

## Interesting or rare Xylariaceae from Thailand

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### Abstract

Twenty three genera of Xylariaceae are currently known from Thailand plus the anamorphic genus *Muscodora* which accounts for close to one third of all known genera. Most of the genera are widely represented throughout the country including *Annulohypoxylon*, *Biscogniauxia*, *Daldinia*, *Hypoxylon*, and *Xylaria*. Others are more restricted, sometimes to a single locality as seen with *C. selangorensis* and others e.g. *Rostrophoxylon*, *Rhopalostroma* and individuals from a number of genera exhibit a northern presence. In common with studies on endophytic fungi in tropical plants Thailand is no exception with many xylariaceous fungi isolated with *Xylaria* species being especially frequent. The family in Thailand is considered to have many currently unknown members.

**keywords :** diversity, endophytes, secondary metabolites, tropical fungi, xylariaceous fungi

### Introduction

Thailand is well known to have a rich and diverse biota (Tantichareon, 2004) and this also applies to the mycota (Jones & Hyde, 2004) so perhaps it is not surprising that members of the ascomycete family Xylariaceae are very well represented (Thienhirun & Whalley, 2004; Suwannasai et al., 2011). In ongoing investigations into the Xylariaceae in Thailand, *Annulohypoxylon* Y.-M. Ju, J.D. Rogers & H.M. Hsieh, *Anthostomella* Sacc., *Astrocystis* Berk., *Biscogniauxia* Kuntze, *Camillea* Fr., *Daldinia* Ces. & De Not., *Emarcea* Duong, R. Jeewon & K.D. Hyde, *Entonaema* A. Möller, *Fasciatispora* K.D. Hyde, *Halorosellinia* (S. Schatz) Whalley, E.B.G. Jones, K.D. Hyde & T. Læssøe, *Hypoxylon* Bull., *Jumillera* J.D. Rogers, Y.-M. Ju & San Martín, *Kretzschmaria* Fr., *Kretzschmariella* Viegas, *Nemania* S.F. Gray, *Podosordaria* Ellis & Holw.,

*Poronia* Willd., *Rhopalostroma* D. Hawksworth, *Rosellinia* De Not., *Rostrophoxylon* J. Fournier, M. Stadler, K.D. Hyde & M.L. Duong, *Sarcoxydon* Cooke, *Whalleya* J.D. Rogers, Y.-M. Ju & San Martín and *Xylaria* Hill ex Schrank, are recorded (Thienhirun, 1997; Thienhirun & Whalley, 2004; Fournier et al., 2010). Twenty three genera of Xylariaceae are now reported as occurring in Thailand. In the 2009 Outline of the Ascomycota (Lumbsch & Huhndorf, 2010) accepted a total of 76 genera of Xylariaceae with 3 or 4 being considered as uncertain. Certainly with new genera being described e.g. *Ruwezoria* J. Fournier, M.S. Stadler, Læssøe & Decock. It suggests that in spite of individual opinions a figure of over 70 genera is realistic and on this basis Thailand is home to almost a third of the currently accepted genera of Xylