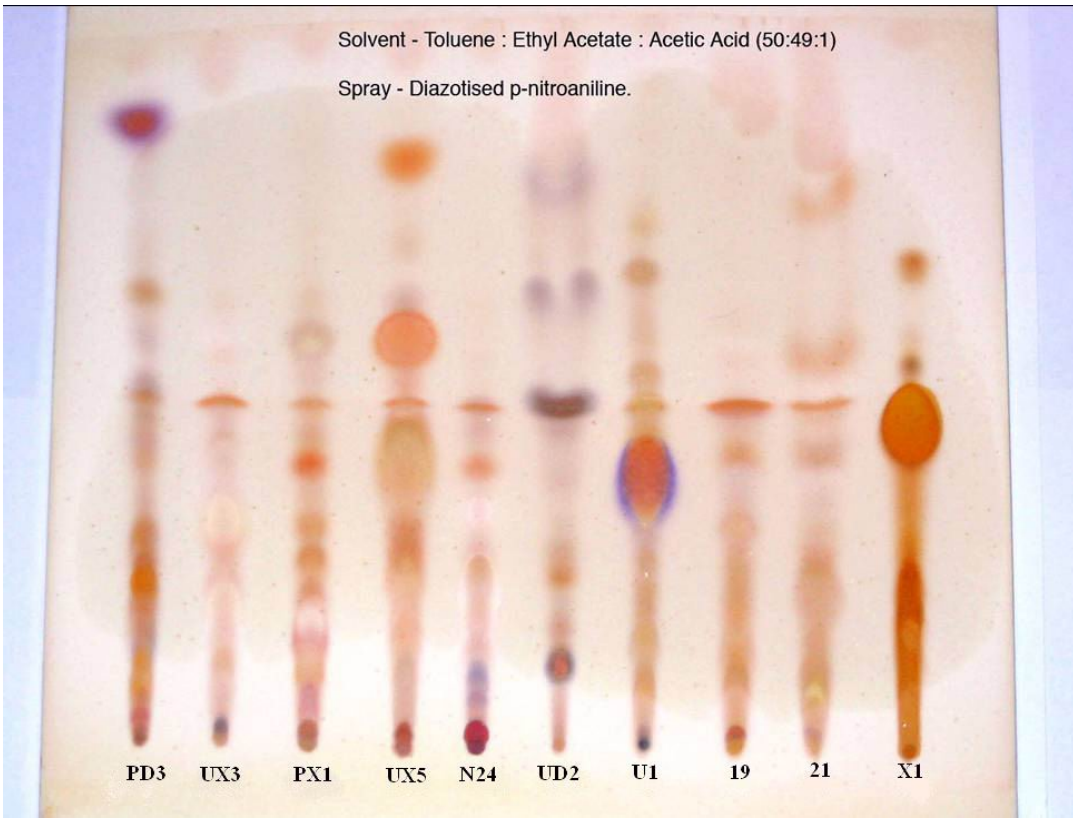

Figure 78. The pattern of secondary metabolites extracted from *X. cubensis* and other fungal endophytes by TLC method. Lanes: PD3, *Nodulisporium* sp. endophyte; UX3, *X. apiculata* endophyte; PX1, *X. longipes* endophyte; UX5, *X. hypoxylon* endophyte; N24, *Rosellinia arcuata* endophyte; UD2, *Daldinia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

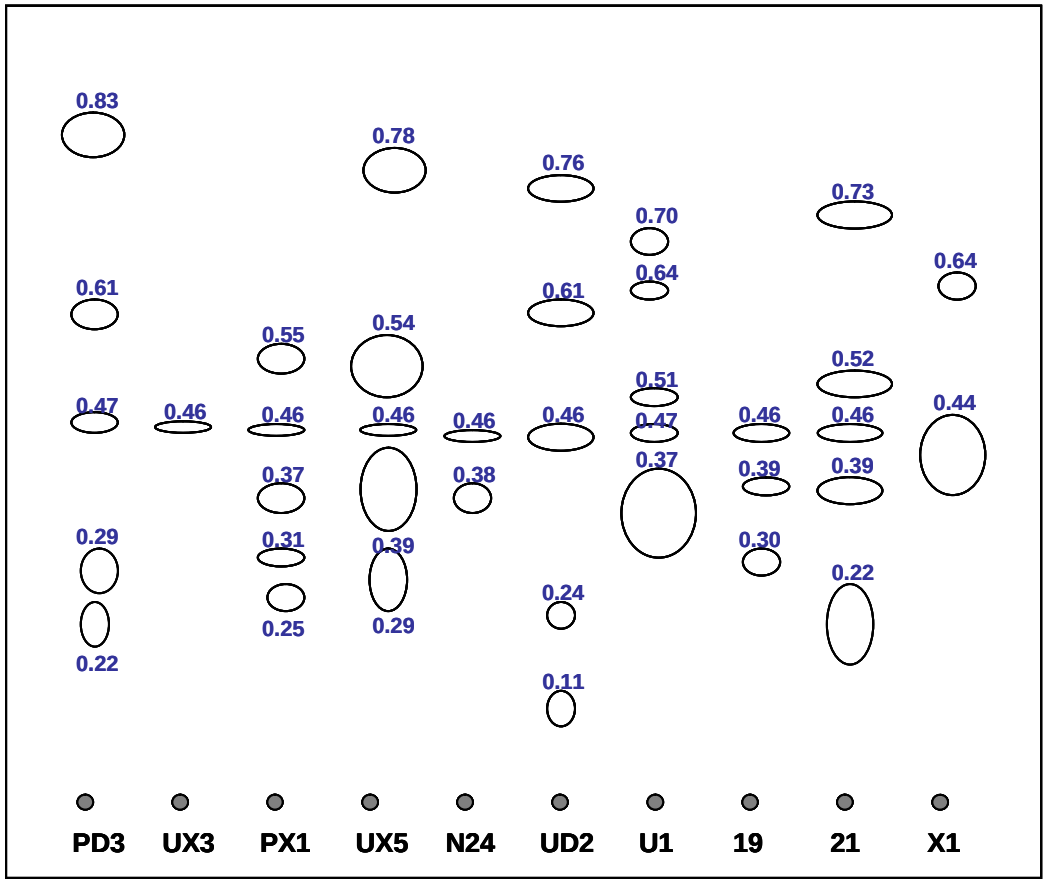

Figure 79. Schematic of secondary metabolites extracted from *Xylaria cubensis* and other endophytes by TLC method. Lanes: PD3, *Nodulisporium* sp. endophyte; UX3, *X. apiculata* endophyte; PX1, *X. longipes* endophyte; UX5, *X. hypoxylon* endophyte; N24, *Rosellinia arcuata* endophyte; UD2, *Daldinia concentrica* endophyte; U1, *Hypoxylon rickii* endophyte; 19, *X. apiculata* endophyte; 21, *X. mali* endophyte; and X1, *X. cubensis* ST2326. The number indicated the R_f 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[a]	Reference
NS4	CTTCCGTCAATTCCTTTAAG	SSU 1150-1131	White *et al*. 1990
SR8R	GAACCAGGACTTTTACCTT	SSU 732-749	Vilgalys, www, 1999

[a] *Saccharomyces cerevisiae* numbering

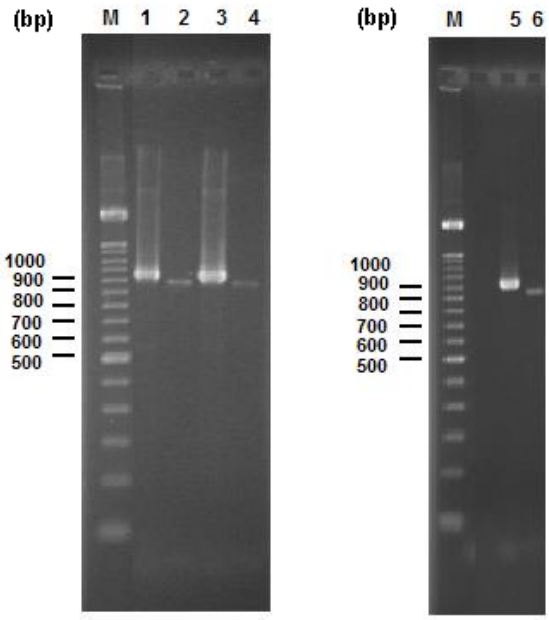

Figure 80. Gel electrophoresis of partial 18S rDNA fragments. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, 3, and 5, *Astrocystis mirabilis* (SUT051), *A. mirabilis* (SUT056) and *Rosellinia* sp. ST2310 using NS1 and NS4 primers respectively; 2, 4, and 6, *Astrocystis mirabilis* (SUT051),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

Species	Location /source	1st fragment (NS1/NS4 primers) (bp)	GenBank accession number	2nd fragment (NS4/NS8 primers) (bp)	GenBank accession number	Total (bp)
A. mirabilis (SUT056)	Ratchaburi Province	1012	DQ322075	1072	DQ322076	2056
A. mirabilis (SUT051)	Ratchaburi Province	1012	DQ322074	NO	ND	ND
Rosellinia sp. (ST2310)	RFD*	1020	DQ322072	1202	DQ322073	2210

* 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. cocoes* (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).

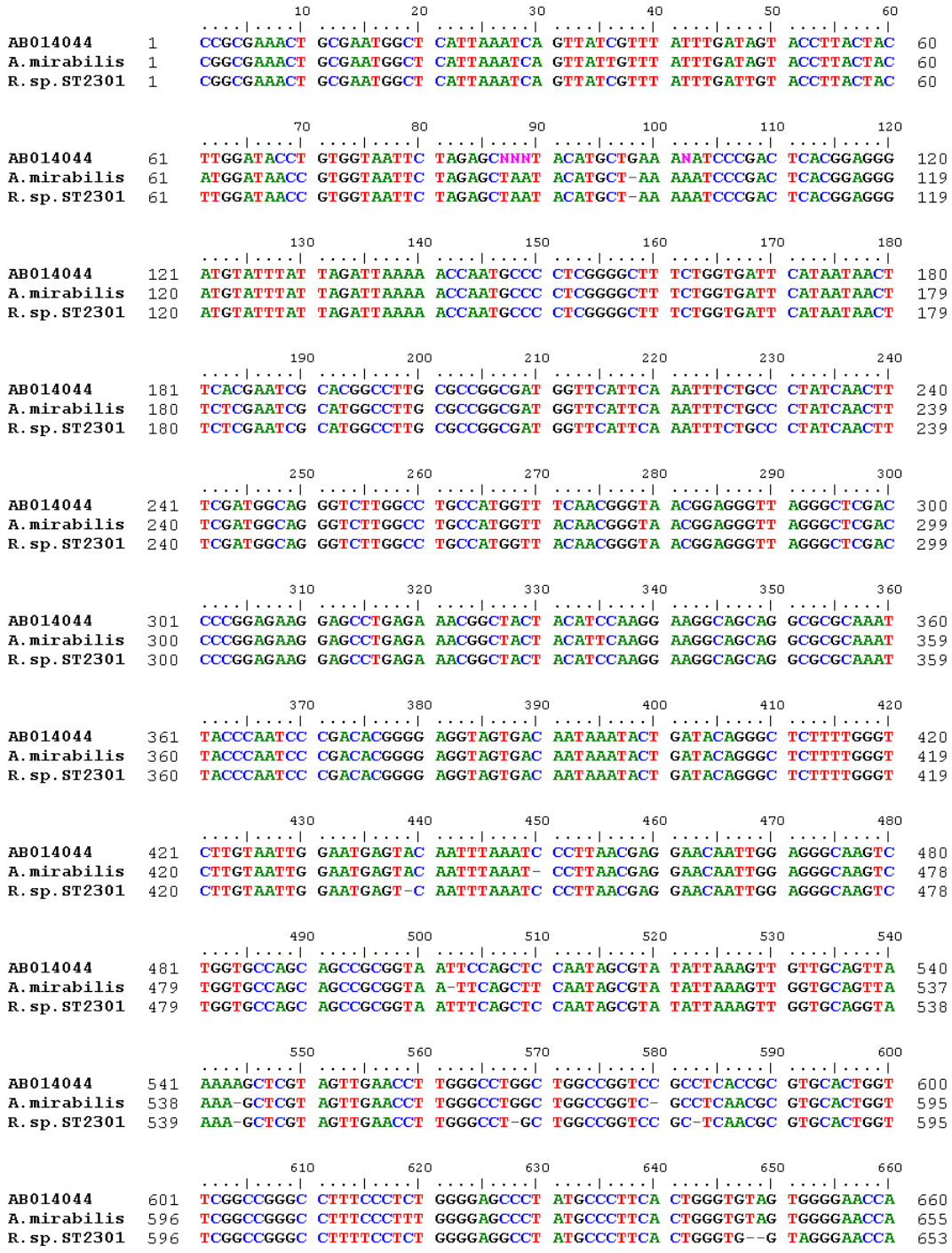

Figure 81. Sequence alignment of 18S rDNA *Rosellinia* sp. (ST2310), *Rosellinia necatrix* (AB014044), and *A. mirabilis* (SUT056).

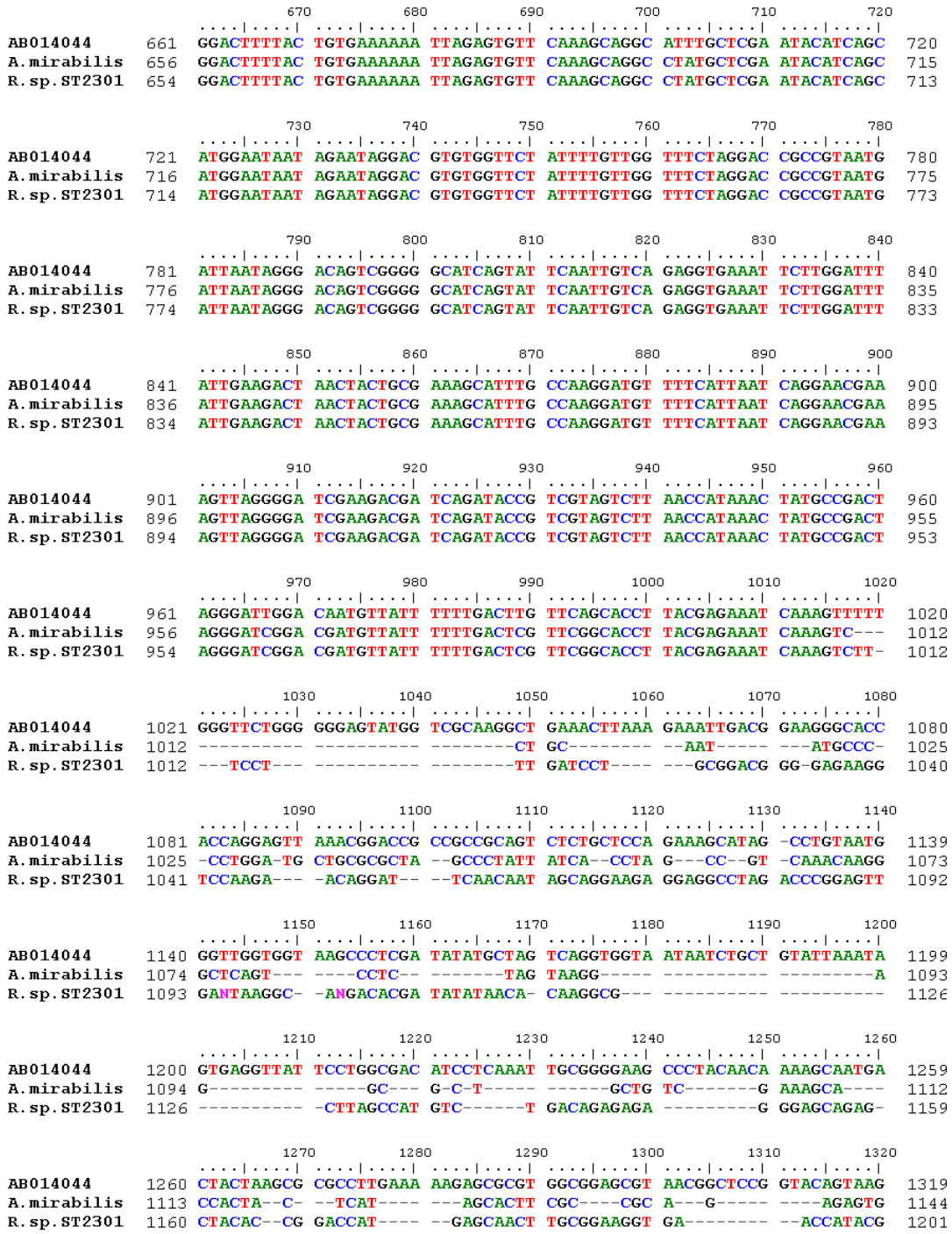

Figure 81. (Continued).

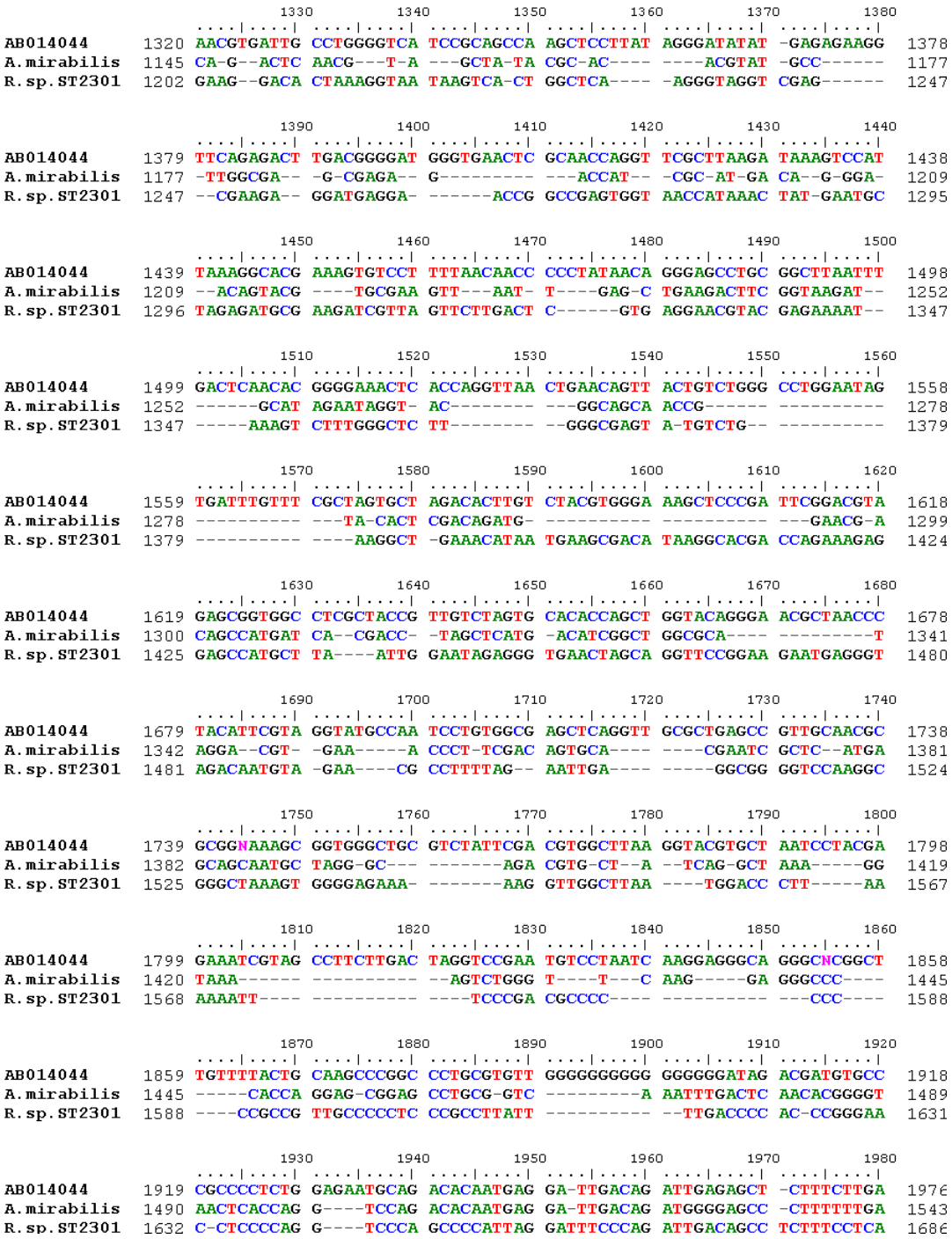

Figure 81. (Continued).

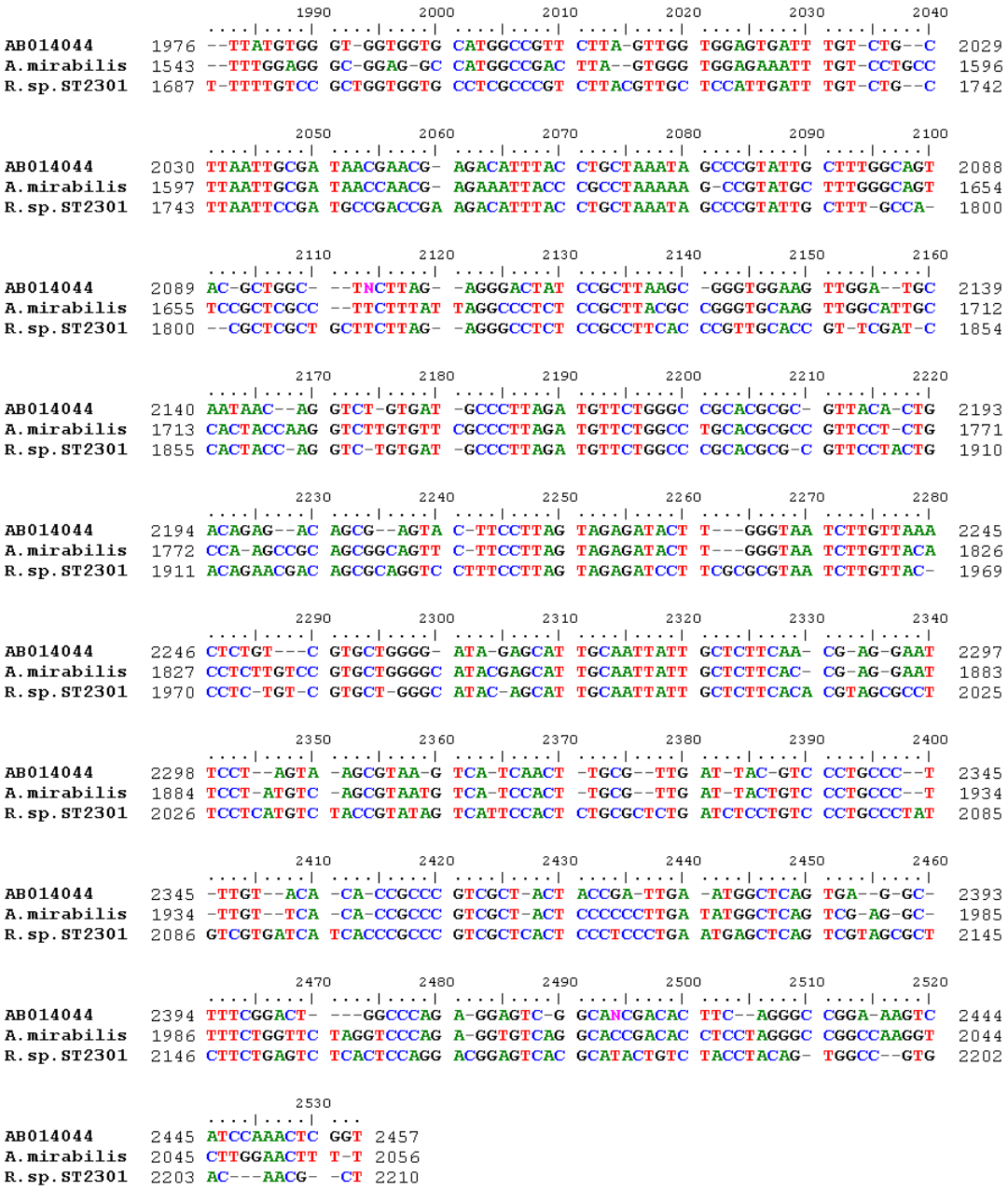

Figure 81. (Continued).

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.

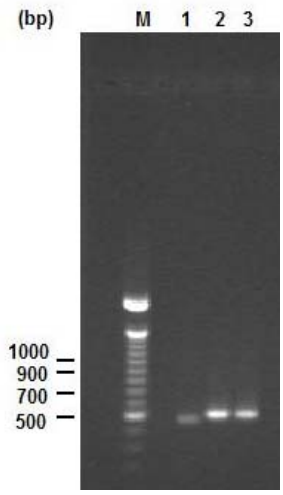

Figure 82. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of *Astrocystis* and *Rosellinia* using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, *Rosellinia* sp. (ST2310); 2, *A. mirabilis* (SUT051); 3, *A. mirabilis* (SUT056).

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 (bp)**	**5.8S (bp)**	**ITS2 (bp)**	**Total (bp)**	**GenBank accession number**
A. mirabilis (SUT051)	Ratchaburi Province	172	155	156	483	DQ322078
A. mirabilis (SUT056)	Ratchaburi Province	172	155	156	483	DQ322076
Rosellinia sp. (ST2310)	RFD*	178	155	160	493	DQ322077

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

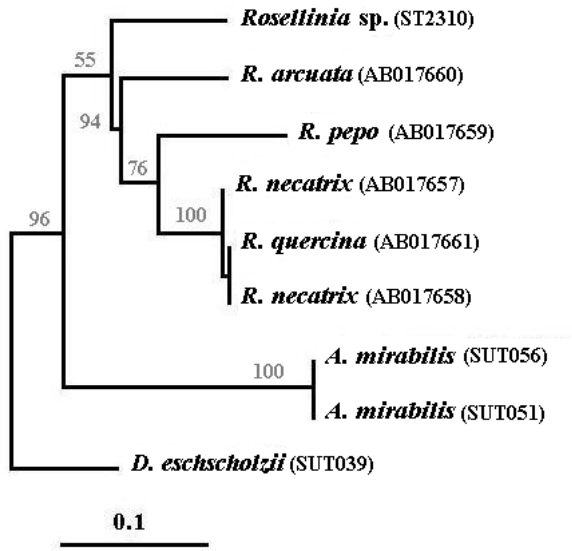

Figure 83. Phylogenetic tree of *Astrocystis* and *Rosellinia* based on ITS1-5.8S-ITS2 sequences constructed by using the neighbour-joining method. *Daldinia eschscholzii* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches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

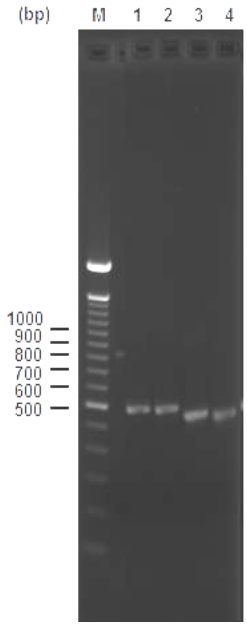

Figure 84. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of *Camillea* using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, *Camillea tinctor* (ST2321); 2, *C. tinctor* (SUT161); 3, *C. tinctor* (SUT260); and 4, *C. selangorensis* (KS15).

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 (bp)	5.8S (bp)	ITS2 (bp)	Total (bp)	GenBank 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 (KS15)	Liverpool John Moores University[b]	175	155	156	486	DQ322083

[a] The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand.
[b] 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.

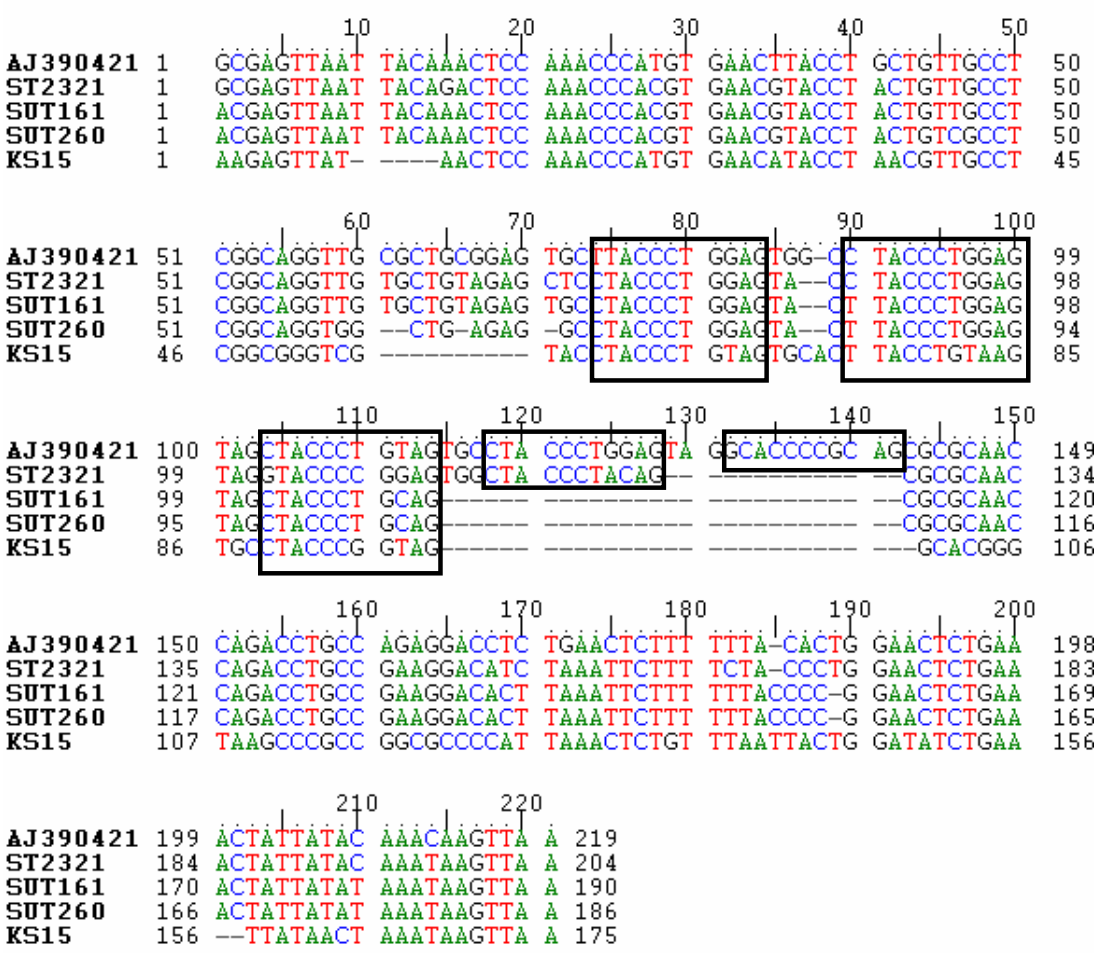

Figure 85. ITS1 sequence alignment of *Camillea tinctor* (AJ390421) from GenBank, *C. tinctor* (ST2321), *C. tinctor* (SUT161), *C. tinctor* (SUT260), and *C. selangorensis* (KS15) by using ClustalX and BioEdit programs. Blocks indicate the short tandem repeat (STR) sequences.

Table 22. The identity matrix of ITS1-5.8S-ITS2 sequence comparison of *Camillea tinctor* and *C. selangorensis*.

Species	***C. tinctor* (AJ390421)**	***C. tinctor* (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

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 changed from 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.

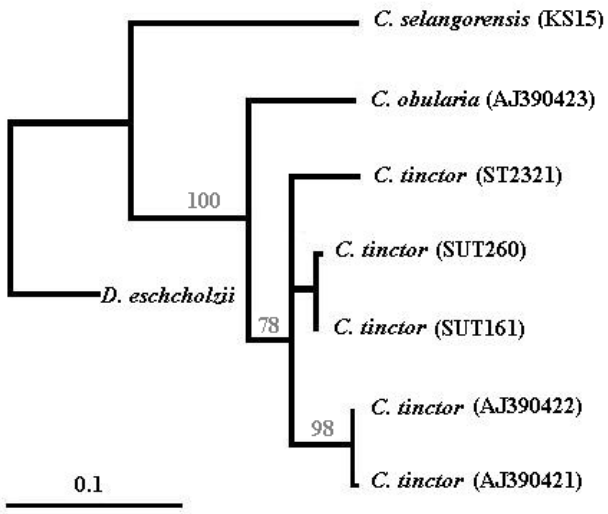

Figure 86. Phylogenetic tree of *Camillea* based on ITS1-5.8S-ITS2 sequences constructed by using the neighbour-joining method. *Daldinia eschscholzii* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values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

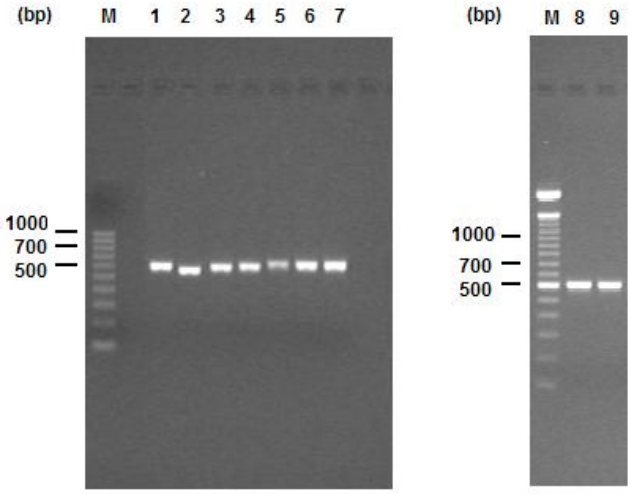

Figure 87. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of *Daldinia* using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, *Daldinia eschscholzii* (SUT038); 2, *D. eschscholzii* (SUT039); 3, *D. eschscholzii* (SUT168); 4, *D. eschscholzii* (SUT169); 5, *D. eschscholzii*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

Table 24. The length of ITS1-5.8S-ITS2 sequences of *Daldinia eschscholzii* and *D. concentrica* obtained from DNA sequence analysis.

Species	Location/Source	ITS1 (bp)	5.8S (bp)	ITS2 (bp)	Total (bp)	GenBank 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 Province	164	155	163	482	DQ322087
D. eschschozii (SUT209)	Trad Province	163	155	163	481	DQ322088
D. eschschozii (SUT278)	Kanchanaburi Province	163	155	159	477	DQ322089
D. eschschozii (SUT322)	Chiang Rai Province	164	155	164	483	DQ322090
D. concentrica (L1)	Liverpool John Moores University*	173	155	171	499	DQ322091
D. concentica (L2)	Liverpool John Moores University*	173	155	171	499	DQ322092

* 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.

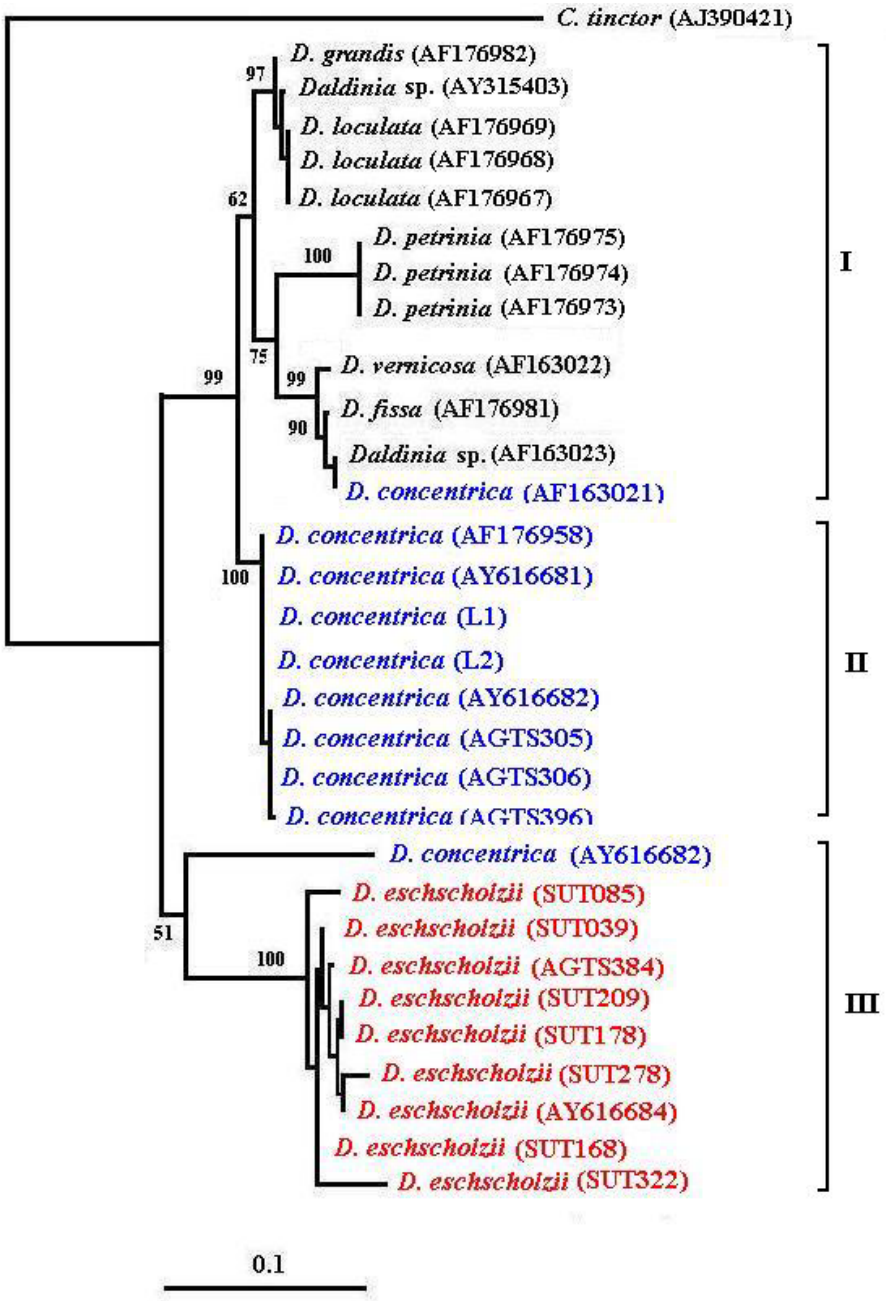

Figure 88. Phylogenetic tree of *Daldinia* species based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000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

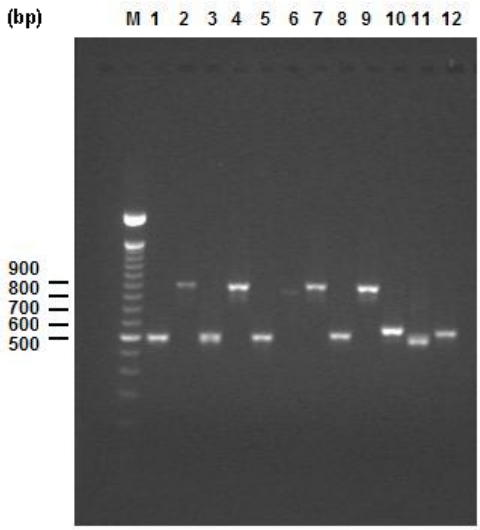

Figure 89. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of *Hypoxylon* sect. *Annulata* using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, *Hypoxylon nitens* (ST2313); 2, *H. stygium*; 3, *H. purpureonitens* (SUT001); 4, *H. atroroseum* (SUT009); 5, *H. purpureonitens* (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* obtained from DNA sequence analysis.

Species	Location/Source	ITS1 (bp)	5.8S (bp)	ITS2 (bp)	Total (bp)	GenBank 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[a]	209	155	161	525	DQ201123
H. cf. *archeri* (ST2527)	RFD[a]	224	155	160	539	DQ201124
H. atroroseum (SUT009)	Nakhon Ratchasima Province	506	155	164	825	DQ223733
H. atroroseum (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[b]	226	155	167	548	DQ201127
H. bovei var. *microspora* (ST2579)	RFD[a]	226	155	167	548	DQ201129
H. bovei var. *microspora* (ST2406)	RFD[a]	225	155	167	547	DQ201128
H. leptascum var. *macrospora* (ST2584)	RFD[a]	248	155	159	562	DQ322097
H. moriforme (SUT220)	Trad Province	230	155	165	550	DQ322129
H. nitens (Ju1)	The University of Taiwan[b]	158	155	166	479	DQ223750
H. nitens (ST2313)	RFD[a]	158	155	166	479	DQ223751
H. nitens (ST2332)	RFD[a]	233	155	166	554	DQ322098
H. nitens (ST2436)	RFD[a]	158	155	166	479	DQ322099
H. nitens (ST2473)	RFD[a]	158	155	166	479	DQ223752
H. purpureonitens (SUT001)	Nakhon Ratchasima Province	225	155	166	546	DQ322100
H. purpureonitens (SUT004)	Nakhon Ratchasima Province	225	155	169	549	DQ223753
H. purpureonitens (SUT005)	Nakhon Ratchasima Province	225	155	165	545	DQ322101
H. purpureonitens (SUT167)	Yasothorn Province	225	155	169	549	DQ223754
H. purpureonitens (SUT262)	Trad Province	225	155	169	549	DQ223755
H. purpureonitens (ST2448)	RFD[a]	225	155	169	549	DQ223756
H. purpureonitens (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

[a] The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand.
[b] 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[a]	176	155	154	489	DQ322104
Hypoxylon taxonomic species 1 (SUT081)	Nakhon Ratchasima Province	560	155	155	870	DQ322105
Hypoxylon taxonomic species 1 (SUT236)	Trad Province	566	155	155	876	DQ322106
Hypoxylon taxonomic species 1 (SUT242)	Trad Province	566	155	155	876	DQ322107
Hypoxylon taxonomic species 1 (SUT244)	Trad Province	566	155	154	875	DQ322108
Hypoxylon taxonomic species 1 (SUT251)	Trad Province	566	155	155	876	DQ322109
Hypoxylon taxonomic species 1 (SUT285)	Kanchanaburi Province	566	155	154	875	DQ322110

[a] The culture was provided by Dr. Surang Thienhirun, The Royal Forest Department, Thailand.
[b] 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 neighbour-joining 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).

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

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.

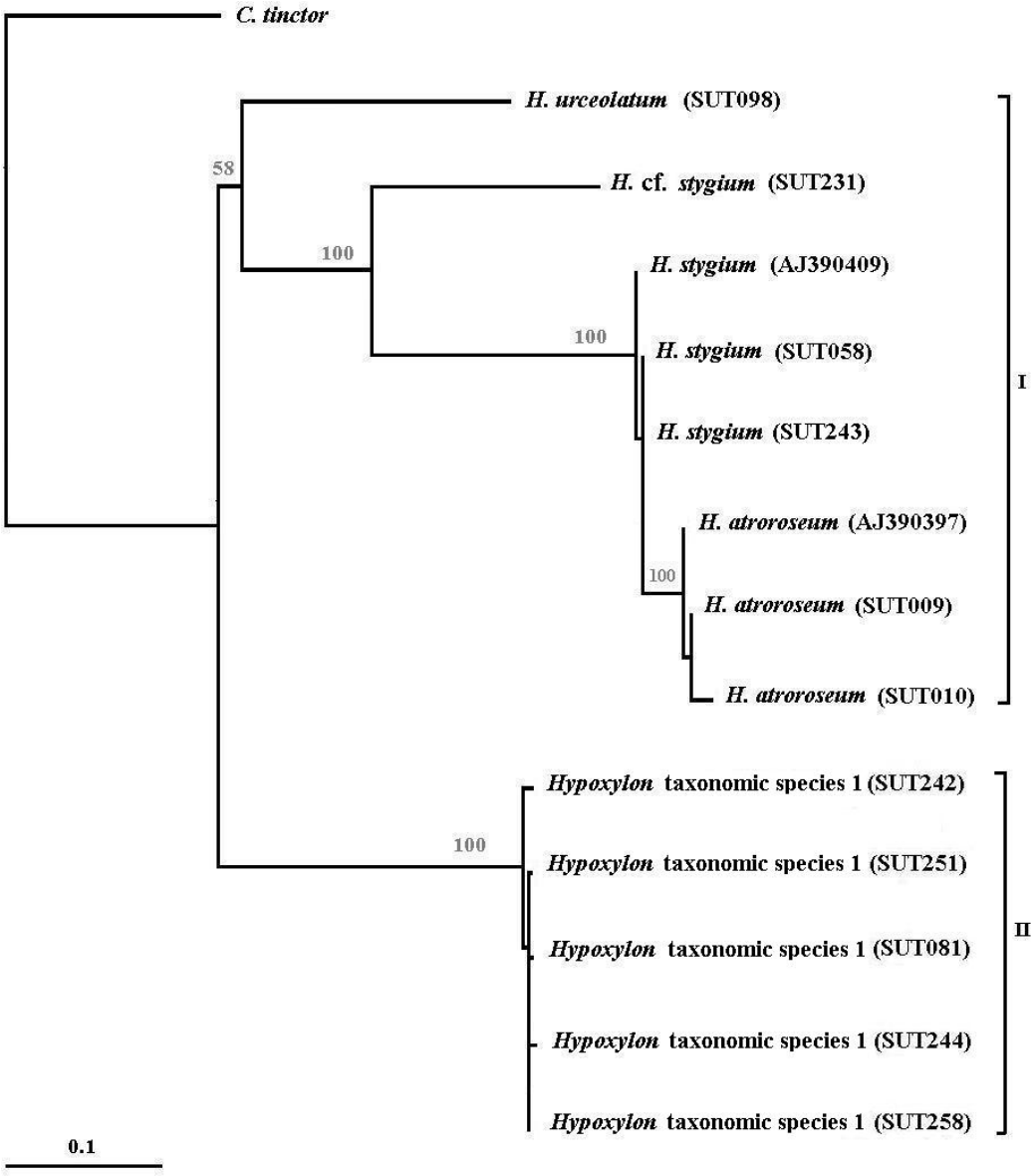

Figure 90. Phylogenetic tree of *Hypoxylon* sect. *Annulata* containing extremely long ITS1 region (398 bp to 588 bp) based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution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

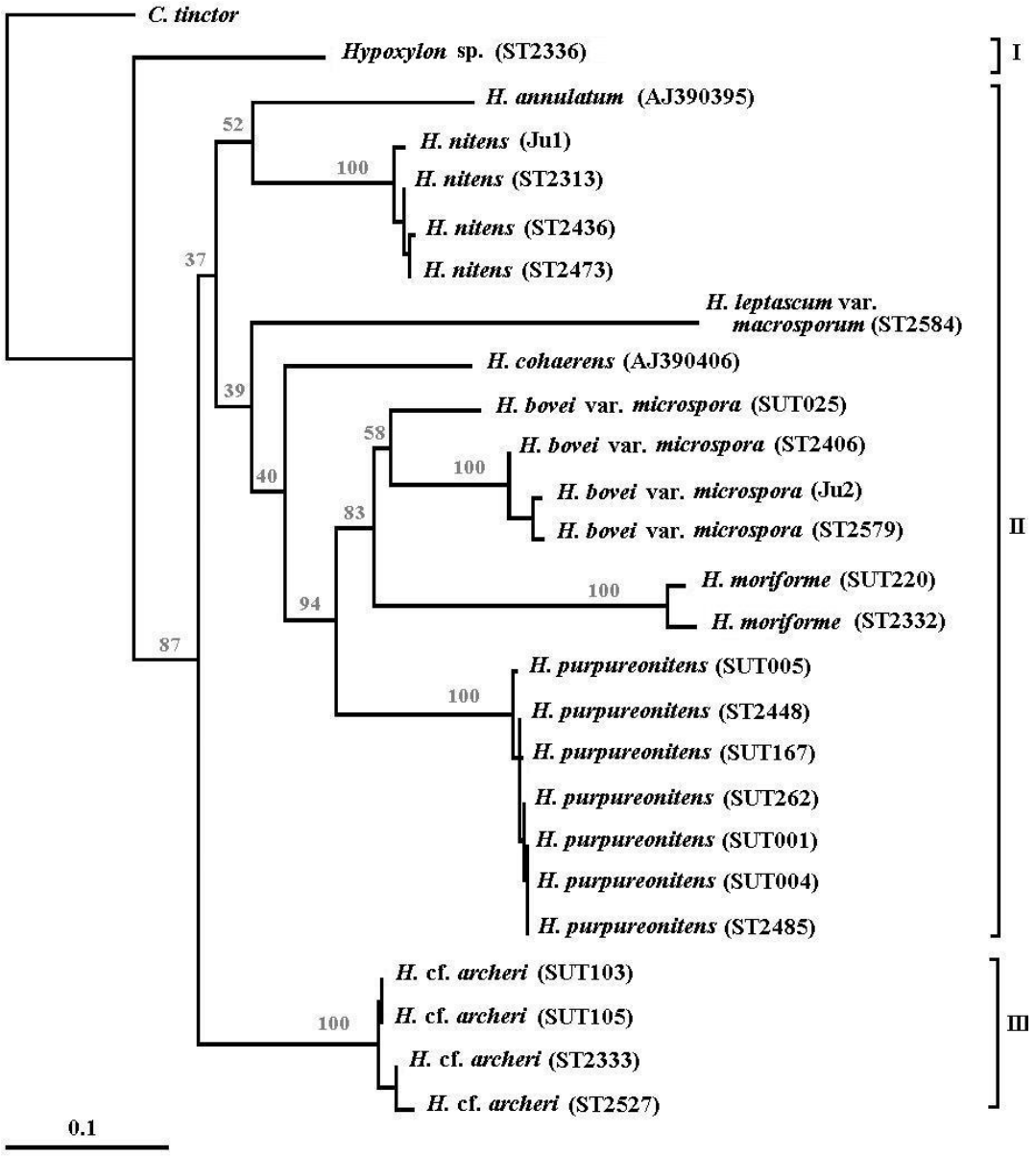

Figure 91. Phylogenetic tree of *Hypoxylon* sect. *Annulata* based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Camillea tinctor* (AJ394021) is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap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

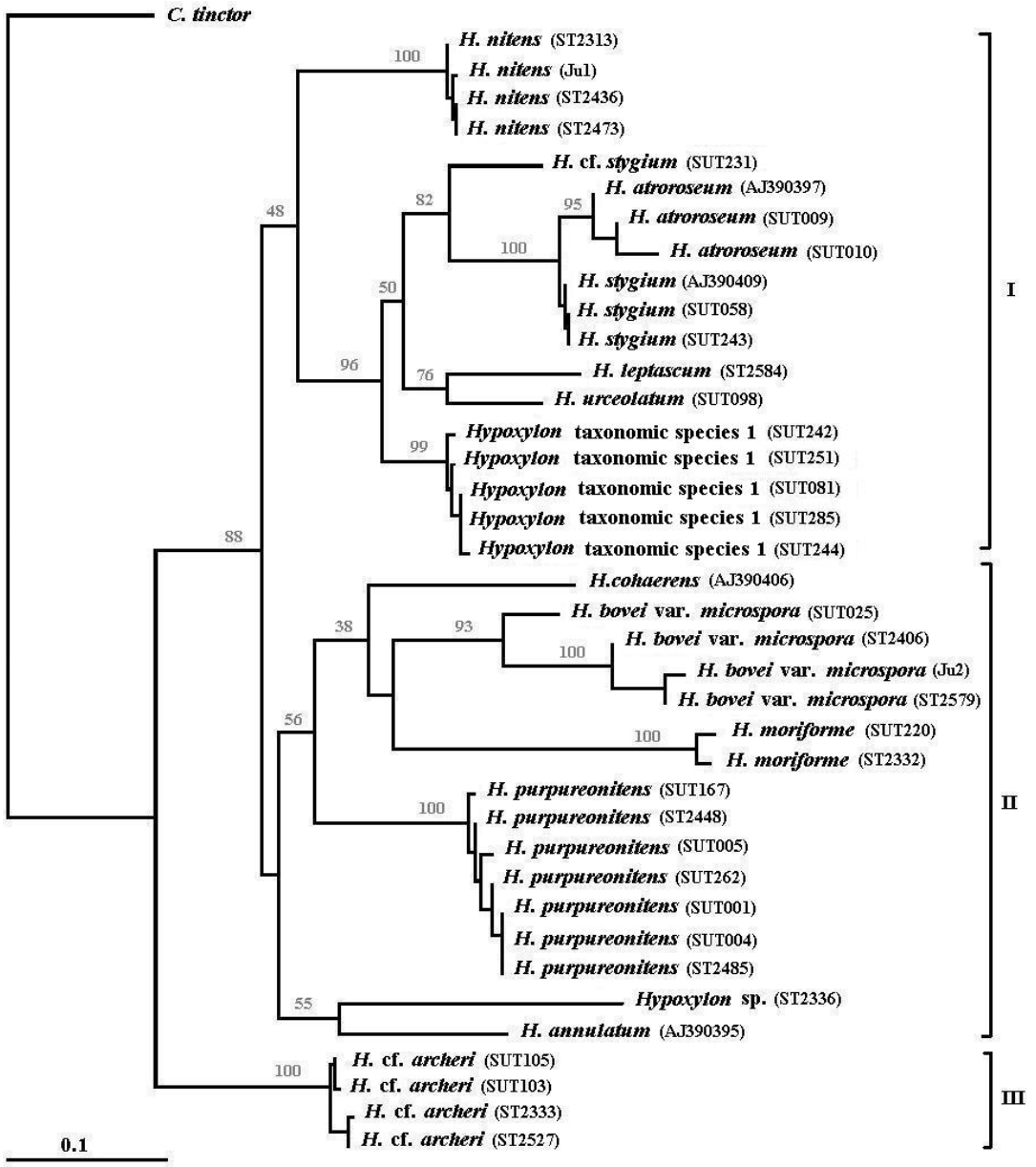

Figure 92. Phylogenetic tree of *Hypoxylon* sect. *Annulata* based on ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000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

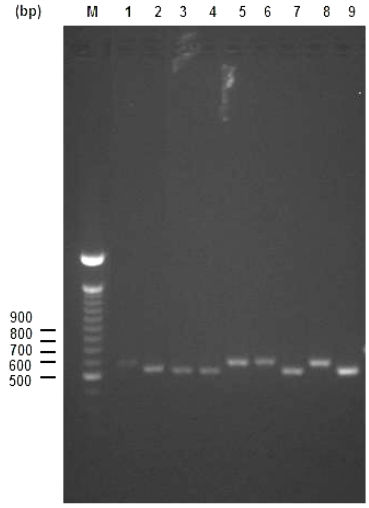

Figure 93. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of *Hypoxylon* sect. *Hypoxylon* using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 bp DNA ladder, Invitrogen); 1, *Hypoxylon investiens* (SUT041); 2, *H. investiens* (SUT063); 3, *H. lenormandii* (SUT046); 4, *H. lenormandii* (SUT180); 5, *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).

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

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

* 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	QD201131
H. cf. *fendleri* (SUT159)	Yasothorn Province	183	155	164	502	QD201132
H. cf. *fendleri* (SUT162)	Yasothorn Province	183	155	165	503	QD223735
H. cf. *fendleri* (SUT165)	Yasothorn Province	182	155	163	500	QD223736
*H.*cf. *fendleri* (SUT280)	Kanchanaburi Province	156	155	163	474	QD223737
H. haematostroma (SUT164)	Yasothorn Province	176	155	161	492	QD223738
H. haematostroma (SUT292)	Kanchanaburi Province	176	155	161	492	QD223739
H. haematostroma (SUT293)	Kanchanaburi Province	176	155	161	492	QD223740
H. hypomiltum (SUT166)	Yasothorn Province	213	155	160	528	QD322114
H. investiens (SUT041)	Ratchaburi Province	276	155	153	584	QD322115
H. investiens (SUT063)	Ratchaburi Province	230	155	155	540	QD322116
H. kanchanapisekii (SUT066) sp. nov.	Ratchaburi Province	209	155	162	526	QD223741
H. kanchanapisekii (SUT068) sp. nov.	Ratchaburi Province	209	155	162	526	QD223742
H. kanchanapisekii (SUT069) sp. nov.	Ratchaburi Province	209	155	162	526	QD223743
H. lenormandii (SUT016)	Burirum Province	188	155	160	503	QD223744
H. lenormandii (SUT046)	Ratchaburi Province	208	155	165	528	DQ322117
H. lenormandii (SUT180)	Nakhon Ratchasima Province	188	155	160	503	QD223745
H. lenormandii (ST2324)	RFD*	188	155	160	503	QD223746
H. monticulosum (SUT042)	Ratchaburi Province	171	155	165	491	QD223747
H. monticulosum (SUT080)	Nakhon Ratchasima Province	171	155	165	491	QD223748
H. monticulosum (SUT116)	Songkhla Province	171	155	165	491	QD223749
H. cf. *perforatum* (SUT020)	Burirum Province	301	155	156	612	QD322118
H. cf. *perforatum* (SUT218)	Trad Province	206	155	186	547	QD322119
H. cf. *perforatum* (SUT294)	Kanchanaburi Province	209	155	161	525	QD322120
H. rubiginosum (SUT215)	Trad Province	178	155	164	497	QD223758
H. rubiginosum (SUT221)	Trad Province	178	155	164	497	QD223759
H. subgilvum var. *microsporum* (SUT104)	Songkhla Province	148	155	164	467	QD322121

* 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
H. sublenormandii sp. nov. (SUT250)	Trad Province	198	155	161	514	QD223762
H. sublenormandii sp. nov. (SUT282)	Kanchanaburi Province	198	155	161	514	QD223763
H. suranareei (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
Hypoxylon taxonomic species 2 (SUT082)	Nakhon Ratchasima Province	130	155	166	451	QD322126
Hypoxylon taxonomic species 3 (SUT158)	Yasothorn Province	173	155	160	488	QD322127
Hypoxylon 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.

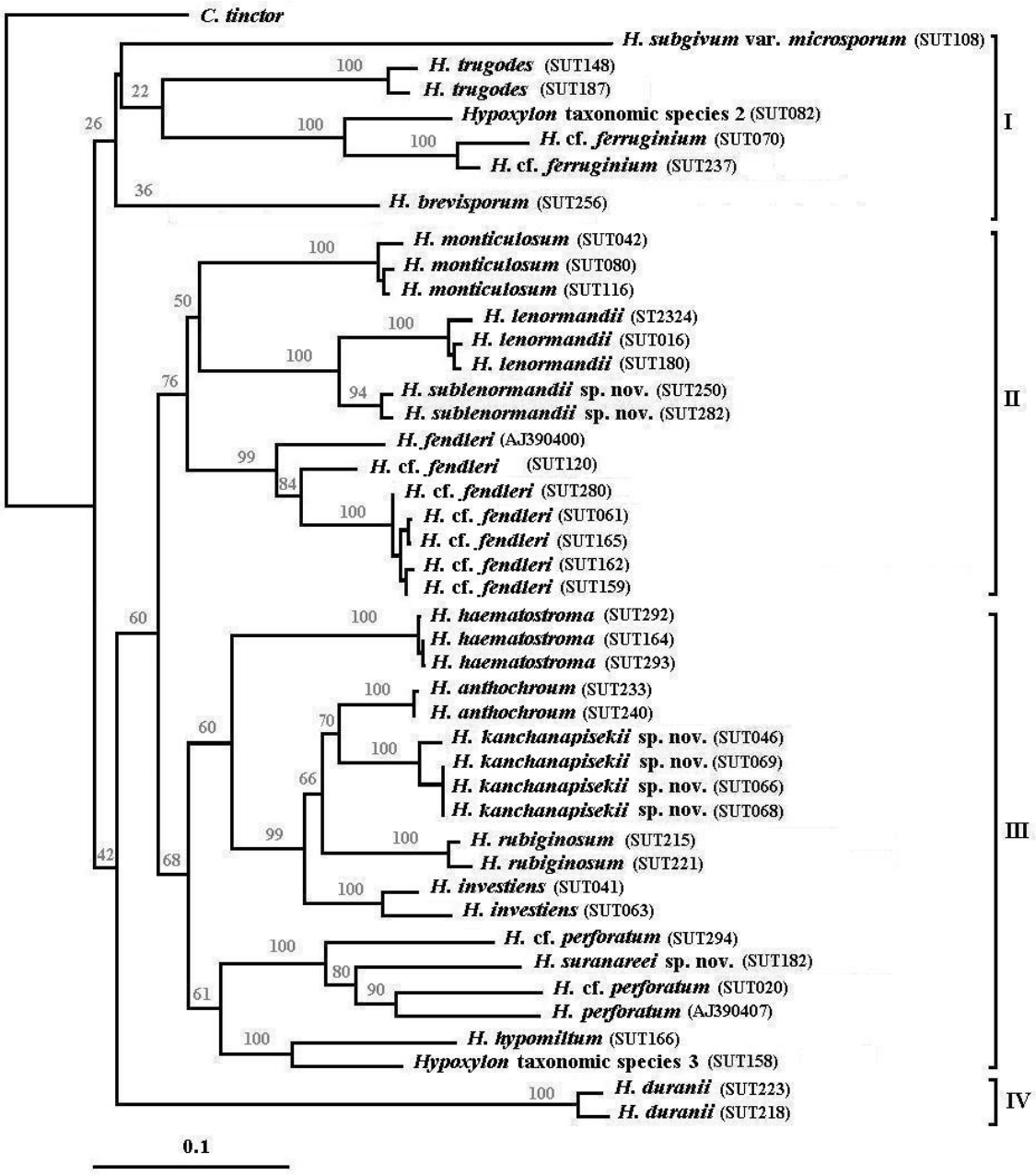

Figure 94. Phylogenetic tree of *Hypoxylon* sect. *Hypoxylon* based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values 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 KOH-extractable 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* (AJ390407) showed 24% and 21% divergence to *H.* cf. *perforatum* (SUT020) and *H.* cf. *perforatum* (SUT294) respectively, which might indicate different taxa. However, further investigations of more collections are required.

Clade IV contained only one species, *H. duranii* (SUT223 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* although most of *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.

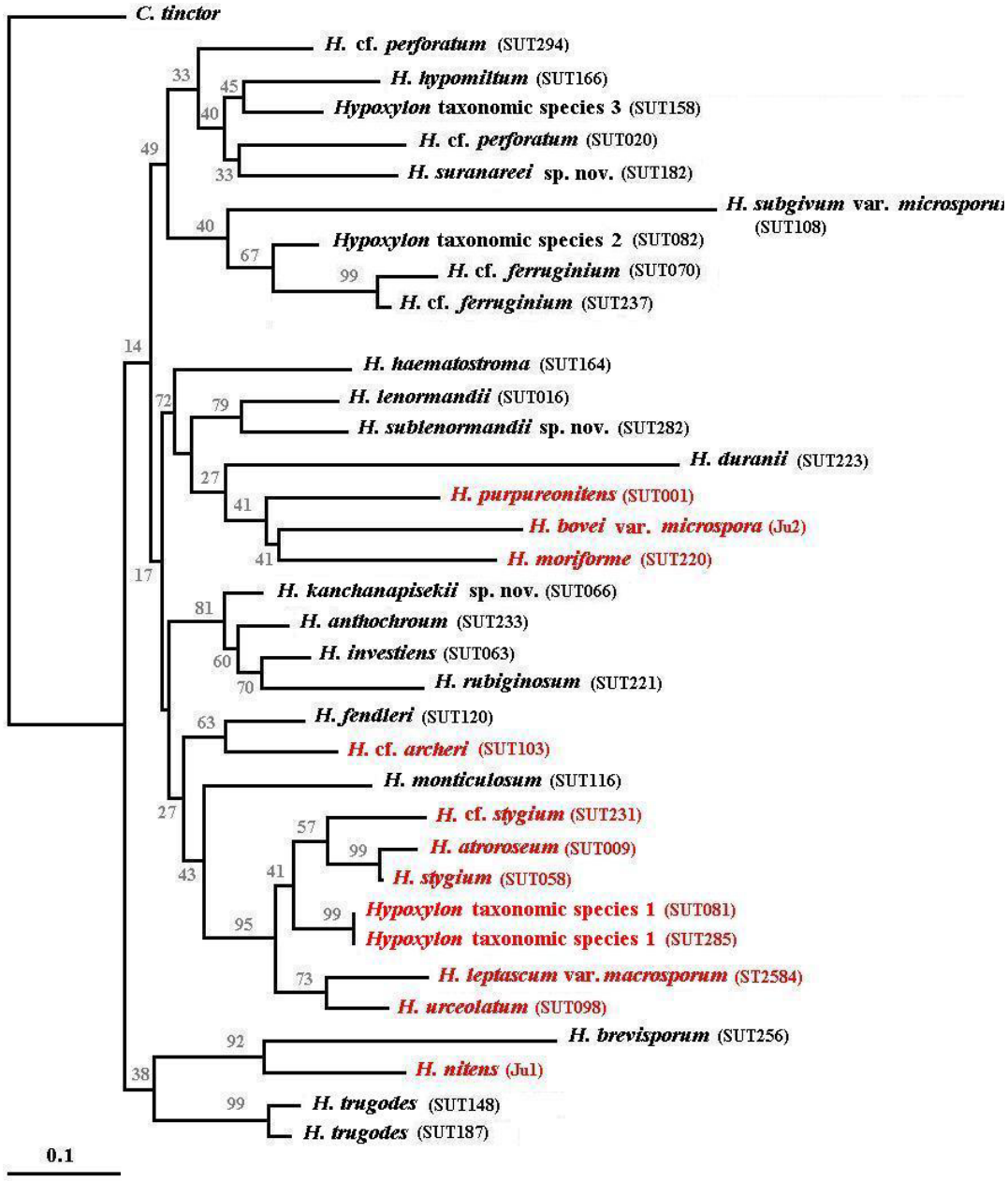

Figure 95. Phylogenetic tree of *Hypoxylon* based on ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replications.

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).

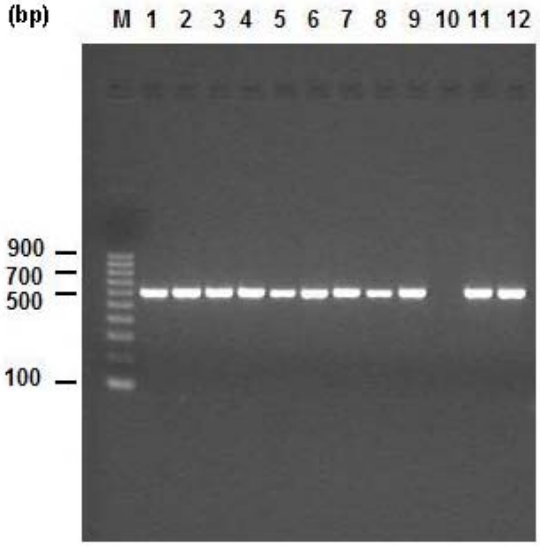

Figure 96. Gel electrophoresis of ITS1-5.8S-ITS2 fragments of *Xylaria* using ITS5 and ITS4 primers. Lanes: M, DNA marker (100 base pair DNA ladder, Invitrogen); 1, *Xylaria* sp. nov. (SUT012); 2, *Xylaria* sp. nov. (SUT014); 3, *X. multiplex* (SUT028); 4, *X. badia* (SUT032); 5, *X. badia* (SUT076); 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).

Table 27. The length of ITS1-5.8S-ITS2 sequences of different species of *Xylaria*, *Kretzschmaria*, *Nemania*, and *Biscogniauxia* found in this study.

Species	Location/Source	ITS1 (bp)	5.8S (bp)	ITS2 (bp)	Total (bp)	GenBank accession number
X. anisopleura (SUT196)	Trad Province	465	155	155	775	DQ322130
X. anisopleura (SUT205)	Trad Province	141	155	155	451	DQ322131
X. anisopleura (ST2329)	RFD*	465	155	155	775	DQ322132
X. cf. *apiculata* (SUT203)	Trad Province	181	155	159	495	DQ322133
X. arbuscula var. *microspora* (ST2372)	RFD*	182	155	162	499	DQ322134
X. badia (SUT032)	Ratchaburi Province	181	155	158	494	DQ322135
X. badia (SUT076)	Ratchaburi Province	179	155	160	494	DQ322136
X. badia (SUT142)	Nakhon Ratchasima Province	181	155	159	495	DQ322137
X. badia (ST2417)	RFD*	181	155	160	496	DQ322138
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	DQ322142
X. cubensis (ST2326)	RFD*	188	155	163	506	DQ322143
X. curta (ST2382)	RFD*	209	155	162	526	DQ322144
X. grammica (ST2348)	RFD*	180	155	159	494	DQ322145
X. grammica (ST2363)	RFD*	180	155	155	490	DQ322146
X. ianthino-velutina (SUT123)	Nakhon Ratchasima Province	177	155	156	488	DQ322147
X. cf. *juruensis* (SUT088)	Songkhla Province	182	155	156	493	DQ322148
X. cf. *juruensis* (SUT140)	Nakhon Ratchasima Province	181	155	162	498	DQ322149
X. juruensis var. *microspora* (SUT129)	Nakhon Ratchasima Province	179	155	159	493	DQ322150
X. juruensis var. *microspora* (SUT138)	Nakhon Ratchasima Province	181	155	159	495	DQ322151
X. juruensis var. *microspora* (SUT139)	Nakhon Ratchasima Province	182	155	159	496	DQ322152
X. maitlandii (SUT177)	Nakhon Ratchasima Province	181	155	159	495	DQ322153
X. multiplex (SUT028)	Ratchaburi Province	178	155	162	495	DQ322154
X. multiplex (ST2298)	RFD*	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 Province	181	155	159	495	DQ322158

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

Table 27. (Continued).

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
Xylaria species 2 (SUT127)	Nakhon Ratchasima Province	174	155	158	487	DQ322162
Xylaria species 2 (SUT130)	Nakhon Ratchasima Province	174	155	157	486	DQ322163
Xylaria 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
Xylaria 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 (SUT207), *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.

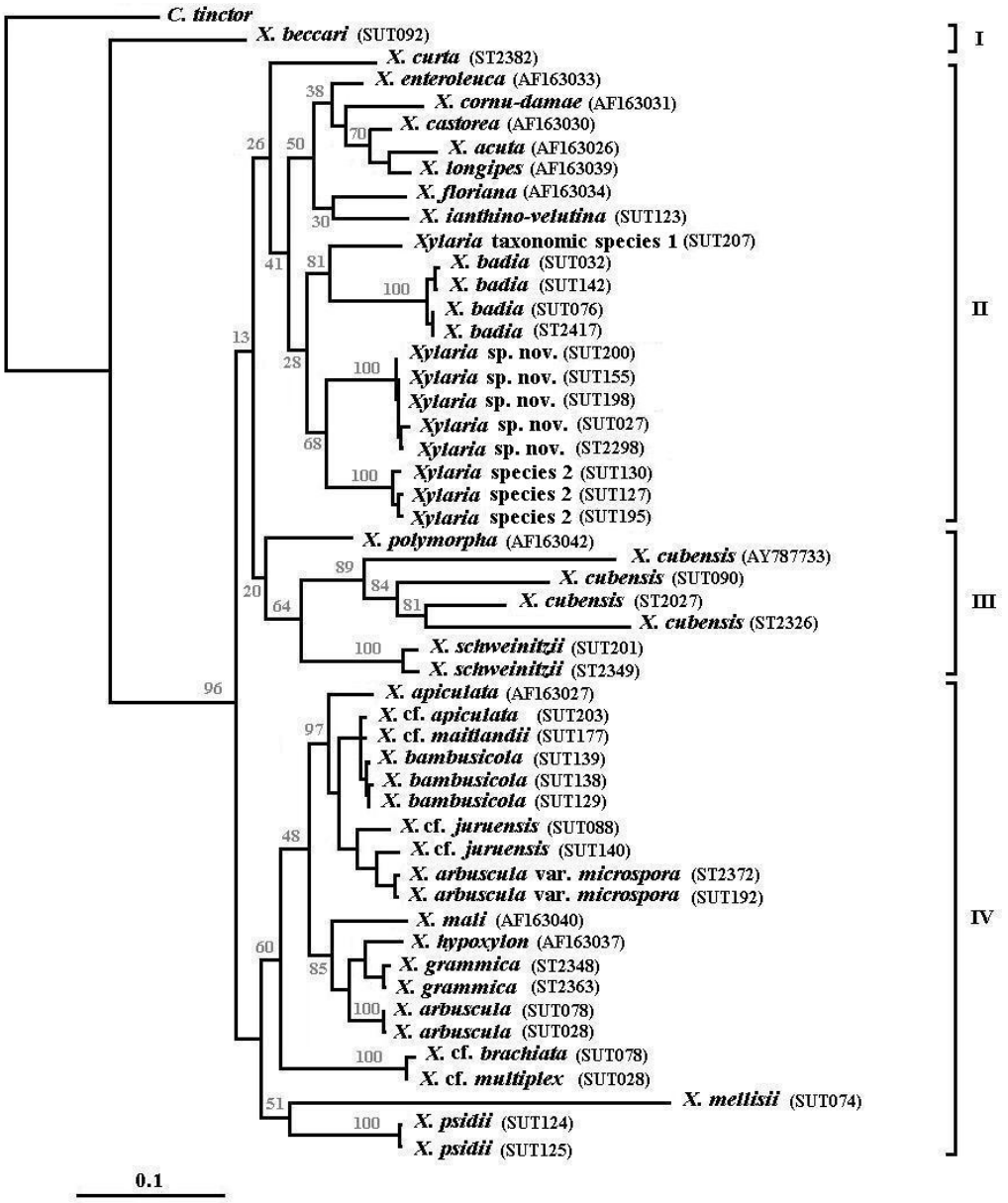

Figure 97. Phylogenetic tree of *Xylaria* based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Camillea tinctor* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000 replications.

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 µm, was smaller than *X. cubensis* (Mont.) Fr., 8-10.5 x 4-5 µm, 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.

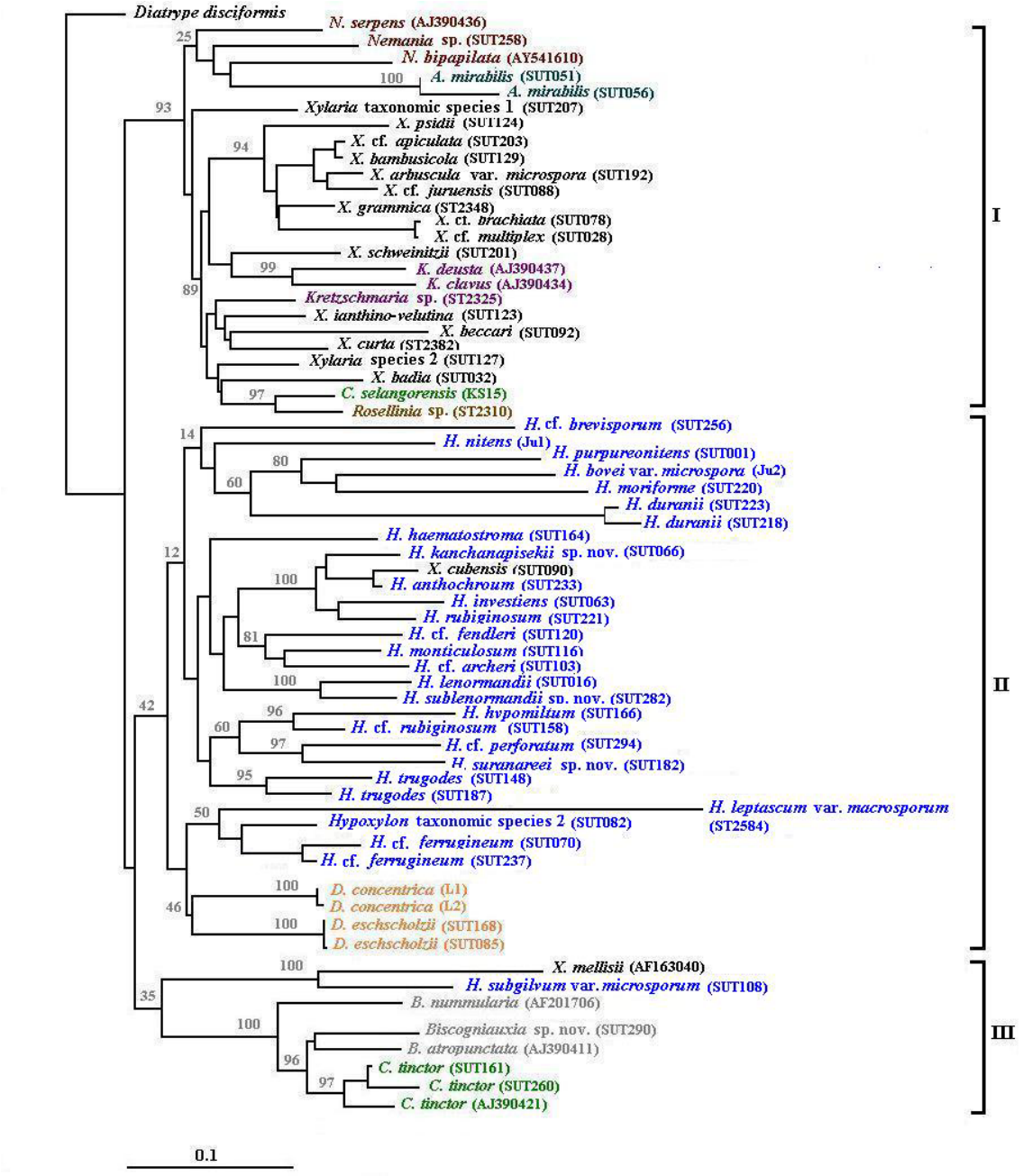

Figure 98. Phylogenetic tree of xylariaceous fungi based on ITS1-5.8S-ITS2 sequences using the neighbour-joining method. *Diatrype disciformis* is the outgroup. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1,000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

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
$MgSO_4.5H_2O$	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
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)

Sodium acetate (CH_3COONa) (Merck)	24.61	g

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

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

3.6 Gel loading buffer (6X)

Bromophenol blue	25.00	g

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

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.

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

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 Ratchasima Province	20-25 September 2003
SUT153 - SUT169	Yasothon Province	15 November 2003
SUT170 - SUT191	Suranaree University of Technology, Nakhon Ratchasima Province	17 November 2003
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 2B. More details of xylariaceous collections.

Code	Species	Stromatal colour	KOH extracted pigments	Ascospore size (µm)
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
SUT006	*Xylaria* sp. nov.	Dark brown to black	Colorless	(7.5)8.8-10x3.8-5
SUT007	*Xylaria* sp. nov.	Dark brown to black	Colorless	8.8-10x3.8-5
SUT008	*Hypoxylon* taxonomic species 1 sp. nov.	Black	Greenish olivaceous	NF
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	*Xylaria* sp.	Brownish black	Colorless	NF
SUT012	*Xylaria* 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	*Xylaria* 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)x5-7.5
SUT018	*H. lenormandii*	Grayish sepia	Red	12.5-15x5-6.3
SUT019	*Eutypa* sp.	-	-	-
SUT020	*H.* cf. *perforatum*	Grayish sepia	Yellowish brown	NF
SUT021	*H. monticulosum*	Brownish vinaceous to black	Colorless	(7.5)8.8-10x5-6.3
SUT022	*H. lenormandii* var. *microspora*	Blackish brown	Red	5-7x2.5-3.8
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	*Xylaria* 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	*Xylaria* sp. nov.	Black	Colorless	NF
SUT031	*Xylaria* sp. nov.	Dark brown to black	Colorless	8.8-10x3.8-5
SUT032	*X. badia*	Silvery brown	Colorless	9.8-12x3.8-5
SUT033	*Xylaria* sp. nov.	Dark brown to black	Colorless	(7.5)8.8-10x3.8-5
SUT034	*Xylaria* 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)11.3-13.8x3.8-5
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
SUT044	*H. monticulosum*	Brownish black	Purple	6.3-7.5x2.5-3.8
SUT045	*H. anthochroum*	Brown vinaceous or chestnut	Dull green	8.8-11.25x3.8-5
SUT046	*H. sublenormandii* sp. nov.	Reddish brown	Reddish brown	(8.8)12.5-15x5-6.3
SUT047	*A. mirabilis*	Black	Colorless	10-13x5
SUT048	*A. mirabilis*	Black	Colorless	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
SUT058	*H. stygium*	Blackish with reddish brown	Greenish olivaceous	3.8-6.3x2.5-3.8

NF = Not found.

Table 2B. (Continued).

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
SUT063	*H. investiens*	Brownish vinaceous	Dull green	(5)7.5-8.8x(2.5)3.8-5
SUT064	*H. haematostroma*	Orange red or rust	Orange red	NF
SUT065	*H. lenormandii*	Grayish sepia	Red	(8.8)10-12.5x3.8-5
SUT066	*H. kanchanabhisakii* sp. nov.	Dull reddish brown	Reddish brown	(8.8)10-11.3x3.8-5
SUT067	*H. kanchanabhisakii* sp. nov.	Dull reddish brown	Reddish brown	(7.5)10-11.3x3.8
SUT068	*H. kanchanabhisakii* 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	*H. kanchanabhisakii* 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	*Xylaria* 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	*Xylaria* 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	*Xylaria* sp. nov.	Dark brown to black	Colorless	7.5-8.8(10)x3.8
SUT088	*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
SUT090	*X. cubensis*	Bronze becoming dark with age	Colorless	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	*Xylaria* 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
SUT104	*H. subgilvum* var. *microsporum*	Dark brick	Orange	(3.8)5-7.5x2.5-3.8
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

NF = Not found.

Table 2B. (Continued).

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	*Xylaria* 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	*Xylaria* 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	*Xylaria* 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	*Xylaria* 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
SUT152	*H.* cf. *fendleri*	Brown	Orange	8.8-10x3.8-5
SUT153	*H. anthochroum*	Brown vinaceous or chestnut	Dull green	NF
SUT154	*H. anthochroum*	Brown vinaceous or chestnut	Dull green	10-12.5x3.8-5
SUT155	*Xylaria* sp. nov.	Dark brown to black	Colorless	7.5-8.8x2.5-3.8

NF = Not found.

Table 2B. (Continued).

Code	Species	Stromatal colour	KOH extracted pigments	Ascospore size (µm)
SUT156	*B. capnodes*	Black	Colorless	12.5-15(18.8)x6.3-7.5
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 layer	Colorless	11.3-12.5x3.8-5
SUT171	*Xylaria* species 2	Dark brown to black	Colorless	10-11.3x2.5-3.8
SUT172	*Xylaria* sp. nov.	Dark brown to black	Colorless	(6.3)7.5-8.8x3.8
SUT173	*Xylaria* 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	*Xylaria* 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
SUT199	*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
SUT201	*X. schweinitzii*	Brownish black to dull black	Colorless	18.8-21.3x6.3-7.5
SUT202	*X. cubensis*	Bronze becoming dark with age	Colorless	6.3-7.5x3.8
SUT203	*Xylaria* taxonomic species 2	Black	Colorless	(7.5)8.8-10x3.8
SUT204	*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	*Xylaria* taxonomic species 4	Copper- to bronze-colored to brown	Colorless	21.3-25x8.8-10

NF = Not found.

Table 2B. (Continued).

Code	Species	Stromatal colour	KOH extracted pigments	Ascospore size (µm)
SUT208	*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
SUT219	*H. atroroseum*	Blackish brown	Greenish olivaceous	5-6.3x2.5
SUT220	*H. moriforme*	Black with shinny	Greenish olivaceous	7.5-8.8x3.8
SUT221	*H. rubiginosum*	Reddish brown	Yellowish 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	NF
SUT236	*Hypoxylon* 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	*Hypoxylon* 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	*Hypoxylon* 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
SUT244	*Hypoxylon* 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	*Hypoxylon* 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
SUT248	*H. duranii*	Brown vinaceous or chestnut	Reddish brown	(6.7)8.1-9.5x4.3-5
SUT249	*H. stygium*	Blackish with reddish brown	Greenish olivaceous	7.5-8.8x3.1-4.4
SUT250	*H. sublenormandii* sp. nov.	Reddish brown	Reddish brown	8.9-11.3x3.4-4.7
SUT251	*Hypoxylon* 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	*Hypoxylon* taxonomic species 1 sp. nov.	Black with shinny	Greenish olivaceous	6.6-8x3.4-4.6
SUT256	*H. brevisporum*	Brown vinaceous or chestnut	Hazel	6.1-7.2x2.7-3.7
SUT257	*H. stygium*	Blackish with reddish brown	Greenish olivaceous	5.6-6.3x2.1-2.8
SUT258	*Nemania* sp.	Black	Colorless	8.9-11.7x4.7-6
SUT259	*H. duranii*	Brown vinaceous or chestnut	Reddish brown	9-10.5x4.6-5.5
SUT260	*C. tinctor*	Black	Colorless	13.3-18.4x5.5-7.3
SUT261	*Nemania* species	Black	Colorless	11.8-14.9x7.3-8.7

NF = Not found.

Table 2B. (Continued).

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
SUT265	*H. monticulosum*	Brownish vinaceous to black	Purple	6.8-9x2.8-3.9
SUT266	*H. monticulosum*	Brownish vinaceous to black	Purple	6.9-8.5x3-3.6
SUT267	*H. monticulosum*	Brownish vinaceous to black	Purple	NF
SUT268	*D. eschscholzii*	Brown vinaceous	Purple	10.8-13.2x5.8-6.6
SUT269	*Xylaria* sp. nov.	Dark brown to black	Colorless	8.9-10.3x3.9-5
SUT270	*X. cubensis*	Bronze becoming dark with age	Colorless	7.5-8.8x3.8-5
SUT271	*X. cubensis*	Bronze becoming dark with age	Colorless	(6.3)7.5-8.8x3.8-5
SUT272	*Xylaria* sp. nov.	Dark brown to black	Colorless	7.5-9x4.1-4.9
SUT273	*Xylaria* sp. nov.	Dark brown to black	Colorless	7.1-9x3.6-4.8
SUT274	*Xylaria* species 2	Dark brown to black	Colorless	9.5-12.1x3.4-4.5
SUT275	*Xylaria* sp. nov.	Dark brown to black	Colorless	7.6-8.8x4-5
SUT276	*Xylaria* taxonomic species 5	Dark brown to black	Colorless	12.5-14.7x4.5-6.2
SUT277	*X. cubensis*	Bronze becoming dark with age	Colorless	7.5-9x3.8-5
SUT278	*D. eschscholzii*	Brown vinaceous	Purple	10-12.3x4.7-6.1
SUT279	*H.* cf. *fendleri*	Brownish vinaceous	Orange	NF
SUT280	*H.* cf. *fendleri*	Brownish vinaceous	Orange	8.4-10.9x3.9-5
SUT281	*H. duranii*	Brown vinaceous or chestnut	Reddish brown	9.7-11.7x4.5-5.5
SUT282	*H. sublenormandii* sp. nov.	Reddish brown	Reddish brown	9-11.8x4.5-5.1
SUT283	*H. lenormandii*	Grayish sepia	Red	9.5-11.9x4.6-5.9
SUT284	*H. duranii*	Brown vinaceous or chestnut	Reddish brown	9-10.9x4-5.3
SUT285	*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
SUT287	*H. monticulosum*	Brownish vinaceous to black	Purple	6.8-8.4x3.3-4
SUT288	*H. nitens*	Black with shinny	Greenish olivaceous	7.2-8.6x3.1-4.3
SUT289	*H. monticulosum*	Brownish vinaceous to black	Colorless	NF
SUT290	*Biscogniauxia* sp.	Black	Colorless	9.2-11.9x5.4-6.7
SUT291	*H.* cf. *fendleri*	Brownish vinaceous	Orange	8.8-10x3.8-5
SUT292	*H. haematostroma*	Orange red or rust	Orange red	13-15.2x6.3-7.9
SUT293	*H. haematostroma*	Orange red or rust	Orange red	15.6-17.9x7.1-8.6
SUT294	*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
SUT296	*H. rubiginosum*	Brown vinaceous	Yellowish brown	(8.8)10-11.3x5-6.3
SUT297	*H. rubiginosum*	Brown vinaceous	Yellowish brown	8.8-10x3.8-5
SUT298	*H. nitens*	Black with shinny	Greenish olivaceous	10-12.5x3.8-5
SUT299	*H. nitens*	Black with shinny	Greenish olivaceous	10-11.3(12.5)x3.8-5(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
SUT302	*H. monticulosum*	Brownish black to black	Colorless	6.3-7.5x3.8
SUT303	*H. sublenormandii* 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
SUT305	*H. sublenormandii* 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
SUT307	*H. lenormandii*	Grayish sepia	Red	8.8-10x2.5-3.8
SUT308	*H.* cf. *fendleri*	Brownish vinaceous	Orange	8.8-10x3.8-5
SUT309	*X. badia*	Silvery brown	Colorless	8.8-11.3x3.8-5
SUT310	*X. badia*	Silvery brown	Colorless	8.8-11.3x3.8-5
SUT311	*H. sublenormandii* sp. nov.	Reddish brown	Reddish brown	(10)11.3-13.8x5-7.5
SUT312	*H. lenormandii*	Grayish sepia	Red	11.3-12.5x3.8-5
SUT313	*H. lenormandii*	Grayish sepia	Red	8.8-11.3x3.8-5
SUT314	*H. fendleri*	Brownish vinaceous	Orange	8.8-12.5x5-6.3
SUT315	*H. monticulosum*	Brownish vinaceous to black	Purple	6.3-7.5x3.8-5
SUT316	*H. nitens*	Black	Greenish olivaceous	10-12.5x3.8-5(6.3)
SUT317	*H. nitens*	Black	Greenish olivaceous	10-12.5x3.8-5(6.3)
SUT318	*H. purpureonitens*	Blackish with reddish brown	Purple	8.8-12.5x3.8-5

NF = Not found.

Table 2B. (Continued).

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

NF = Not found.

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.

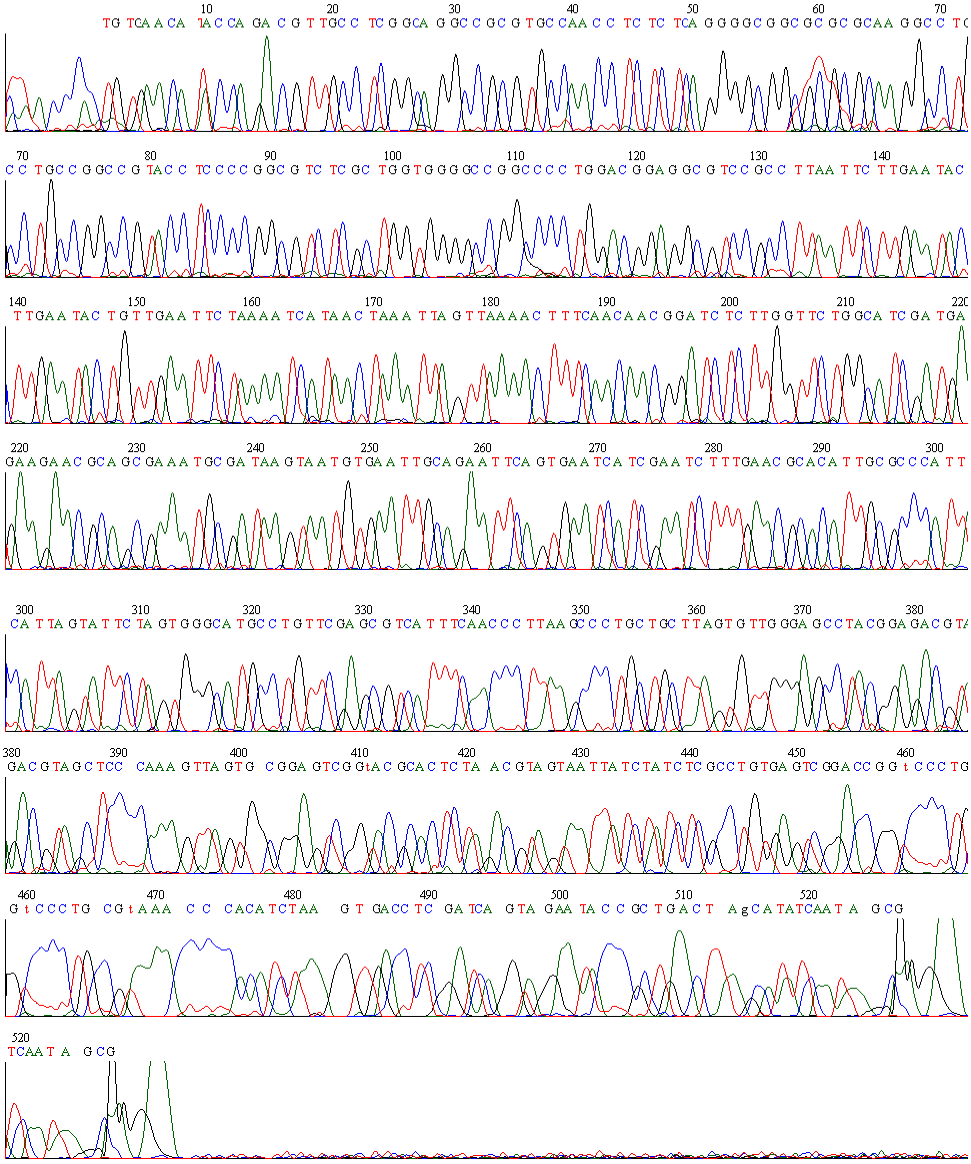

Figure 1C. Sequence electrophenogram of ITS1-5.8S-ITS2 region of *Astrocystis mirabilis* (SUT051) using ITS5 primer.

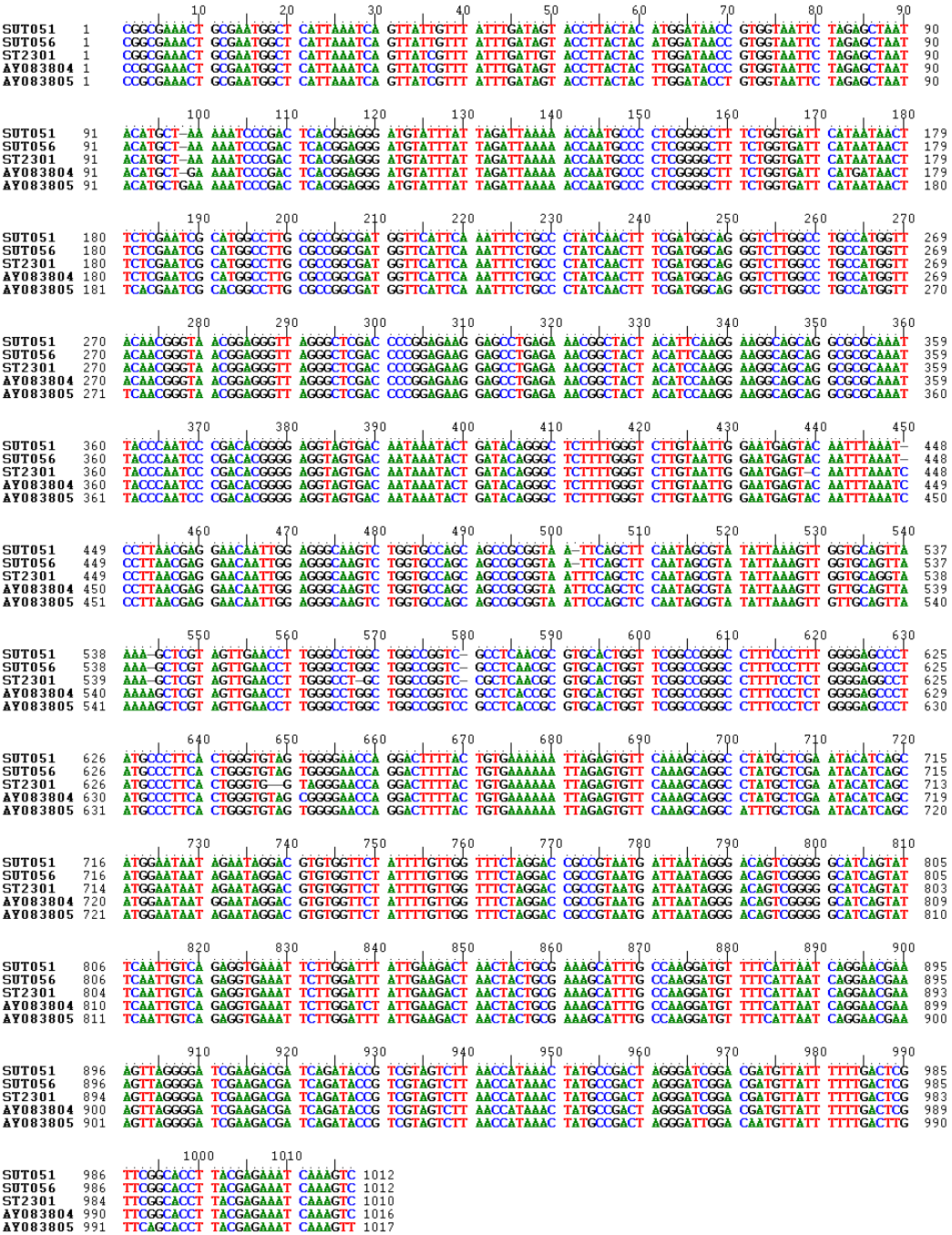

Figure 2C. Multiple sequence alignment of partial 18S rDNA using NS1 and NS4 primers of *Astrocystis mirabilis* (SUT051, SUT056) and *Rosellinia* sp. (ST3201) examined compared to DNA sequences from GenBank database, *R. necatrix* (AY083805) and *A. cocoes* (AY083804), by using ClustalX and BioEdit program.

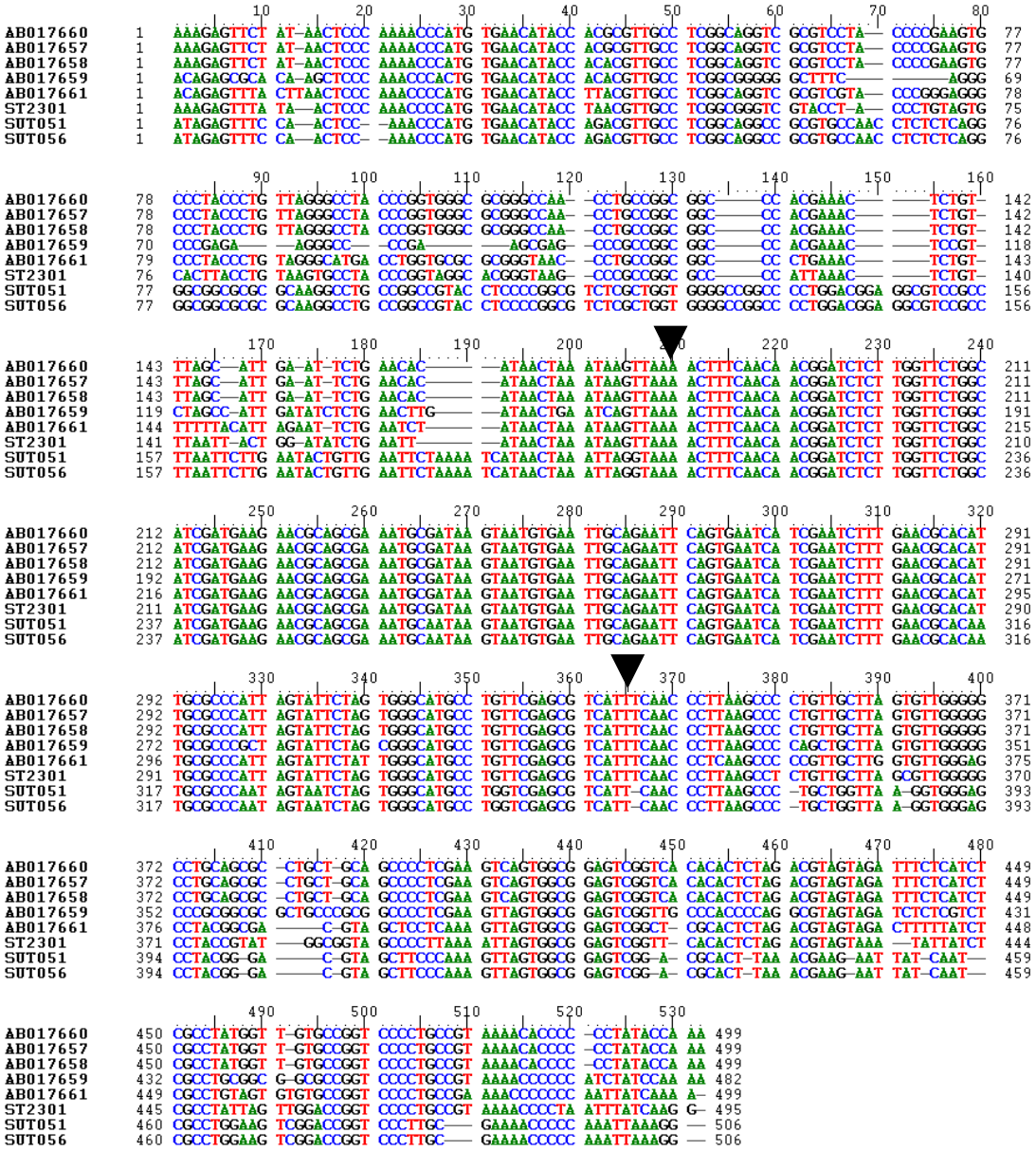

Figure 3C. The ITS1-5.8S-ITS2 sequence alignment of *Astrocystis mirabilis* (SUT056, SUT051) and *Rosellinia* sp. (ST2301) examined compared to DNA sequences from GenBank database, *R. arcuata* (AB017660), *R. pepo* (AB017659), *R. quercina* (AB017661), and *R. necatrix* (AB017657 and AB017658), by using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

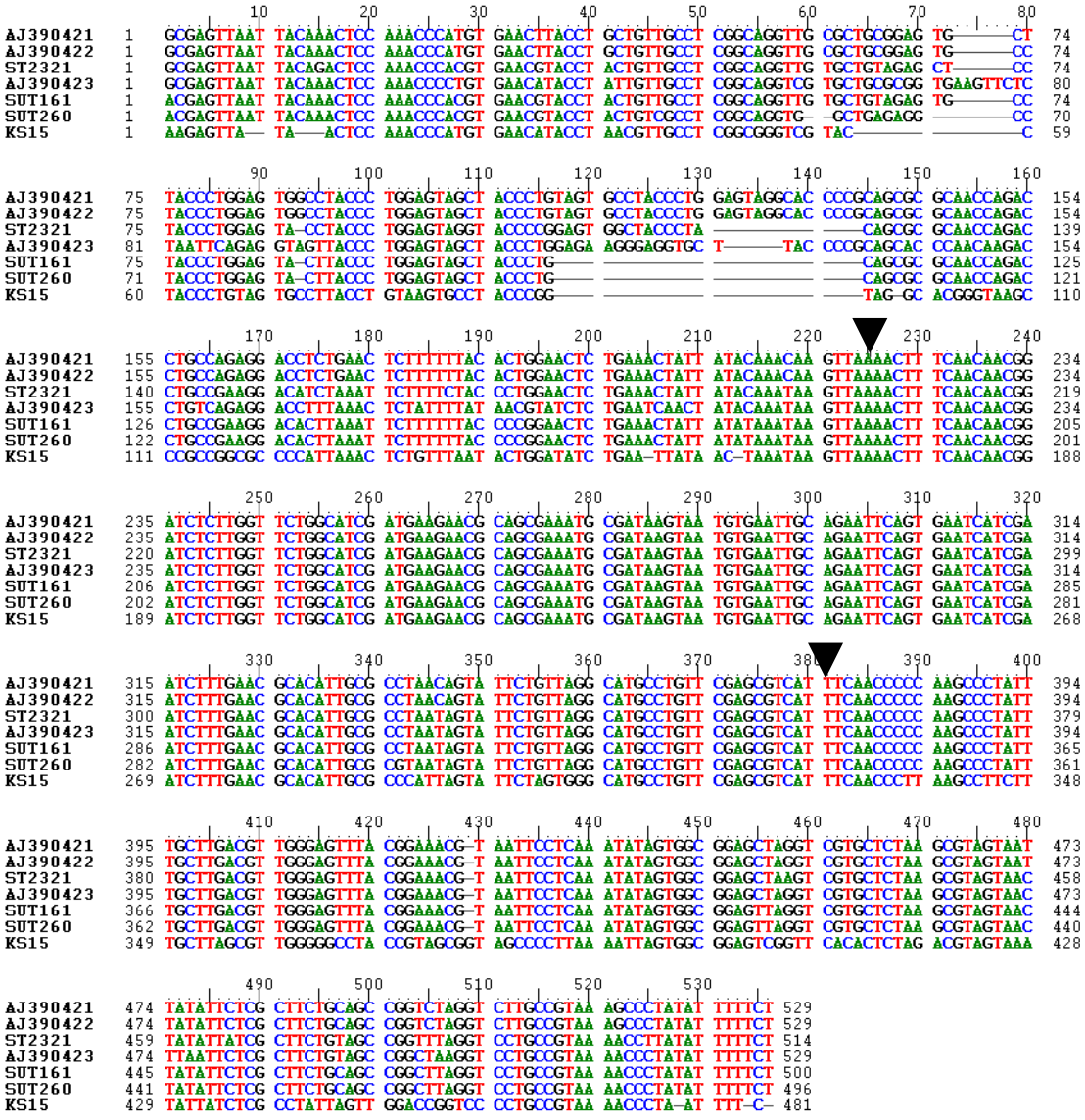

Figure 4C. The ITS1-5.8S-ITS2 sequence alignment of *Camillea tinctor* (AJ390421, AJ390422), *C. tinctor* (ST2321), *C. obularia* (AJ390423), *C. tinctor* (SUT161), *C. tinctor* (SUT260), and *C. selangorensis* (KS15) by using ClustalX and BioEdit programs for phylogenetic tree construction in Figure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

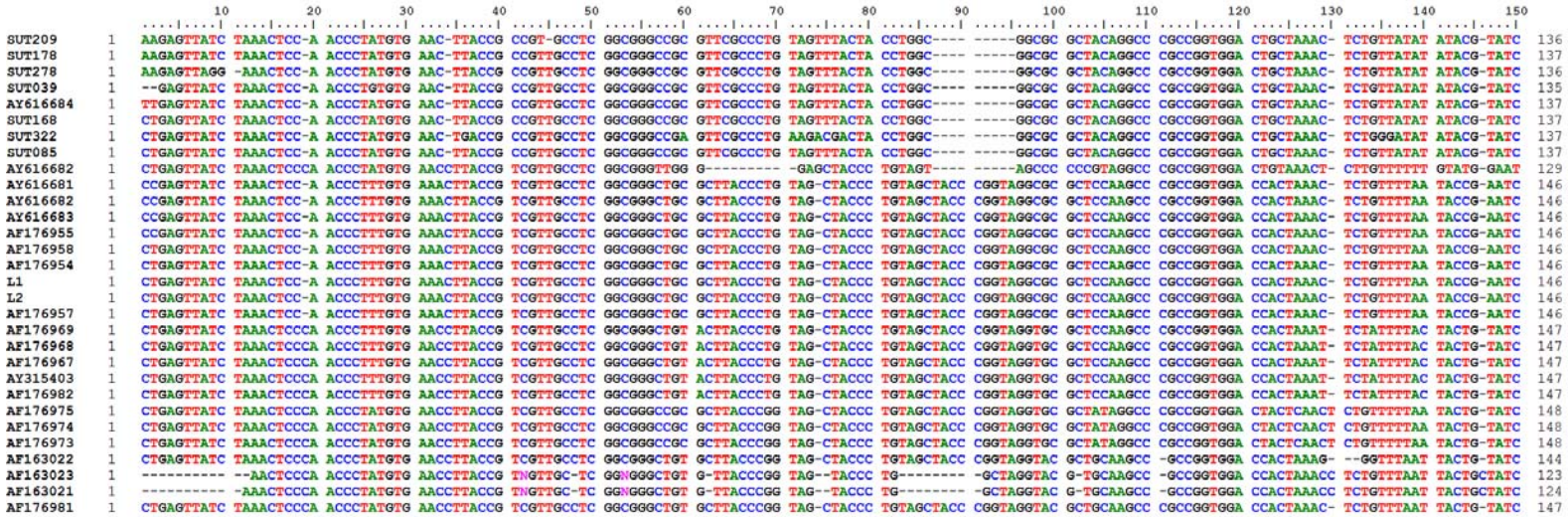

Figure 5C. The ITS1-5.8S-ITS2 sequence alignment of *Daldinia eschscholzii* (SUT209, SUT178, SUT278, SUT039, SUT168, SUT322, SUT085, AY616684) and *D. concentrica* (L1 and L2) examined compared to DNA sequences from GenBank database, *D. concentrica* (AY616682, AY616681, AY616683, AY176955, AY176958, AY17695854), *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.

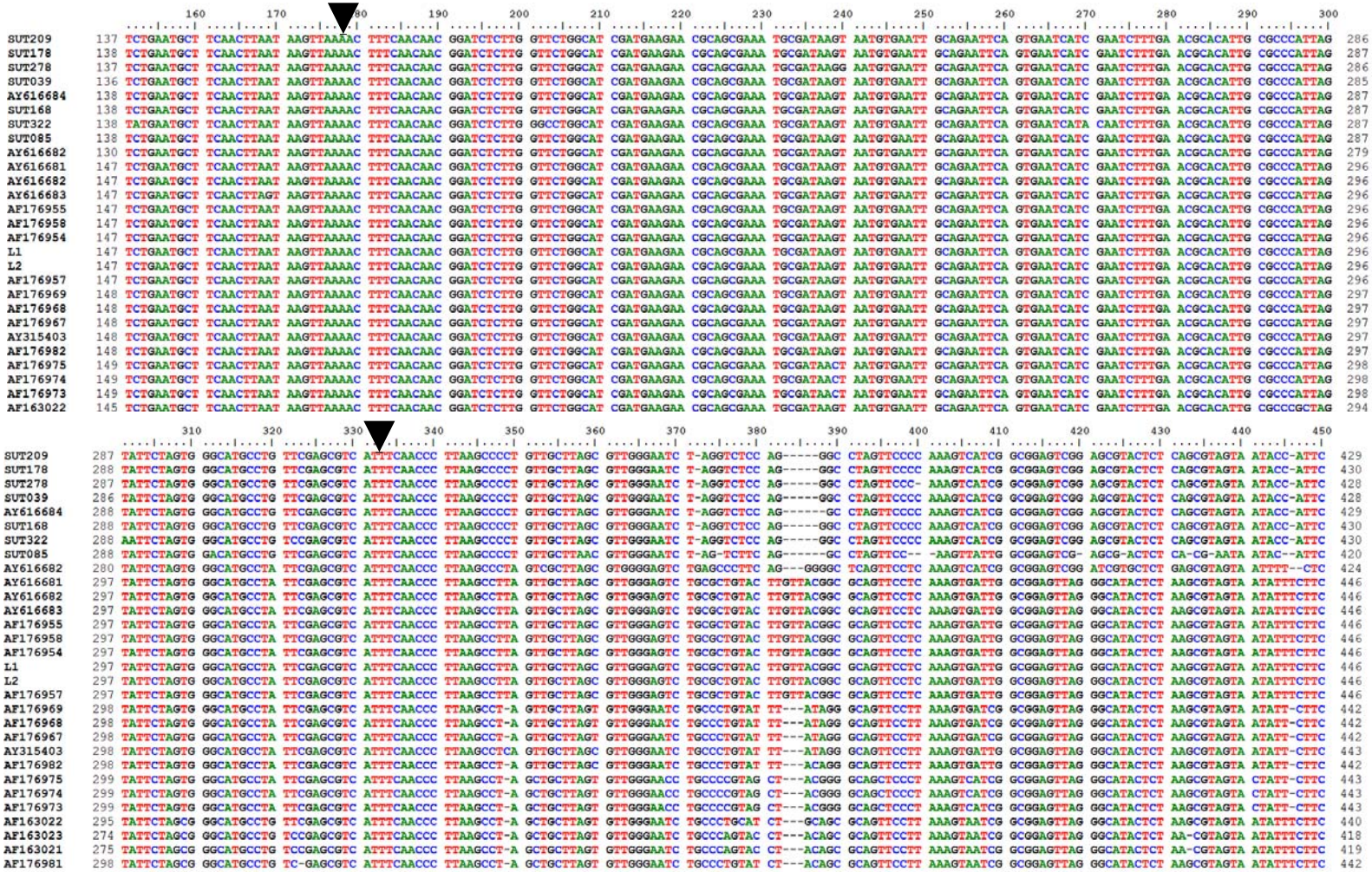

Figure 5C. (Continued).

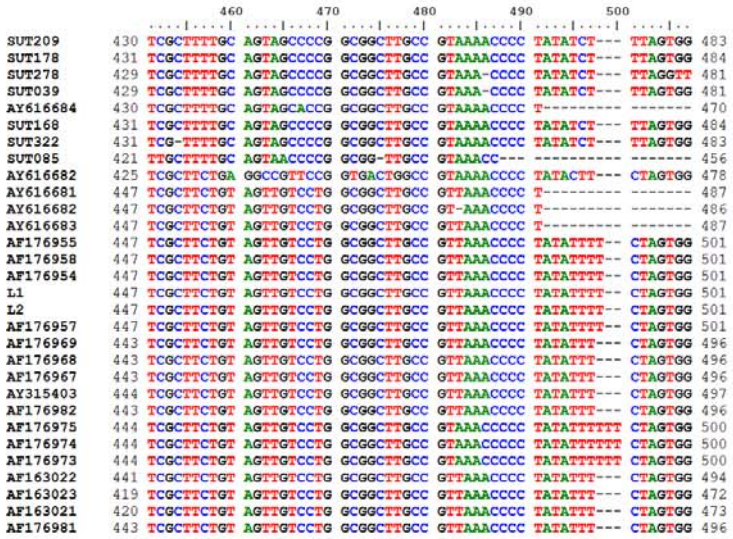

Figure 5C. (Continued).

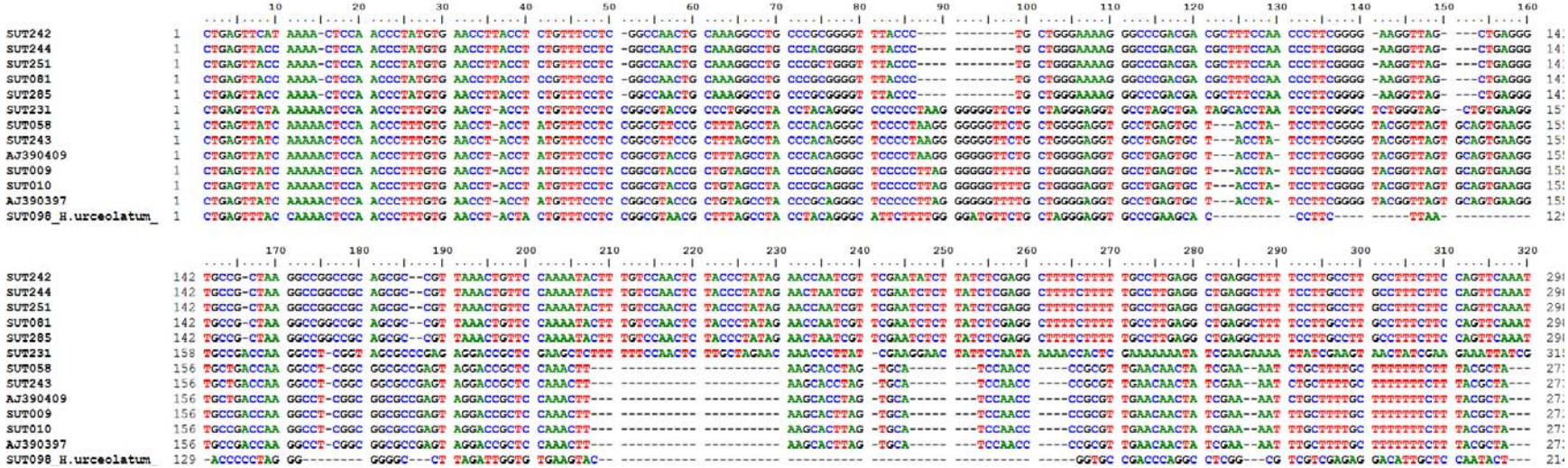

Figure 6C. The ITS1-5.8S-ITS2 sequence alignment of *Hypoxylon* sect. *Annulata*, *Hypoxylon* cf. *nitens* (SUT242, SUT244, SUT251, SUT081, SUT285), *H.* cf. *stygium* (SUT231), *H. stygium* (SUT058, SUT243, AJ390409), *H. atroroseum* (SUT009, SUT010, AJ390397), *H. urceolatum* (SUT098), by using ClustalX and BioEdit programs for phylogenetic tree construction in Figure 90. Arrows indicate the start and the end of 5.8S rDNA sequences.

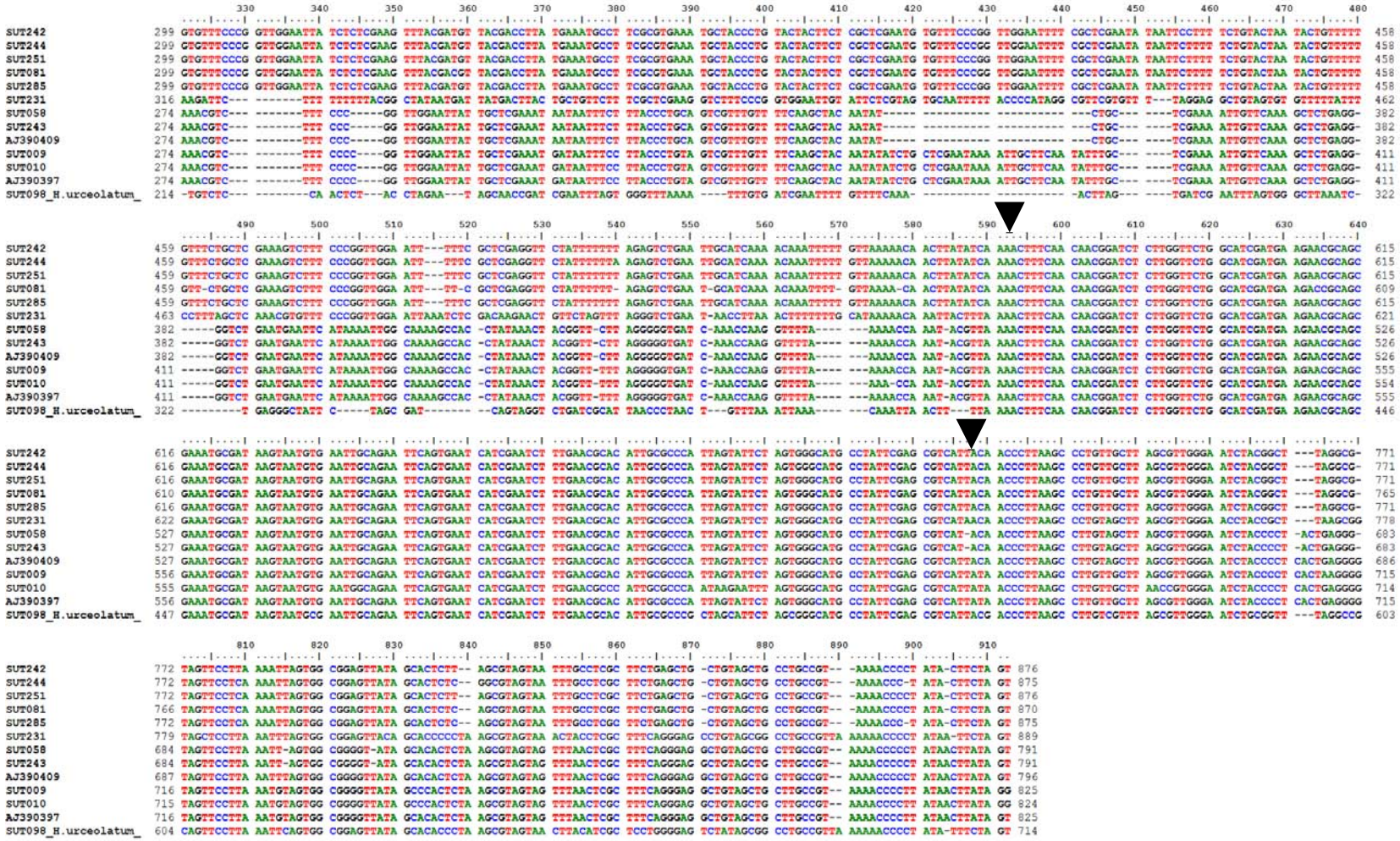

Figure 6C. (Continued).

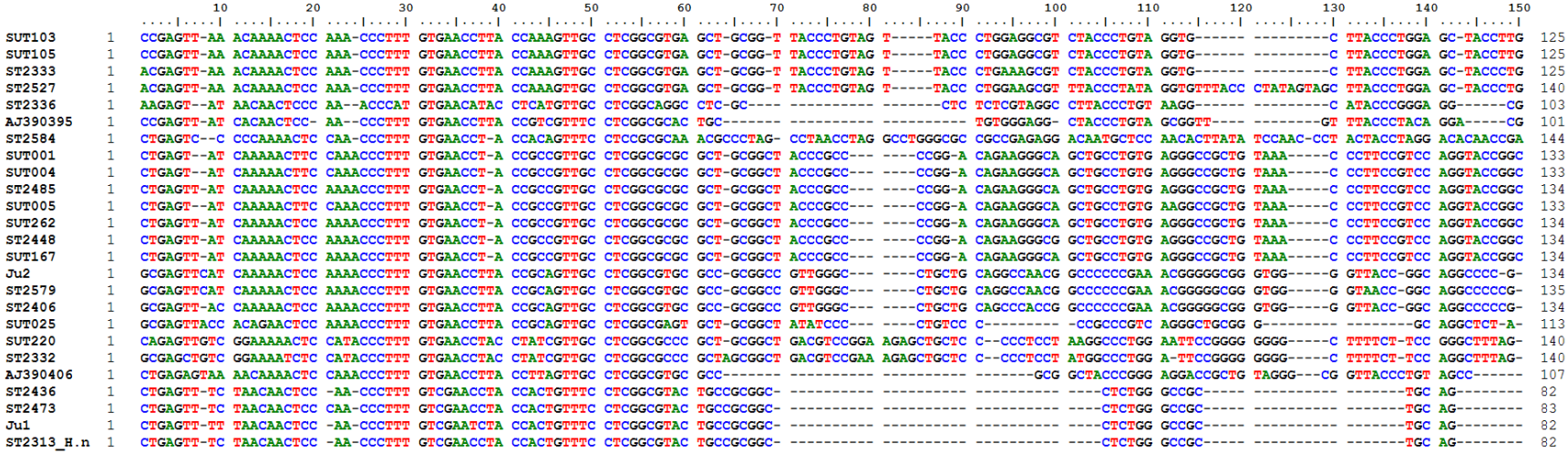

Figure 7C. The ITS1-5.8S-ITS2 sequence alignment of *Hypoxylon* sect. *Annulata*, *Hypoxylon* cf. *archeri* (SUT103, SUT105, ST2333, ST2527), *Hypoxylon* sp. ST2336, *H. annulatum* (AJ390395), *H. leptascum* var. *microsporum* (ST2584), *H. purpureonitens* (SUT001, SUT004, SUT005, SUT167, SUT262, ST2485, 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.

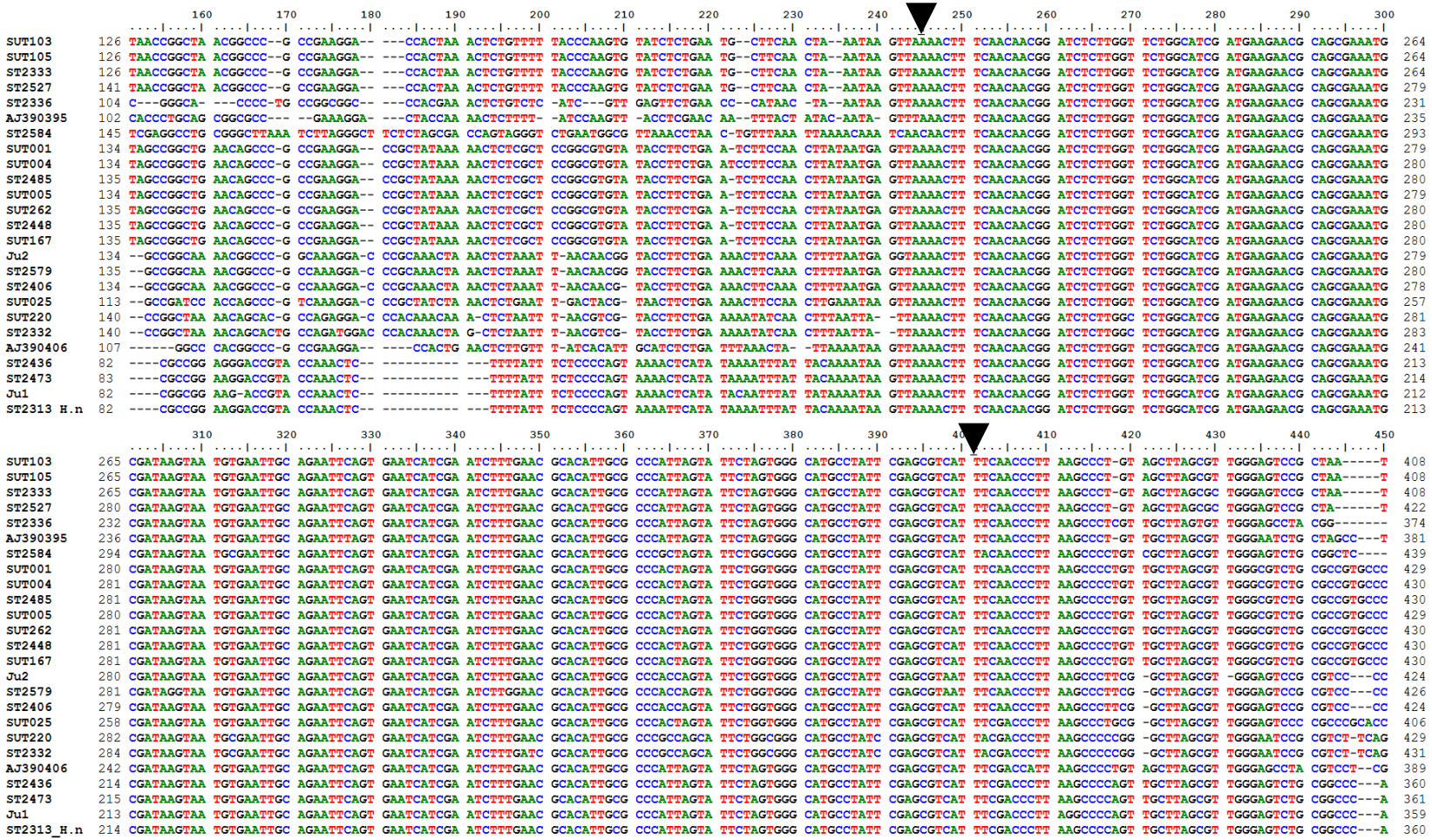

Figure 7C. (Continued).

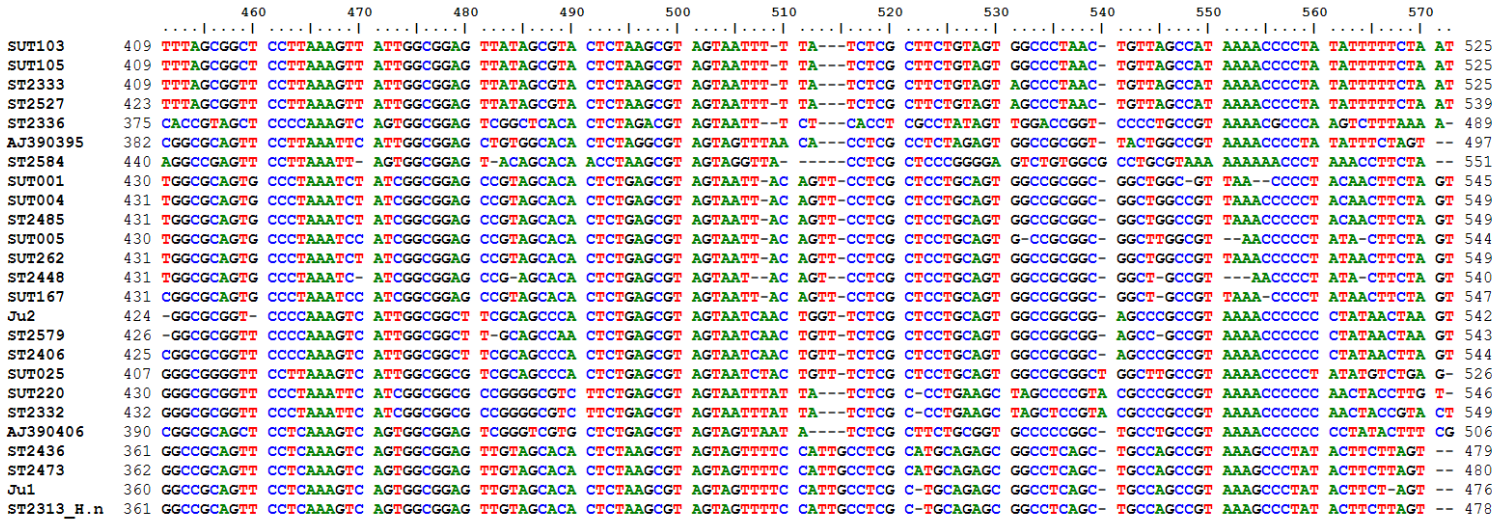

Figure 7C. (Continued).

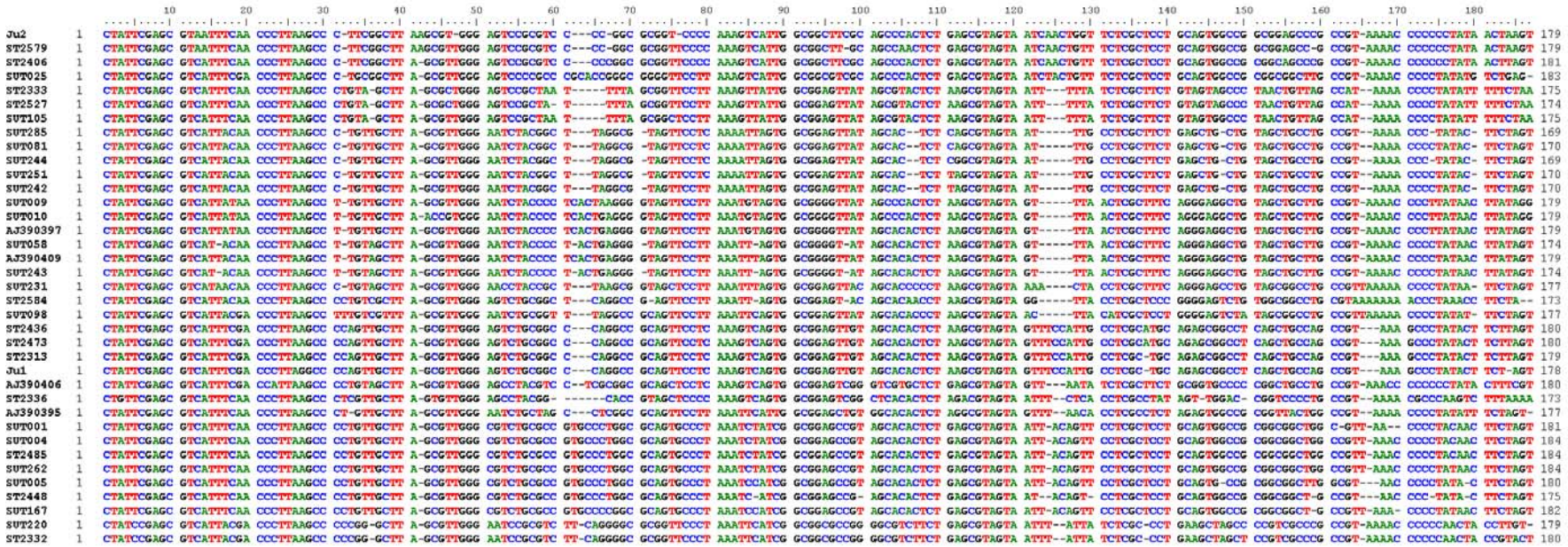

Figure 8C. The ITS2 sequence alignment of *Hypoxylon* sect. *Annulata* for phylogenetic tree construction in Figure 95 by using ClustalX and BioEdit programs. Arrows indicate the start and the end of 5.8S rDNA sequences.

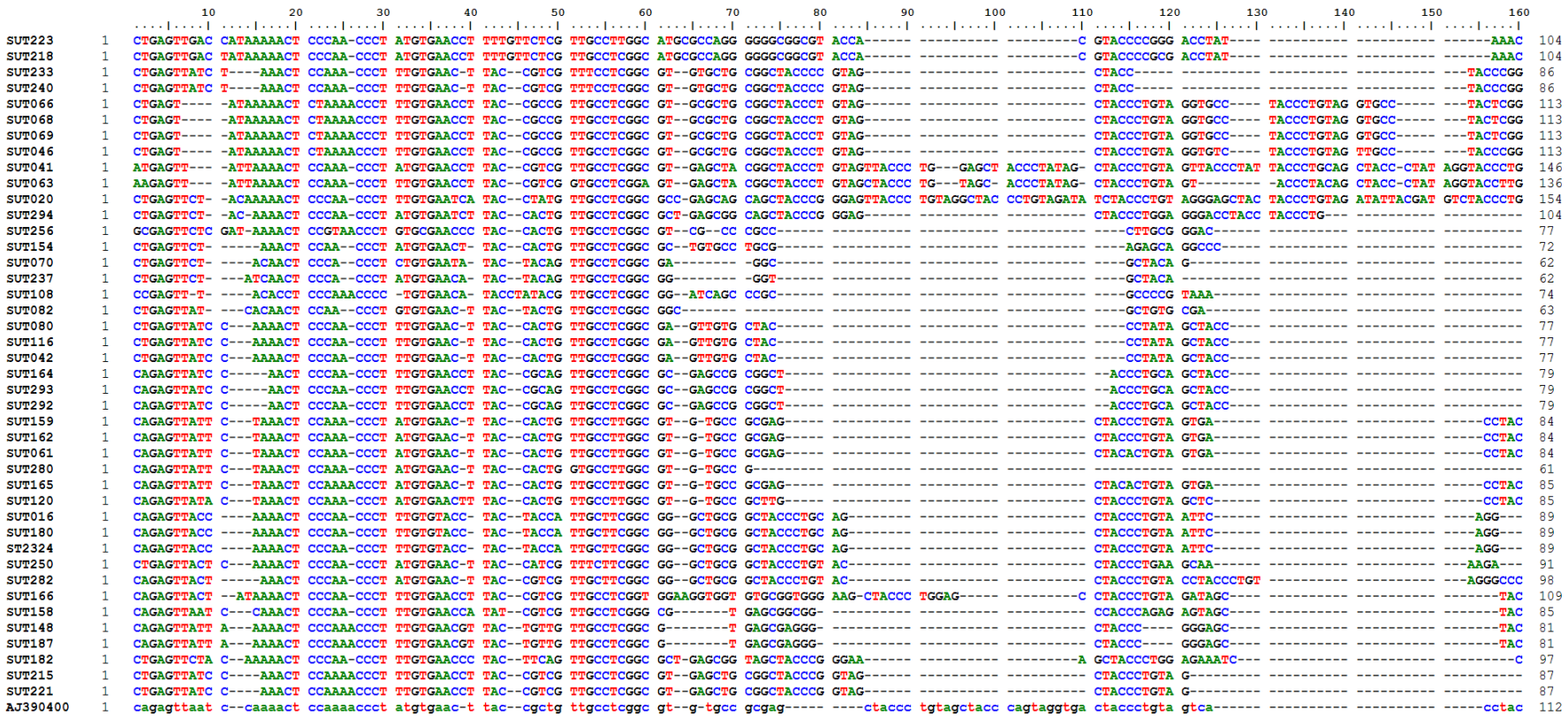

Figure 9C. The ITS1-5.8S-ITS2 multiple sequence alignment of *Hypoxylon* sect. *Hypoxylon* for phylogenetic tree construction in Figure 94 by using ClustalX and BioEdit programs. Arrows indicate the start and the end of 5.8S rDNA sequences.

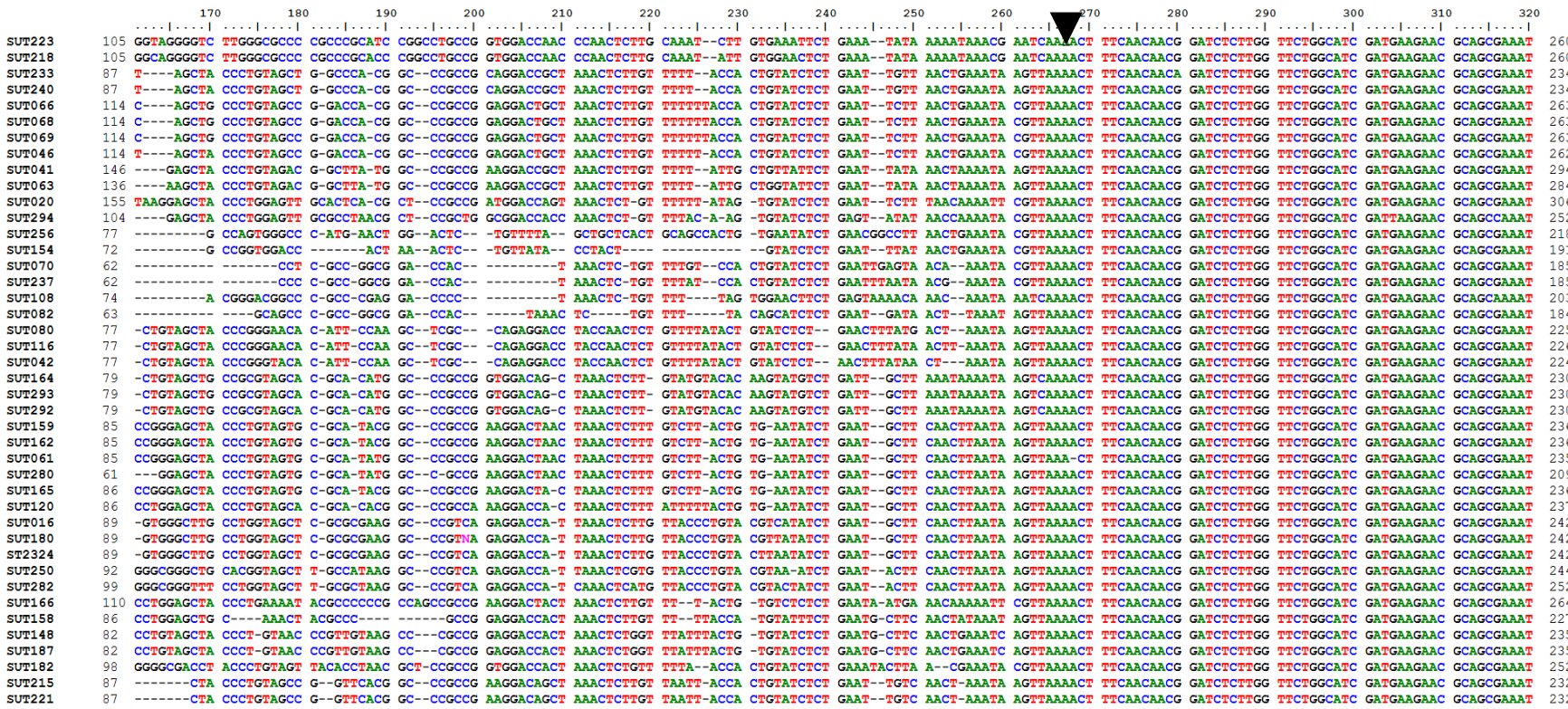

Figure 9C. (Continued).

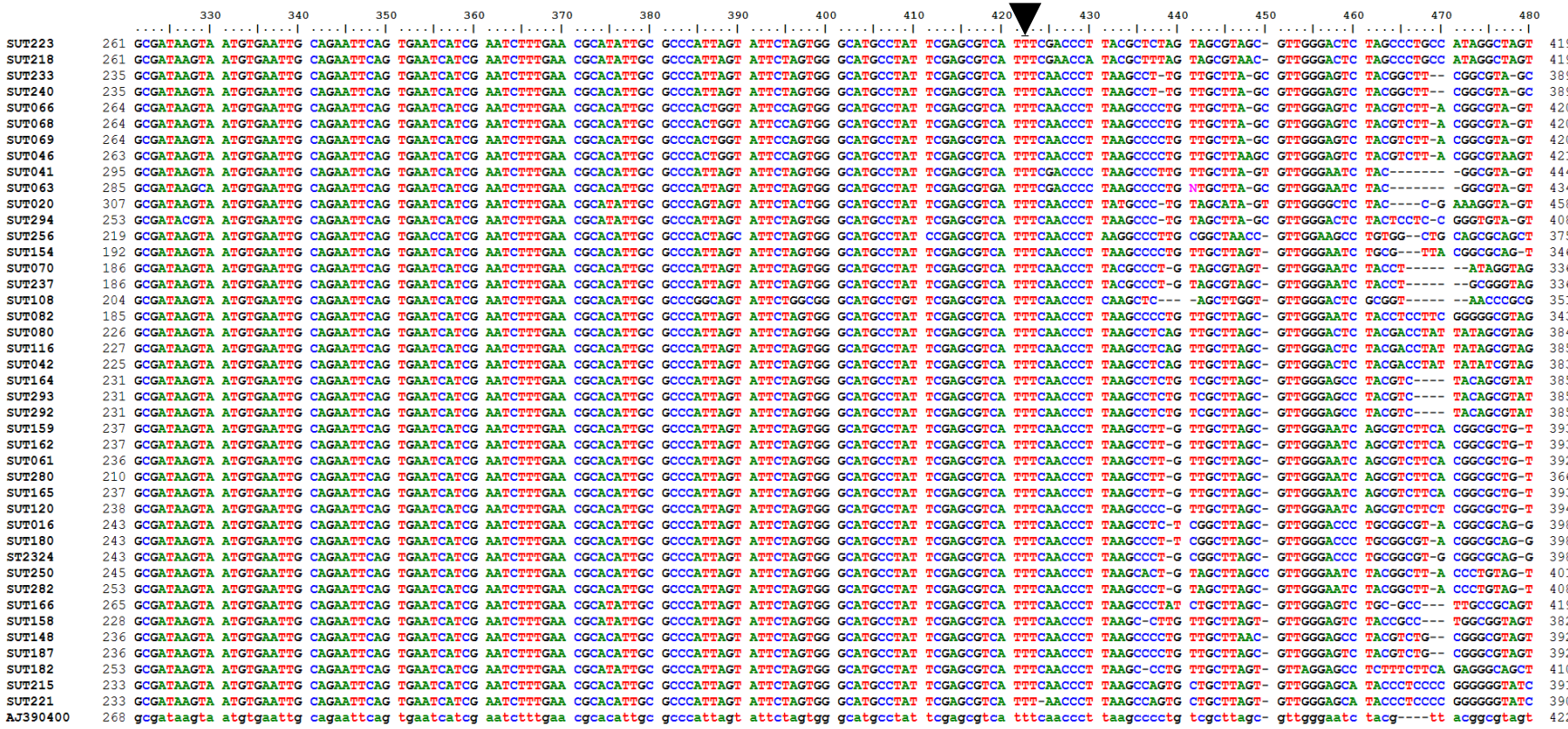

Figure 9C. (Continued).

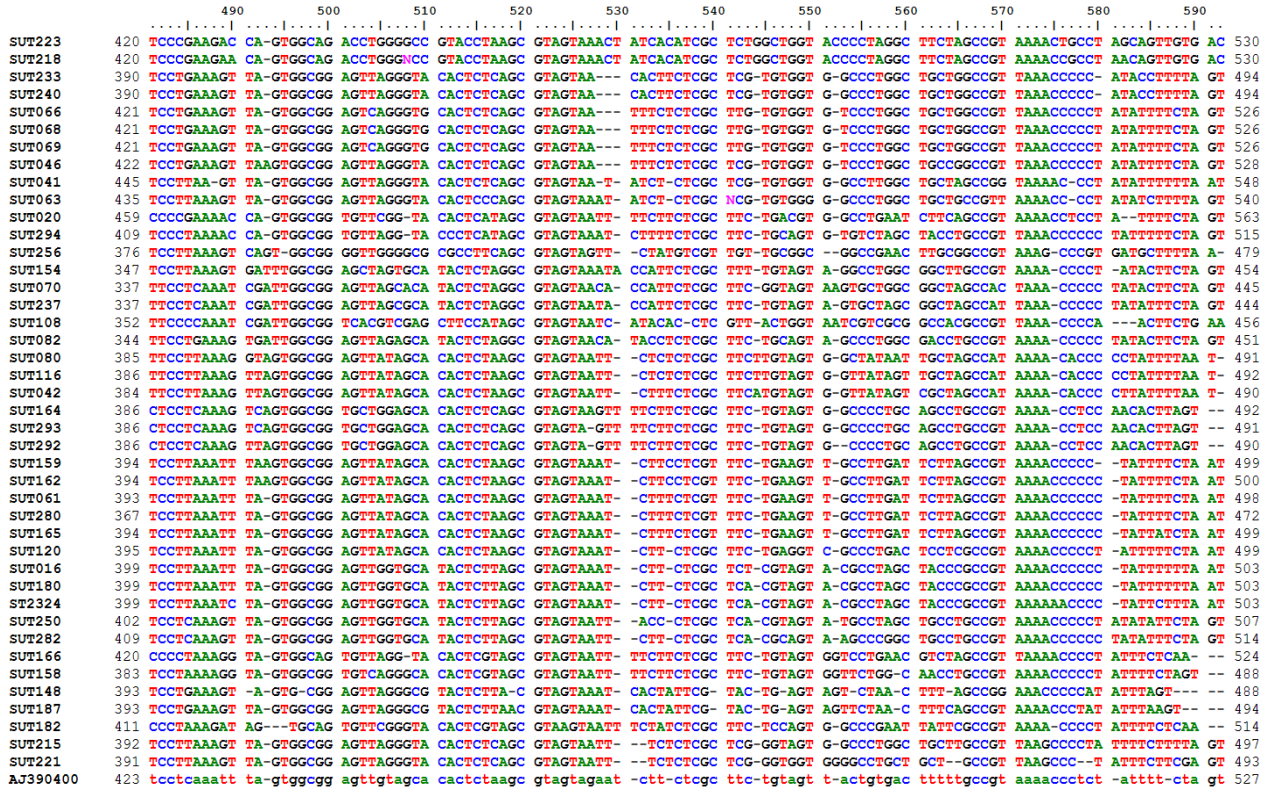

Figure 9C. (Continued).

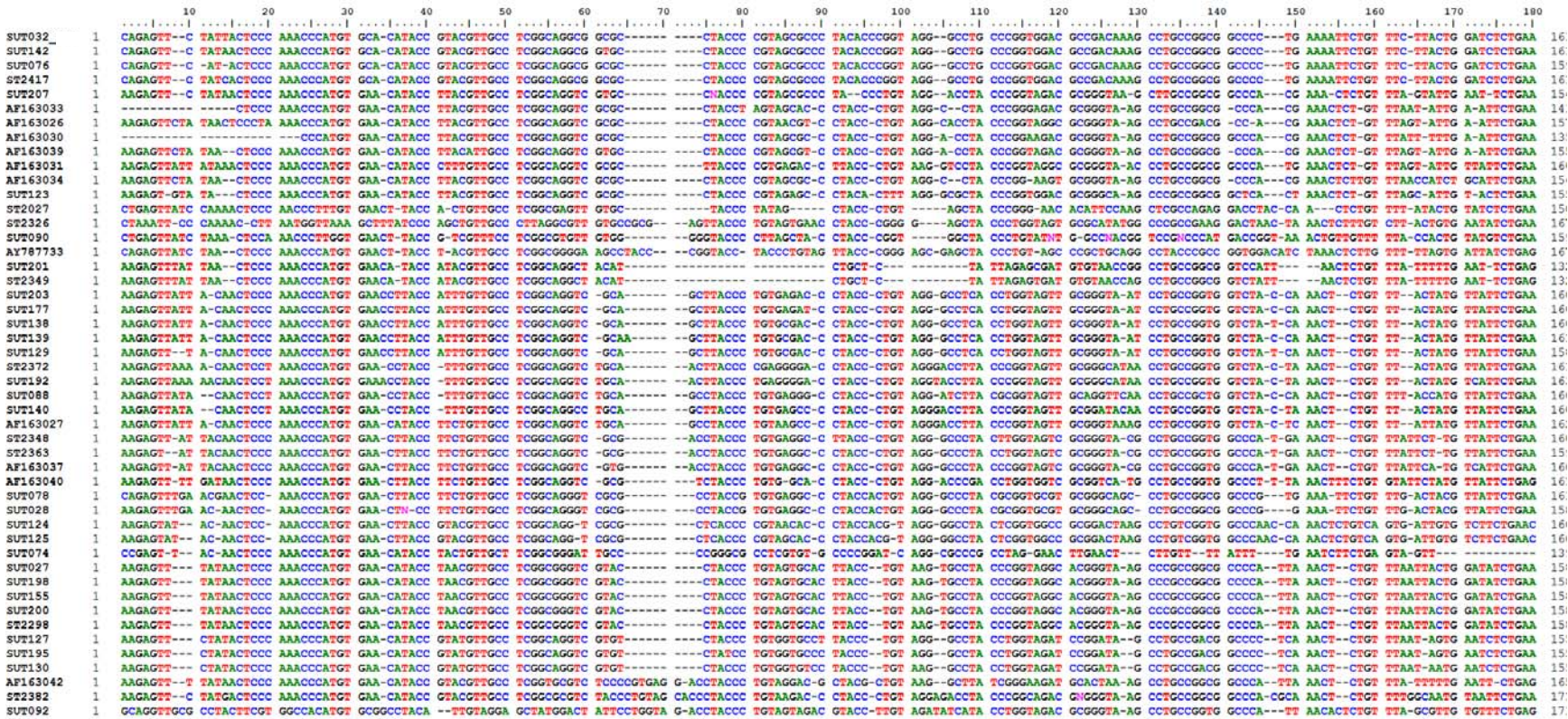

Figure 10C. The ITS1-5.8S-ITS2 sequence alignment of *Xylaria* specimens for phylogenetic construction in Figure 97 by using ClustalX and BioEdit programs. Arrows indicate the start and the end of 5.8S rDNA sequences.

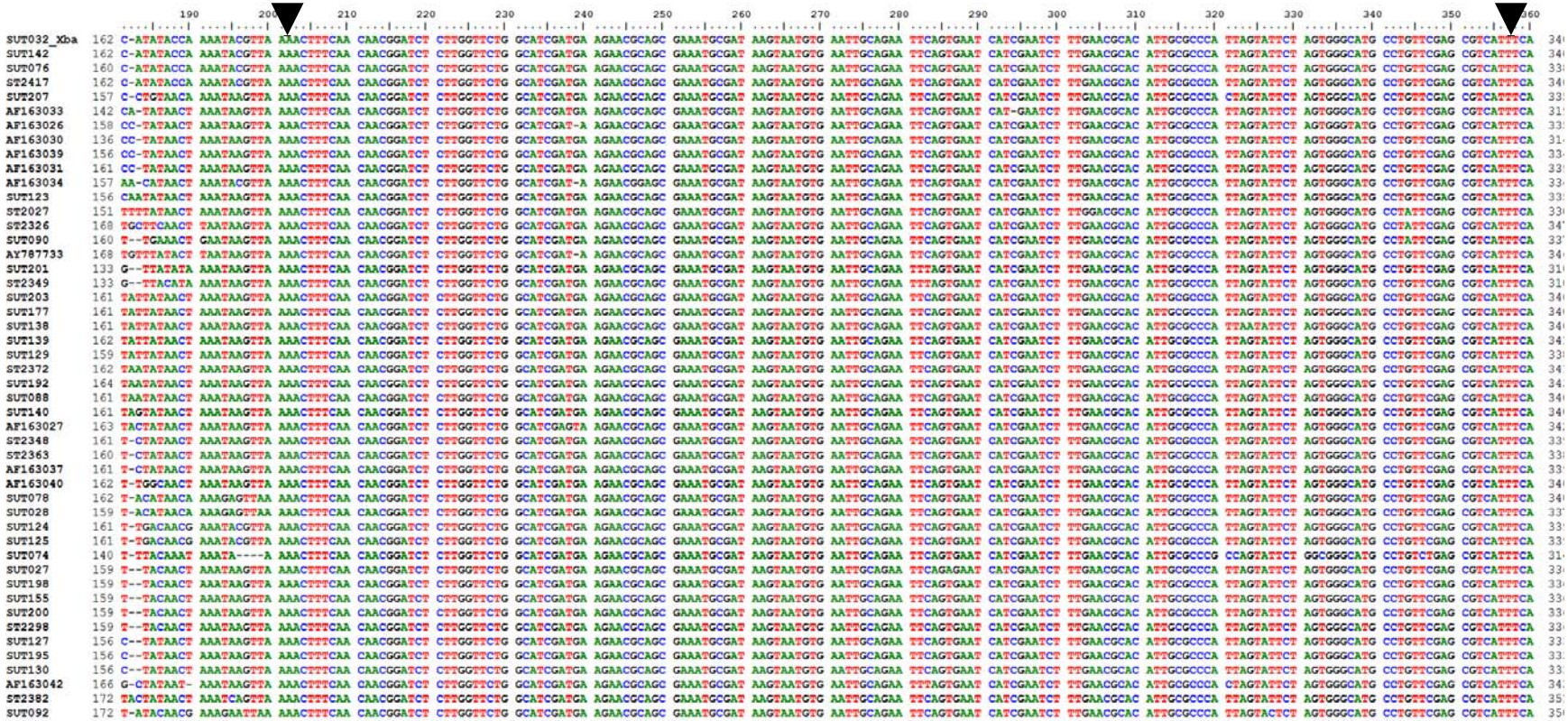

Figure 10C. (Continued).

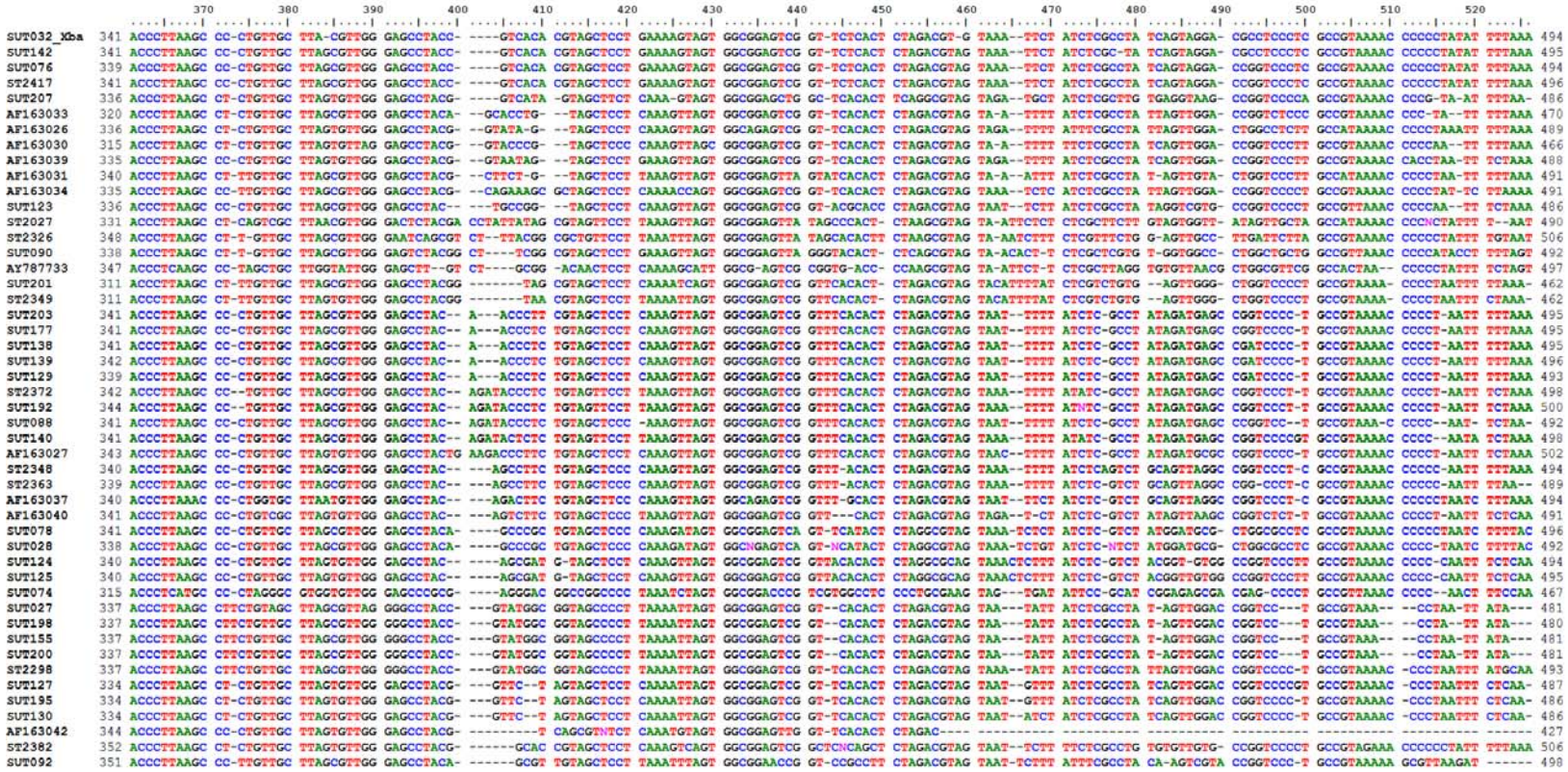

Figure 10C. (Continued).

Figure 11C. (Continued).

Figure 11C. (Continued).

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)**	***R. necatrix* (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
Rosellinia sp. (ST2301)				1.000	0.983
R. necatrix (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)**	***R. necatrix* (AB014044)**
A. mirabilis (SUT056)	1.000	0.703	0.687
Rosellinia sp. (ST2301)		1.000	0.673
R. necatrix (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

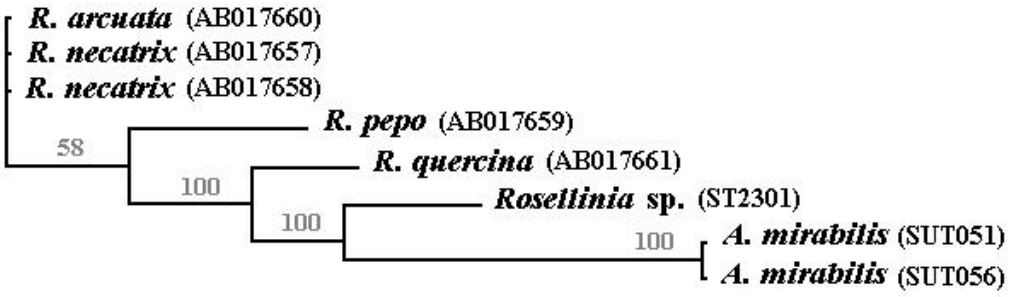

Figure 1D. Phylogenetic tree of *Rosellinia* and *Astrocystis* constructed by the maximum parsimony method using PAUP* program based on ITS1-5.8S-ITS2 sequences. Tree length = 246; Consistent index (CI) = 0.8984; Homoplasy index (HI) = 0.1016; Retention index (RI) = 0.8663. Numbers of the branches indicate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

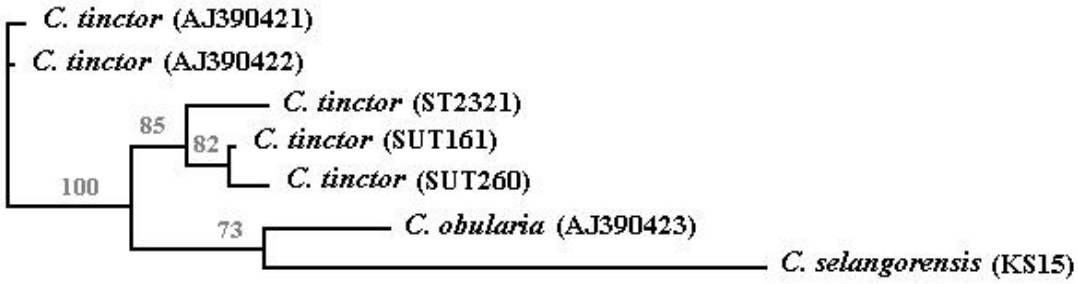

Figure 2D. Phylogenetic tree of *Camillea* constructed by the maximum parsimony method using PAUP* program based on ITS1-5.8S-ITS2 sequences. Tree length = 181; Consistent index (CI) = 0.9337; Homoplasy index (HI) = 0.0663; Retention index (RI) = 0.7600. Numbers of the branches indicate the bootstrap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

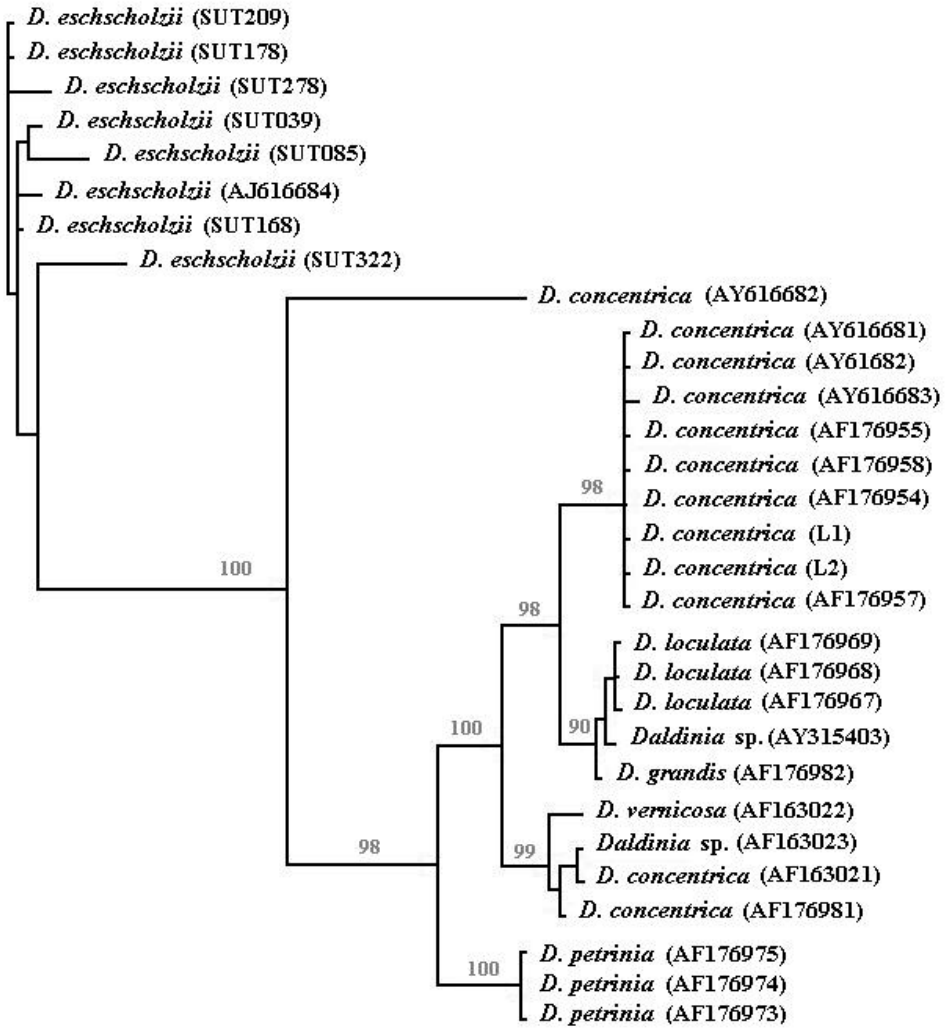

Figure 3D. Phylogenetic tree of *Daldinia* constructed by the maximum parsimony method using PAUP* program based on ITS1-5.8S-ITS2 sequences. Tree length = 200; Consistent index (CI) = 0.8400; Homoplasy index (HI) = 0.1600; Retention index (RI) = 0.9489. Numbers of the branches indicate the bootstrap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

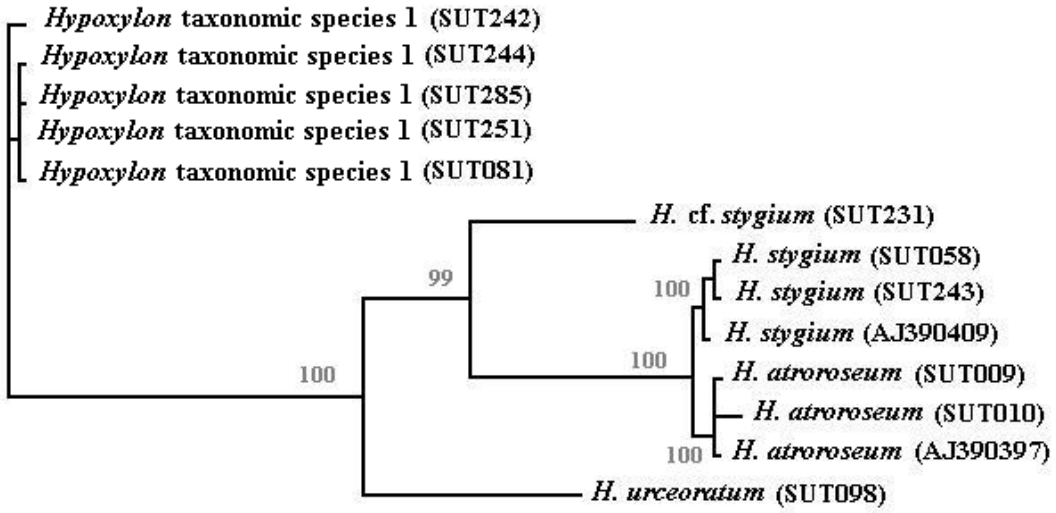

Figure 4D. Phylogenetic tree of *Hypoxylon* sect. *Annulata* constructed by the maximum parsimony method using PAUP* program based ITS1-5.8S-ITS2 sequences. Tree length = 613; Consistent index (CI) = 0.9396; Homoplasy index (HI) = 0.0604; Retention index (RI) = 0.9693. Numbers of the branches indicate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

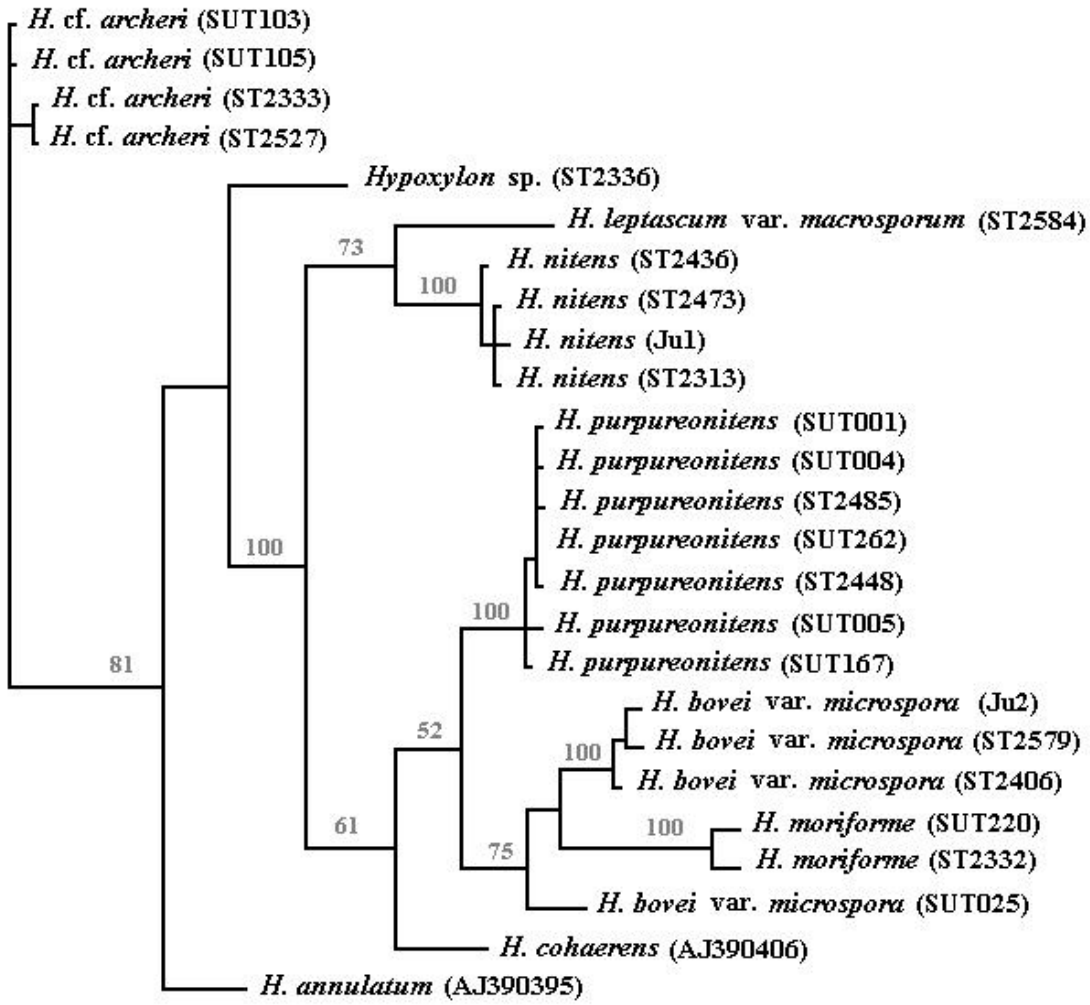

Figure 5D. Phylogenetic tree of *Hypoxylon* sect. *Annulata* constructed by the maximum parsimony method using PAUP* program based on ITS1-5.8S-ITS2 sequences. Tree length = 867; Consistent index (CI) = 0.6586; Homoplasy index (HI) = 0.3414; Retention index (RI) = 0.8087. Numbers of the branches indicate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

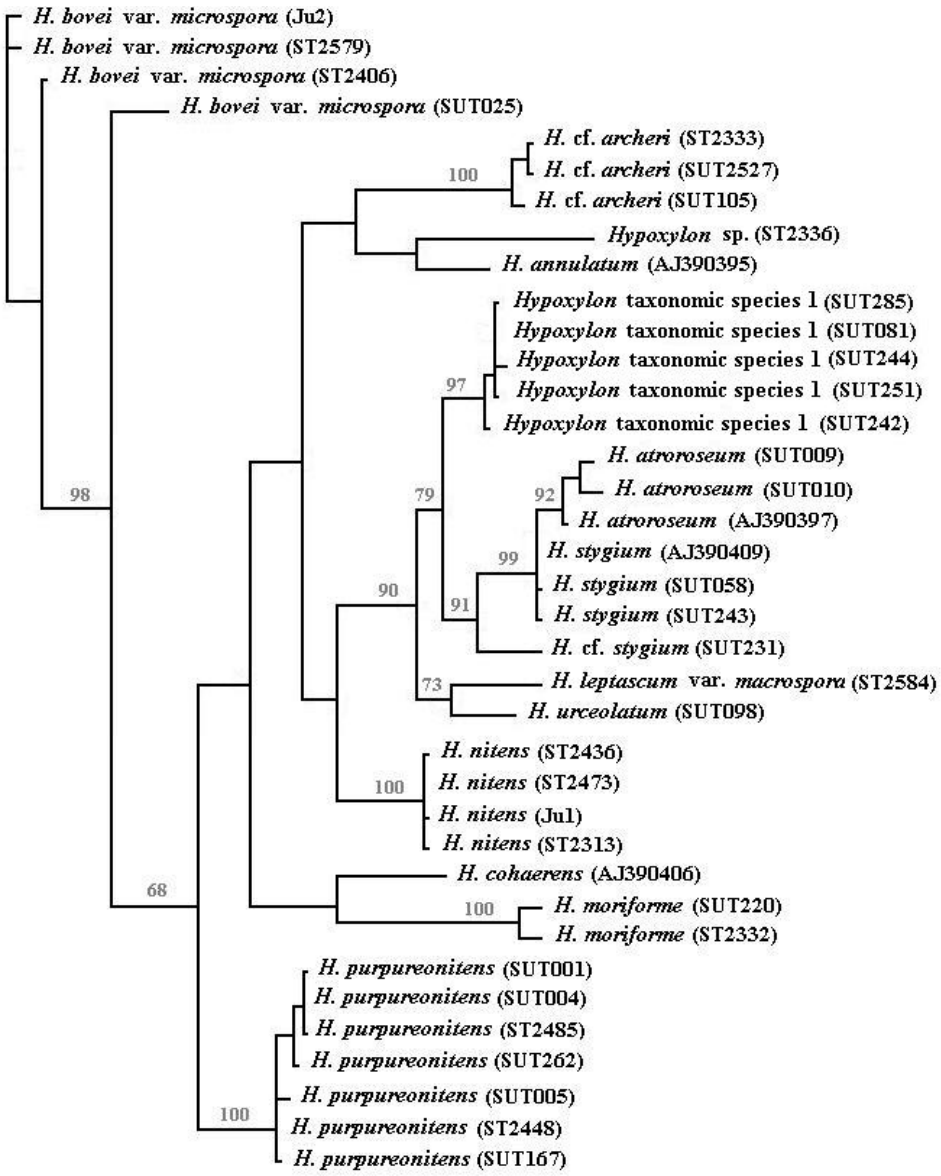

Figure 6D. Phylogenetic tree of *Hypoxylon* sect. *Annulata* constructed by the maximum parsimony method using PAUP* program based on ITS2 sequences. Tree length = 330; Consistent index (CI) = 0.6242; Homoplasy index (HI) = 0.3758; Retention index (RI) = 0.8545. Numbers of the branches indicate the bootstrap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

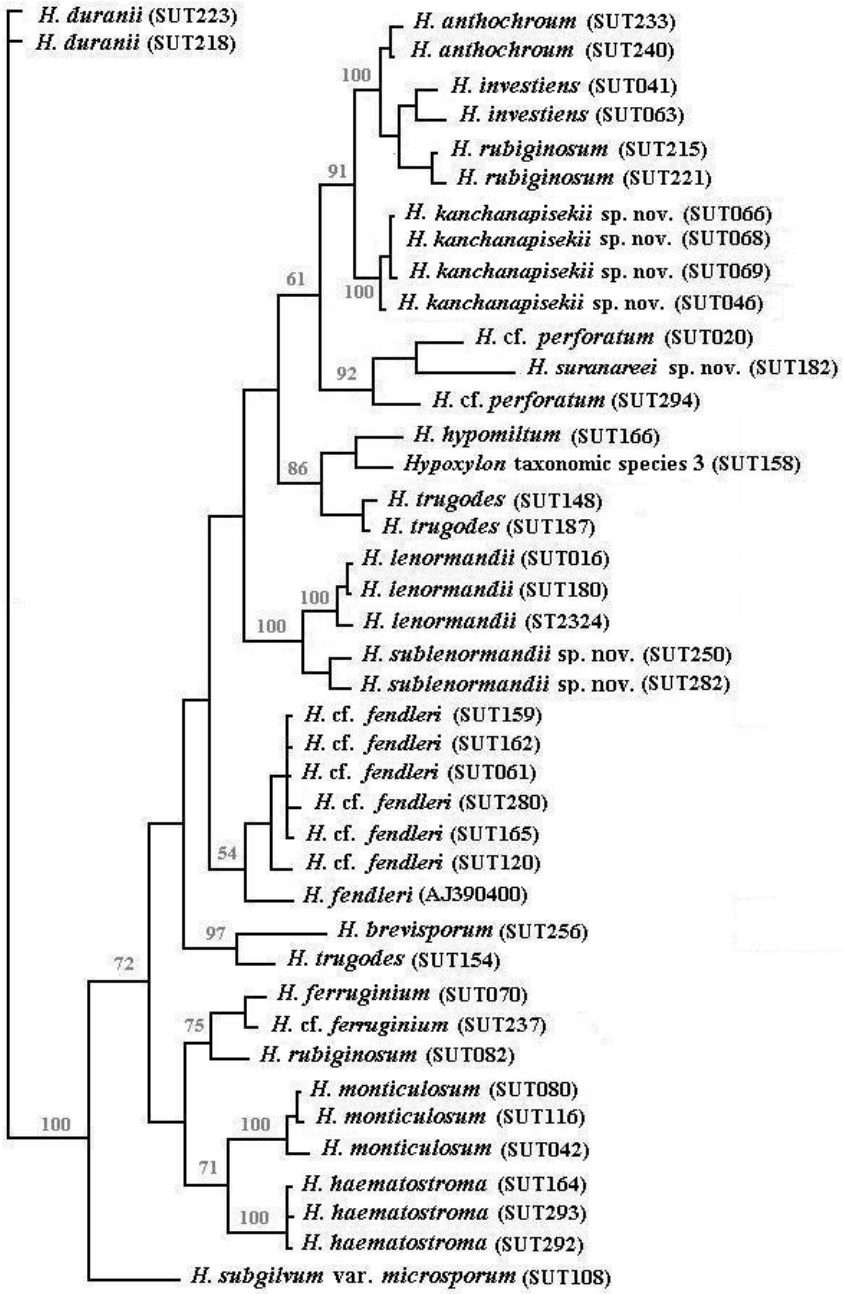

Figure 7D. Phylogenetic tree of *Hypoxylon* sect. *Hypoxylon* constructed by the maximum parsimony method using PAUP* program based on ITS1-5.8S-ITS2 sequences. Tree length = 1437; Consistent index (CI) = 0.4878; Homoplasy index (HI) = 0.5122; Retention index (RI) = 0.7126. Numbers of the branches indicate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

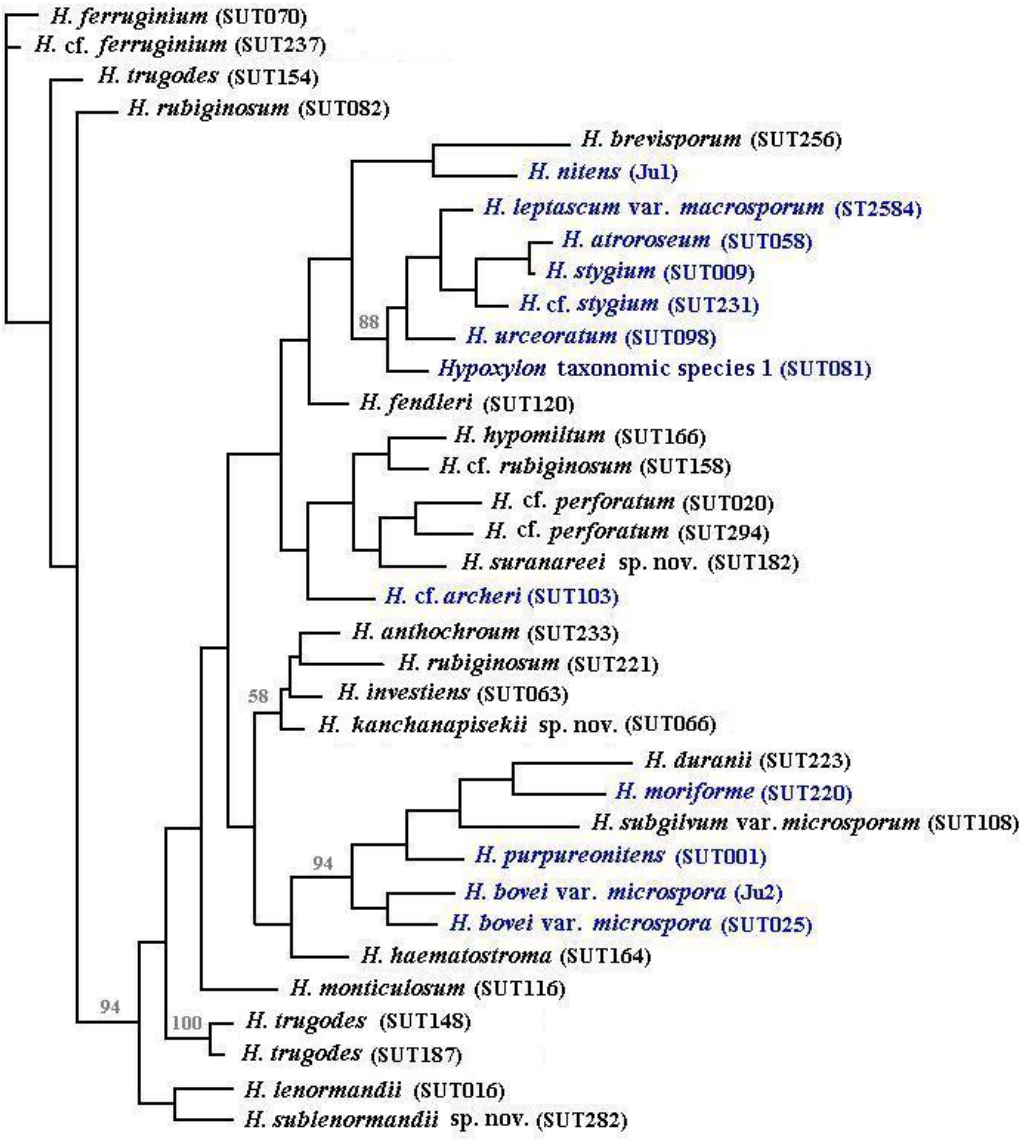

Figure 8D. Phylogenetic tree of *Hypoxylon* constructed by the maximum parsimony method using PAUP* program based on ITS2 sequences. *Hypoxylon* sect. *Hypoxylon* is black whilst sect. *Annulata* is blue. Tree length = 798; Consistent index (CI) = 0.3797; Homoplasy index (HI) = 0.6203; Retention index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

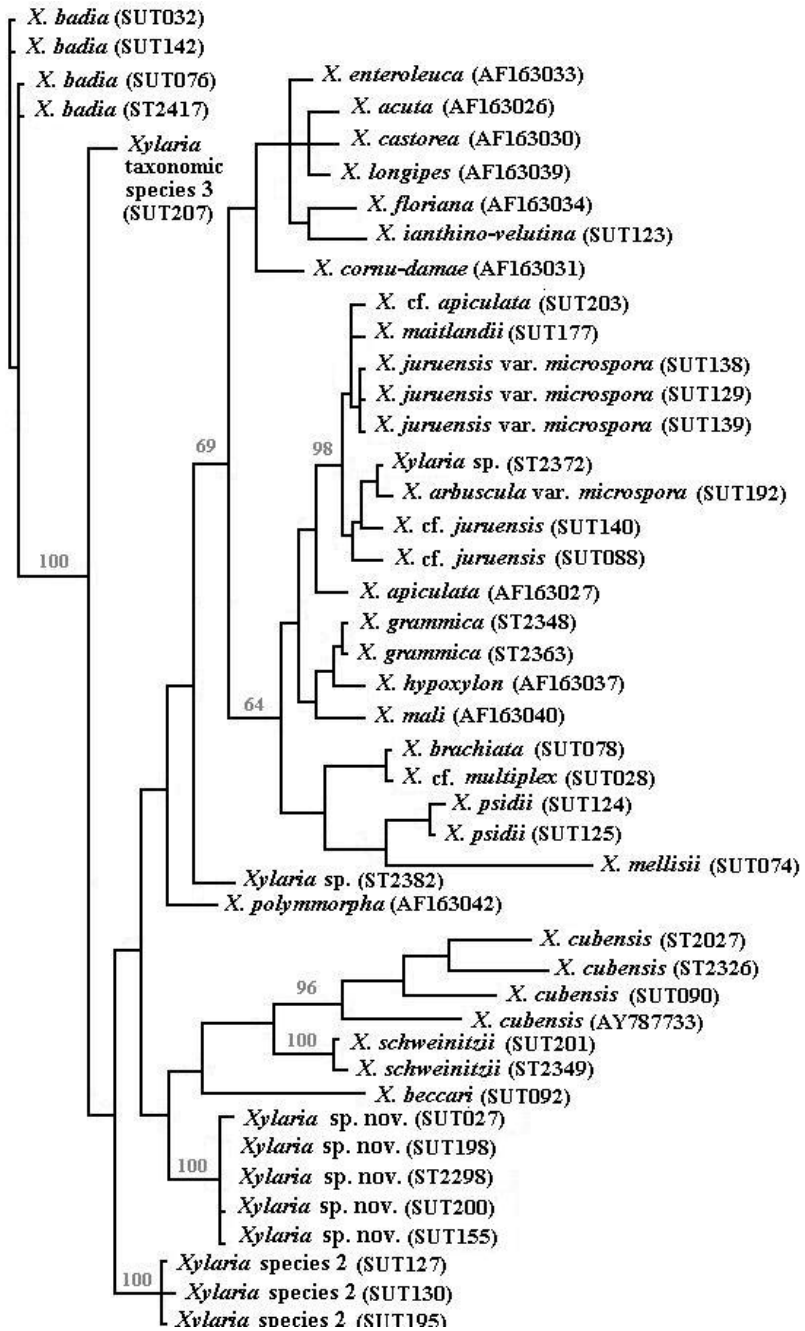

Figure 9D. Phylogenetic tree of *Xylaria* constructed by the maximum parsimony method using PAUP* program based on ITS2 sequences. Tree length = 1230; Consistent index (CI) = 0.4886; Homoplasy index (HI) = 0.5114; Retention index (RI) = 0.6921. Numbers of the branches indicate the bootstrap values resulting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

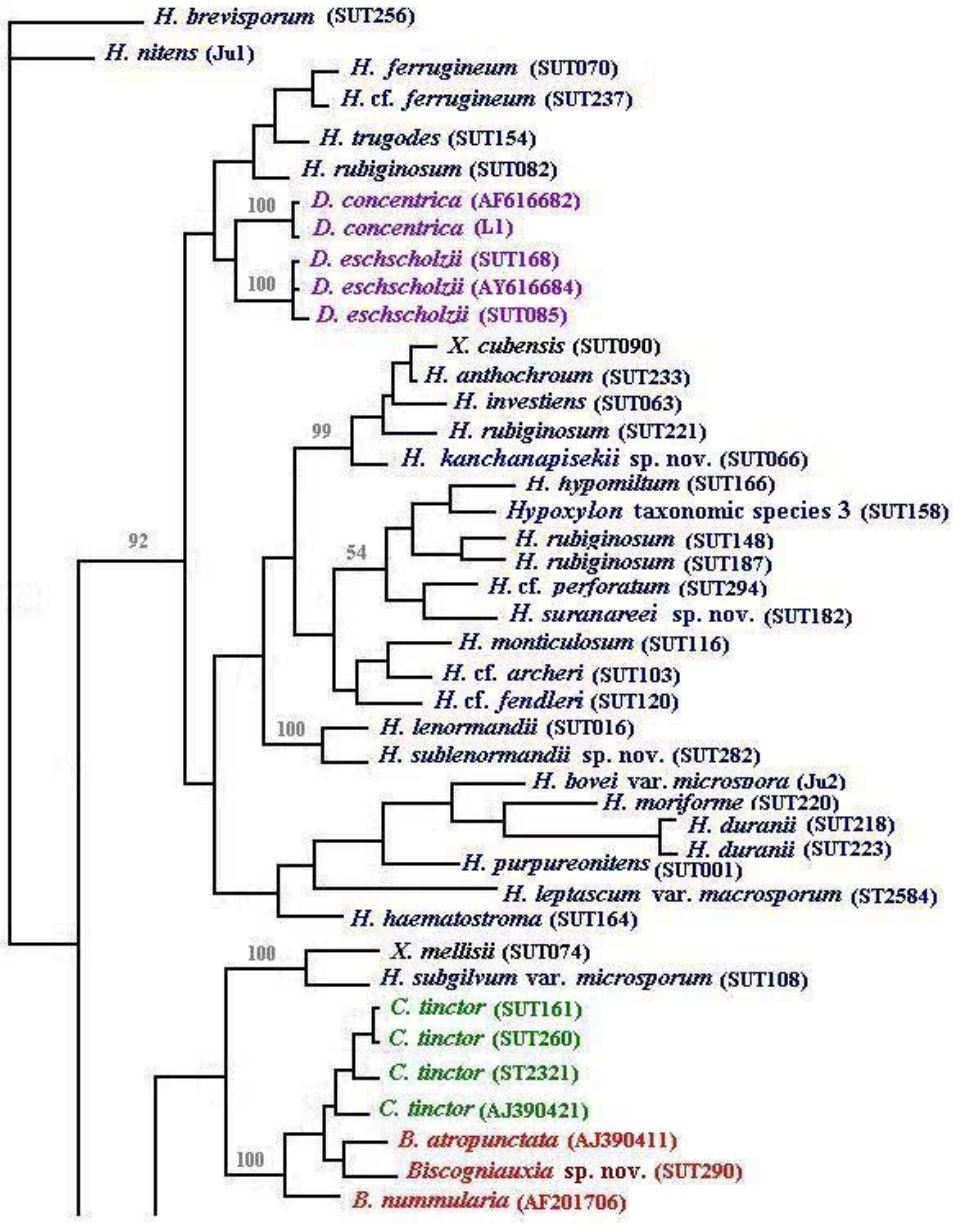

Figure 10D. Phylogenetic tree of xylariaceous fungi constructed by the maximum parsimony method using PAUP* program based on ITS2 sequences. Tree length = 3244; Consistent index (CI) = 0.3203; Homoplasy index (HI) = 0.6797; Retention index (RI) = 0.5708. Numbers of the branches indicate the bootstrap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

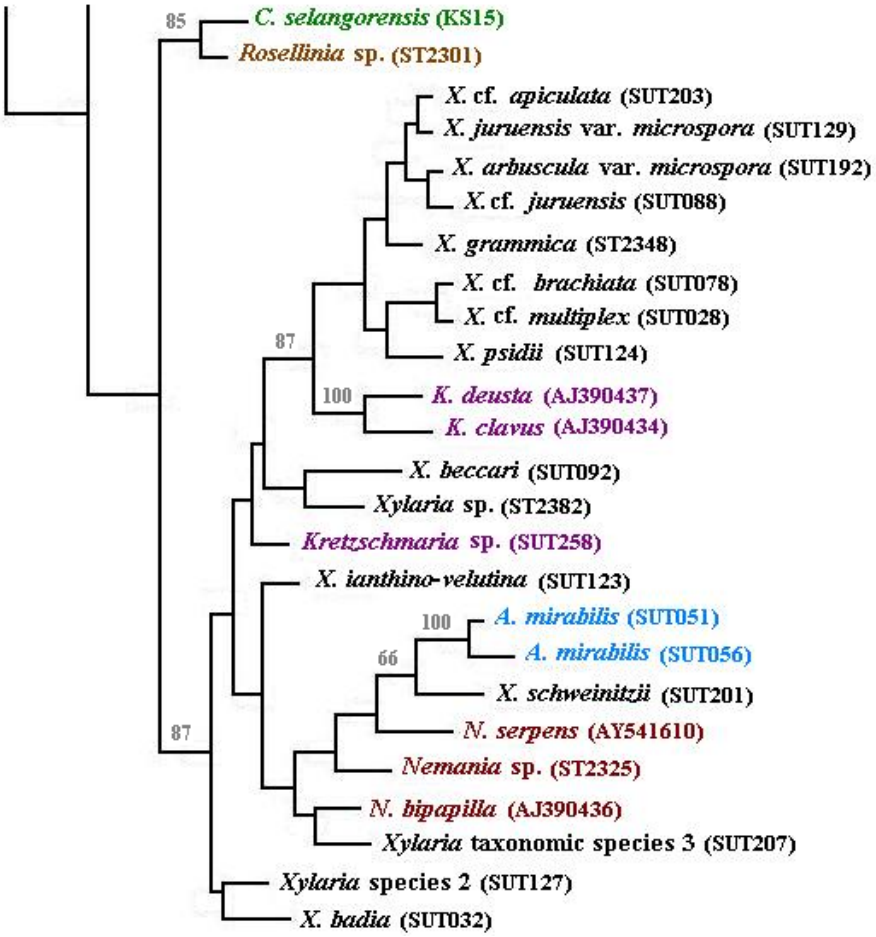

Figure 10D. (Continued).

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.

Thienhirun, S., Rodtong, S., Phukhawan, N., and Suwannasai, N. **Xylariaceous Fungus in Phu Hin Rongkra National Park, Thailand 2003**. (2003). Technology for Life, July 17-21, 2003, Pattaya Exhibition and Conventional Hall, Thailand.

ณัฏฐิกา สุวรรณาศรัย, สุรีลักษณ์ รอดทอง, สุรางค์ เธียรหิรัญ, และ Whalley, A.J.S. (2547). **ข้อมูลทางชีววิทยาโมเลกุลเพื่อการอนุกรมวิธานของเชื้อราสกุล *Hypoxylon* (Molecular and Biology data for the Taxonomy Study of Hypoxylon)**. การประชุมความหลากหลายทางชีวภาพ "งานวิจัยจากอดีตสู่อนาคต", 30 สิงหาคม – 3 กันยายน 2547, โรงแรมเวียงอินท์ จ.เชียงราย ประเทศไทย

Suwannasai, N., Rodtong, S., Thienhirun, S., and Whalley, A.J.S. (2004). **ITS Sequence Heterogeneity of *Xylaria* Species and Some Other Xylariaceous Genera**. 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.

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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 purpureonitens*, *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 ที่มีบทบาทสำคัญในการย่อยสลายซากพืชในระบบนิเวศและบางชนิดยังเป็นสาเหตุของโรคพืช ในการศึกษาอนุกรมวิธานของเชื้อราสกุลนี้อาศัยลักษณะทางสัณฐานเป็นหลักได้แก่ ลักษณะรูปร่าง ขนาด และ สี ของ Stroma, Perithecia, Ascospore, Germ slit และ Apical apparatus รวมถึงการเกิดปฏิกิริยาเคมีของ Stroma กับสารละลาย KOH 10% ซึ่งลักษณะทางสัณฐานดังกล่าวมีความผันแปรสูงและยากต่อการใช้เพื่อให้ได้ผลการศึกษาอนุกรมวิธานของ *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 และมีลำดับนิวคลีโอไทด์ที่สามารถใช้เพื่อช่วยระบุและจัดจำแนกชนิดของ *Hypoxylon* ได้อย่างชัดเจนและยังเป็นข้อมูลสำคัญที่ใช้เพื่ออธิบายความสัมพันธ์และวิวัฒนาการของเชื้อราสกุลนี้ได้ด้วย

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) ประกอบด้วยอย่างน้อย 130 ชนิด (Ju & Rogers, 1996) ซึ่งเป็นที่รู้จักและพบได้ในหลายประเทศโดยเฉพาะในเขตร้อนและเขตอบอุ่น ในประเทศไทยมีรายงานว่าพบเชื้อราสกุลนี้อย่างน้อย 47 ชนิด (Thienhirun, 1997) ซึ่งพบได้บนท่อนไม้และกิ่งไม้

เชื้อราสกุลนี้มีบทบาทสำคัญในการย่อยสลายซึ่งช่วยรักษาสมดุลของระบบนิเวศ และยังพบว่าบางชนิดเป็นสาเหตุของโรคพืช เช่น *Hypoxylon rubiginosum* และ *Hypoxylon mammata* (คือ *Entoleuca mammata* ตาม Edward และคณะ (2003) ซึ่งก่อให้เกิดโรค Canker ในการศึกษาอนุกรมวิธานของเชื้อราสกุลนี้อาศัยลักษณะทางสัณฐานเป็นหลักได้แก่ ลักษณะรูปร่าง ขนาด และ สี ของ Stroma, Perithecia, Ascospore, Germ slit และ Apical apparatus รวมถึงการเกิดปฏิกิริยาเคมีของ Stroma กับสารละลาย KOH 10% ซึ่งลักษณะทางสัณฐานดังกล่าวมีความผันแปรสูงโดยเฉพาะอย่างยิ่งชนิดที่ใกล้เคียงกันของ *Hypoxylon* Section *Annulata* ซึ่งพบ 1/3 ของเชื้อสกุล *Hypoxylon* มีลักษณะเด่นคือ Ostioles อยู่ในระดับที่สูงกว่าผิวของ Stroma และมีลักษณะเป็นแผ่นกลม (Disc) อยู่ข้างบน ซึ่งยากต่อการศึกษาเพื่อให้ได้ข้อมูลทางอนุกรมวิธานที่ถูกต้อง ดังนั้นในการศึกษาครั้งนี้จึงได้นำเทคนิคทางชีววิทยาโมเลกุลโดยการหาลำดับนิวคลีโอไทด์ของดีเอ็นเอในส่วน Internal transcribed spacer (ITS) regions 1 และ 2 รวมทั้งส่วน 5.8S ribosomal RNA gene มาช่วยในการระบุและจัดจำแนกชนิดเพื่อเป็นข้อมูลในการศึกษาอนุกรมวิธานของเชื้อรากลุ่มนี้ได้อย่างเชื่อมั่นและชัดเจนต่อไป

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% ของเชื้อราสกุล *Hypoxylon* Section *Annulata* จำนวน 38 ตัวอย่าง ที่รวบรวมได้จากพื้นที่ในประเทศไทย (ตารางที่ 1)

2. การศึกษาเพื่อให้ได้ข้อมูลทางชีววิทยาโมเลกุลของเชื้อราสกุล *Hypoxylon*

แยกให้ได้เชื้อบริสุทธิ์จากสปอร์ โดยเพาะเลี้ยงบนอาหาร Potato Dextrose Agar (PDA) และ สกัดดีเอ็นเอจากเส้นใยโดยใช้วิธีที่ประยุกต์จาก 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) ตรวจสอบขนาดของชิ้นดีเอ็นเอที่ได้ (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

จากการศึกษาลักษณะทางสัณฐานเพื่อการระบุและจัดจำแนกชนิดของเชื้อรา *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 พบว่าบางตัวอย่างไม่สามารถจัดจำแนกชนิดได้ เนื่องจากลักษณะทางสัณฐานมีความผันแปรสูงคือสีและขนาดของ Stroma, Ostiolar disc, Ascospores รวมทั้งการเกิดปฏิกิริยาของ Stroma กับ KOH 10% ใกล้เคียงกันมากดังตัวอย่างในตารางที่ 1 และรูปที่ 1

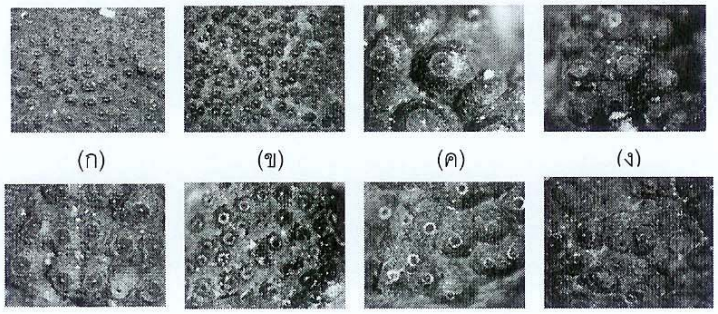
2. ข้อมูลทางชีววิทยาโมเลกุลของเชื้อราในสกุล *Hypoxylon*

ในการเพิ่มจำนวนดีเอ็นเอในส่วน ITS regions ของเชื้อรา *Hypoxylon* พบว่ามีขนาดแตกต่างกันอยู่ในช่วง 500 ถึง 900 คู่เบส (รูปที่ 2) ซึ่งใช้เพื่อหาลำดับนิวคลีโอไทด์และความสัมพันธ์ เมื่อนำลำดับนิวคลีโอไทด์ที่ได้มาจัดแนวความสัมพันธ์ที่เหมาะสม (Alignment) พบความผันแปรสูงที่สุดในส่วน ITS1 โดยเฉพาะ *H. stygium* และ *H. atroroseum* (รูปที่ 3) ซึ่งมีลักษณะทางสัณฐานใกล้เคียงกันมาก แต่พบความต่างของลำดับนิวคลีโอไทด์ในส่วน ITS1 (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 รวมทั้งมีลำดับนิวคลีโอไทด์ที่แตกต่างกัน ทำให้สามารถแยก *Hypoxylon* แต่ละชนิดออกจากกันได้อย่างชัดเจน (รูปที่ 4) โดยหาความสัมพันธ์ทางพันธุกรรมในรูปของ Phylogenetic tree (รูปที่ 5) และเชื้อ *Hypoxylon* บางตัวอย่างที่ยังไม่สามารถระบุและจัดจำแนกชนิดได้เช่น *Hypoxylon* sp. SUT103, *Hypoxylon* sp. ST2345 และ *Hypoxylon* sp. ST2406 พบว่าสามารถระบุแยกเชื้อราดังกล่าวออกมาจากกลุ่มของ *Hypoxylon* ชนิดอื่น อาจนำมาซึ่งการค้นพบเชื้อราชนิดใหม่ใน *Hypoxylon* ก็เป็นได้ โดยลำดับนิวคลีโอไทด์ที่ได้จากการศึกษาครั้งนี้สามารถนำมาใช้ออกแบบ Primers และ/หรือ Probes ที่จำเพาะต่อเชื้อแต่ละชนิดเพื่อช่วยในการศึกษาอนุกรมวิธานและยังเป็นข้อมูลที่สำคัญเพื่อใช้อธิบายความสัมพันธ์และวิวัฒนาการของเชื้อราสกุลนี้อีกด้วย

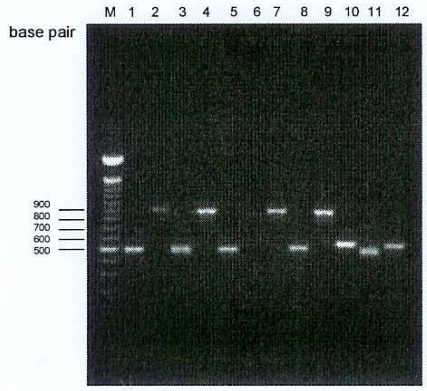
ตารางที่ 1 ความหลากหลายของลักษณะทางสัณฐานและการเกิดปฏิกิริยาของ Stroma กับ KOH 10% ของเชื้อราสกุล *Hypoxylon* Section *Annulata* ที่พบในประเทศไทย

ชนิดของเชื้อรา	สีของ Stroma	ขนาดของ Ostiolar disc (Ø/mm)	ขนาดของ Ascospores (µm)	สีจากปฏิกิริยาของ Stroma กับ KOH 10%	พื้นที่ของจังหวัดที่พบเชื้อ	จำนวนตัวอย่าง
Hypoxylon stygium	ดำมัน	0.1	3.75-6.25 x 2.5-3.75	เขียว	ตราด ราชบุรี	10
H. atroroseum	ดำ-ชมพู	0.1	4.6-6.25 x 2.5-3.75	เขียว	นครราชสีมา	2
H. nitens	ดำมัน	0.3-0.4	7.6-9.1 x 3.4-4.0	เขียว	ตราด นครราชสีมา	8
H. bovei var. *microspora*	ดำ	0.3-0.6	7.5-10.0 x 3.75-5.0	เขียว	นครราชสีมา	5
H. purpureonitens	ดำมัน	0.2-0.5	7.5-10.0 x 3.75-5.0	ม่วง	ตราด นครราชสีมา สงขลา	6
H. urceolatum	ดำ	0.2-0.3	10.0-12.5 x 2.5-5.0	ม่วง	สงขลา	1
*H. moriforme**	ดำ	0.2-0.5	6.0-9.0 x 2.5-4.0	เขียว	-	1
H. sp. SUT103	ดำ	0.1	8.75-10.0-(11.5)x2.5-3.75	เหลือง-น้ำตาล	สงขลา	3
H. sp. ST2345*	ดำ	0.1	6.3-7.5 x 3.8	เขียว	-	1
H. sp. ST2406*	ดำ	0.3	7.5-8.8 x 3.8-5	เขียว	-	1

* เชื้อจากสำนักวิจัยการจัดการป่าไม้และผลิตผลป่าไม้ กรมป่าไม้ กรุงเทพฯ (ดร.สุรางค์ เธียรหิรัญ ผู้เก็บและดูแลตัวอย่างเชื้อรา)

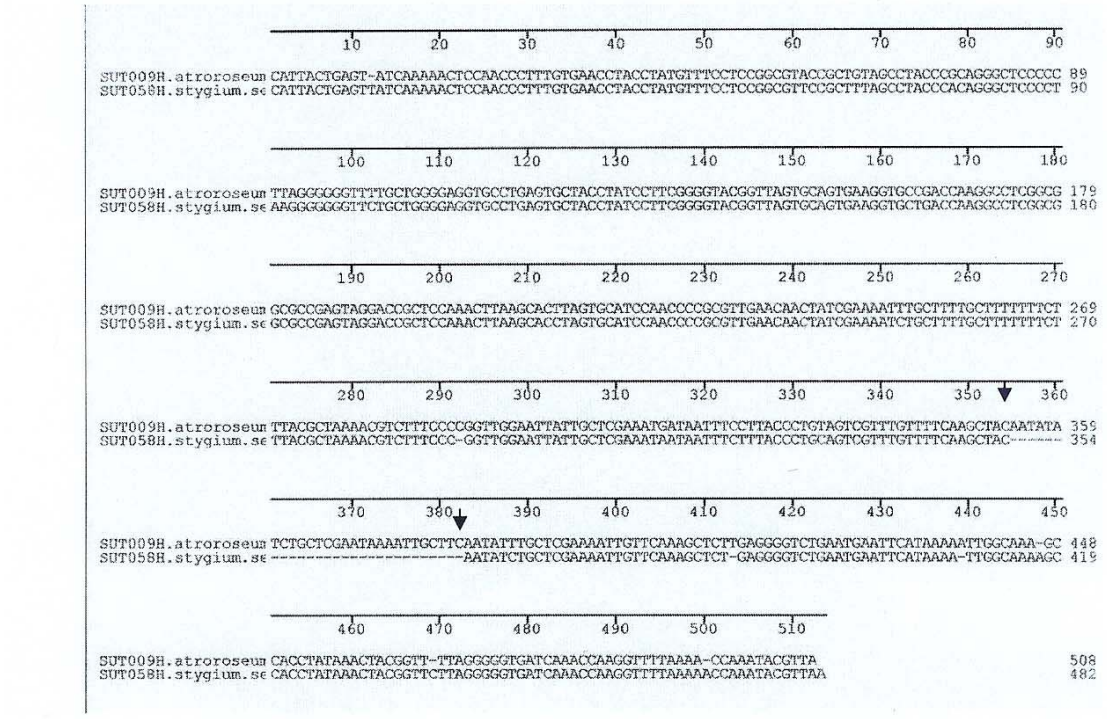


รูปที่ 1 ตัวอย่างลักษณะ Stroma (กำลังขยาย 40 เท่า) ของเชื้อราสกุล *Hypoxylon* ใน Section *Annulata* (ก) *Hypoxylon stygium*, (ข) *H. atroroseum*, (ค) *H. nitens*, (ง) *H. bovei* var. *microspora*, (จ) *H. purpureonitens*, (ฉ) *H. urceolatum*, (ช) *Hypoxylon* sp. SUT103, (ซ) *Hypoxylon* sp. SUT251

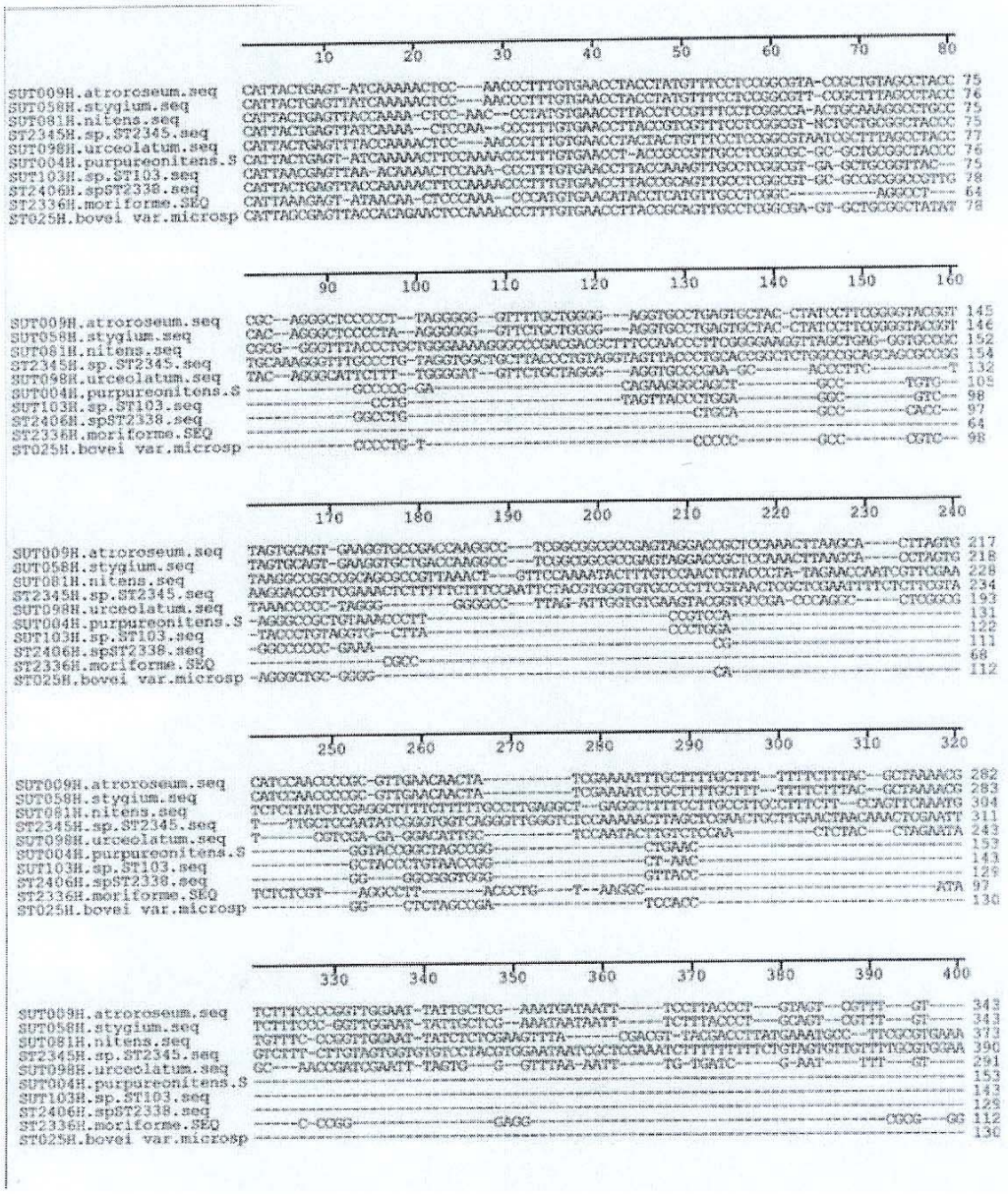


รูปที่ 2 ผลผลิตจาก PCR ในส่วนของ ITS1-5.8S-ITS2 ของเชื้อรา *Hypoxylon* Section *Annulata* เมื่อตรวจหาด้วยเทคนิคเจลอิเล็กโทรโฟรีซีส (Agarose 1.5%)

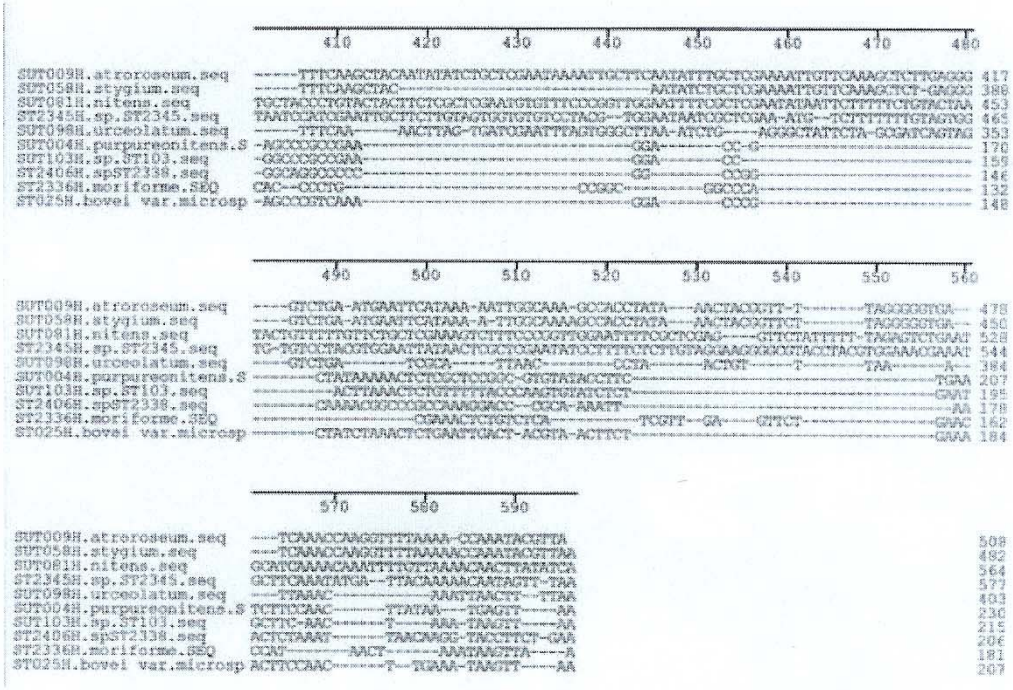
ช่อง 1: M, Molecular weight marker (100 base pairs DNA Ladder, Promaga); 1, 5 และ 8, *Hypoxylon purpureonitens*; 2 และ 4, *H. atroroseum* ; 3, *H. bovei* var. *microspora*; 6, 7 และ 9, *H. nitens*; 10 และ 12, *Hypoxylon* sp. SUT103; 11, *H. moriforme*



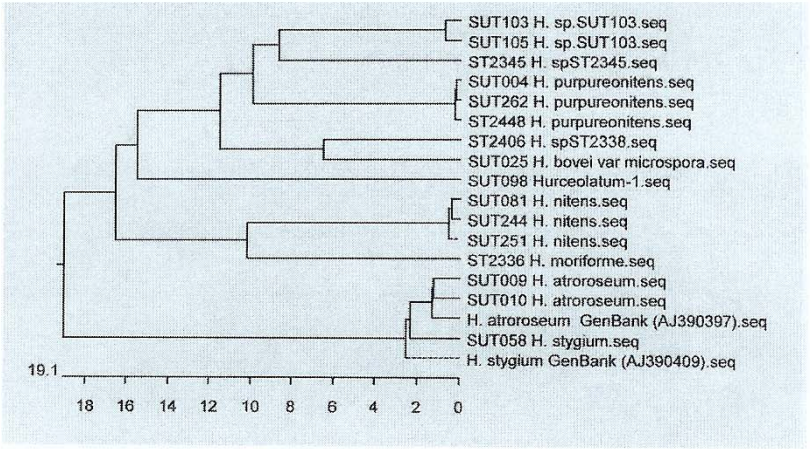
รูปที่ 3 การจัดแนวความสัมพันธ์ที่เหมาะสมของลำดับนิวคลีโอไทด์ในส่วน ITS1 ของเชื้อ *Hypoxylon atroroseum* กับ *H. stygium* (ลูกศรแสดงช่วงของลำดับนิวคลีโอไทด์ที่ต่างกัน) ซึ่งพบความเหมือน (homology) 90 %



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*)



รูปที่ 4 (ต่อเนื่อง)



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

กิตติกรรมประกาศ: งานวิจัยนี้ได้รับทุนสนันสนุนการวิจัยจากโครงการปริญญาเอกกาญจนาภิเษก สำนักงานกองทุนสนับสนุนการวิจัย และการสนับสนุนสถานที่และเครื่องมือจากมหาวิทยาลัยเทคโนโลยีสุรนารีและกรมป่าไม้

เอกสารอ้างอิง:

(1) Ju, Y.-M. and Rogers, J.D. (1996) **A revision of the genus *Hypoxylon***. Minnesota: American Phytopathological Society Press.

(2) Edwards, R.D., Jonglaekha, N., Kshirsagar, A., Maitland, D.J., Mekkamol, S., Nugent, L.K., et al. (2003) **The Xylariaceae as phytopathogens**. Recent Research Develop Plant Science. 1: 1-19.

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AP59

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

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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.

22nd MST Annual Conference Journal of Microscopy Society of Thailand 2005, 19(1): 207-208

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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5. Whalley MA, Whalley AJS, Jones EBG. *Camillea selangorensis* sp. nov. from Malaysia. *Sydowia* 1996, 48: 145-151.

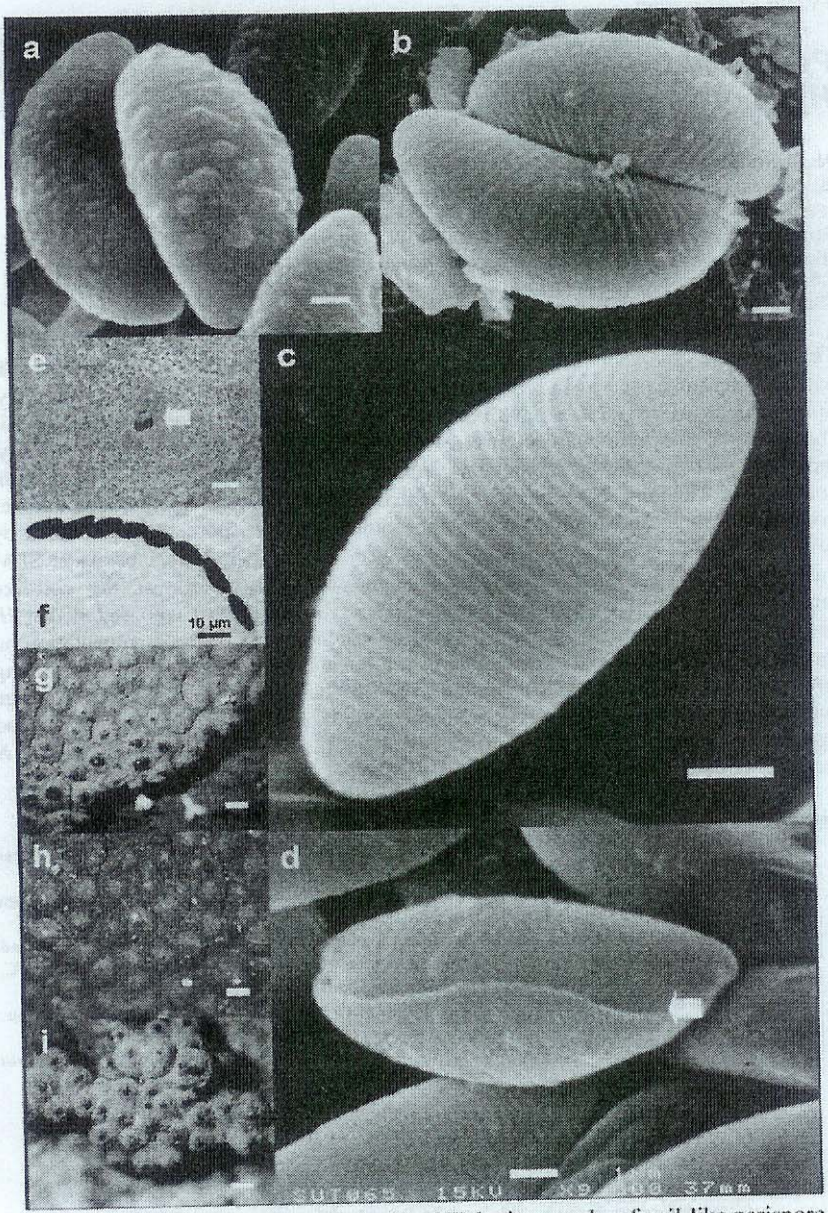

Figure 1. The fungus genus *Hypoxylon*. SEM micrographs of coil-like perispore ornamentation of *H.* cf. *lenormandii* (a), *H. retpela* (b), and *H. fendleri* (c), germ slit of *H.* cf. *lenormandii* (arrow) (d); and ostiole of *H.* cf. *lenormandii* (arrow) (e). Light microscope micrographs of *H. 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 rDNA 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 *Biscogniauxia*, *Camillea*, *Daldinia* and *Hypoxylon*. The data also supported a clear distinction between *Astrocystis* 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 groupings.

Please identify which mode of presentation your prefer and the session relevant to your work, by checking the boxes below.

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Session 2		Session 5	
Session 3		Howard Eggins Session	

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P-ST-34

แนวโน้มการพบเชื้อราชนิดใหม่ในกลุ่ม Xylariaceae ในประเทศไทย

TREND IN THE FINDING OF NEW XYLARIACEOUS FUNGAL SPECIES IN THAILAND

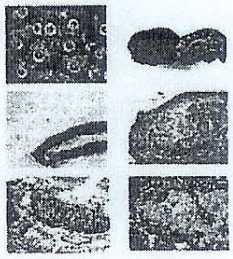
ณัฏฐิกา สุวรรณาศรัย[1] สุรีลักษณ์ รอดทอง[1] สุรางค์ เธียรหิรัญ[2] และ Anthony Whalley[3]

[1] สาขาวิชาจุลชีววิทยา สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยเทคโนโลยีสุรนารี นครราชสีมา 30000, [2] สำนักวิจัยการจัดการป่าไม้และผลิตผลป่าไม้ กรมป่าไม้ กรุงเทพฯ, [3] School of Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK; e-mail address: d4410126@ccs.sut.ac.th

บทคัดย่อ: เชื้อราวงศ์ Xylariaceae เป็นเชื้อวงศ์ใหญ่ใน Ascomycetes ประกอบด้วย 45 สกุล (genera) พบได้บนท่อนไม้ ใบไม้ ผลไม้ มูลสัตว์ และบนดิน และมีบทบาทสำคัญในการย่อยสลายซึ่งช่วยรักษาสมดุลของระบบนิเวศ นอกจากนี้ยังพบว่าบางชนิดเป็นสาเหตุของโรคพืช และบางชนิดสามารถเจริญอยู่ร่วมกับพืชโดยไม่ก่อให้เกิดโรคและอาจสร้างสารต้านแมลงศัตรูพืชได้อีกด้วย (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 ที่มีแนวโน้มว่าจะเป็นชนิดใหม่ คือมีวงแหวนสีขาวอยู่บนผิวของ Stroma ซึ่งเป็นลักษณะเด่นในการระบุว่าเป็น *Hypoxylon michelianum* ที่พบในแถบยุโรปเท่านั้น (1) แต่เชื้อที่พบนี้มีลักษณะดังกล่าวแต่ขนาดของสปอร์เล็กกว่าประมาณ 1 เท่าซึ่งยังไม่เคยมีการรายงานมาก่อน

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

ผลการวิจัย อธิปราย และสรุปผลการวิจัย: จากตัวอย่างที่รวบรวมได้จำนวน 320 ตัวอย่าง สามารถจัดอยู่ในสกุล *Hypoxylon* 35 ชนิด *Xylaria* 25 ชนิด *Daldinia* 1 ชนิด *Astrocystis* 1 ชนิด *Camellea* 1 ชนิด *Biscogniauxia* 3 ชนิด *Rosellinia* 1 ชนิด *Kretzschmaria* 1 ชนิด *Nemania* 1 ชนิด และพบเชื้อ *Hypoxylon* 1 ชนิด ที่มีแนวโน้มว่าจะเป็นชนิดใหม่คือมีวงแหวนสีขาวอยู่บนผิวของ Stroma ซึ่งเป็นลักษณะจำเพาะของ *Hypoxylon michelianum* ที่พบในแถบยุโรปเท่านั้น (1) แต่เชื้อที่พบนี้มีขนาดสปอร์เล็กกว่าประมาณ 1 เท่า ซึ่งยังไม่เคยมีการรายงานมาก่อน ทั้งนี้การศึกษาลักษณะทางสัณฐานมีข้อจำกัดในการระบุและจัดจำแนกชนิดได้อย่างเชื่อมั่นและชัดเจน ดังนั้นจึงได้ดำเนินการศึกษาต่อเนื่องโดยการใช้เทคนิคทางชีวโมเลกุลเพื่อการระบุชนิดที่แน่นอน สร้างฐานข้อมูล และพัฒนาวิธีการตรวจจับเชื้อแต่ละชนิดต่อไป



รูปที่1. ตัวอย่างเชื้อราในกลุ่ม Xylariaceae

คำนิยม: งานวิจัยนี้ได้รับทุนวิจัยจากโครงการปริญญาเอกกาญจนาภิเษก สำนักงานกองทุนสนับสนุนการวิจัย และการสนับสนุนสถานที่และเครื่องมือจากมหาวิทยาลัยเทคโนโลยีสุรนารี

เอกสารอ้างอิง: (1) Ju, Y.-M. and Rogers, J.D. (1996) A revision of the genus *Hypoxylon*. Minnesota: American Phytopathological Society Press.

(2) Thienhirun, S. (1997) A Preliminary Account of the Xylariaceae of Thailand: Liverpool John Moores University.

IV ASIA-PACIFIC MYCOLOGICAL CONGRESS

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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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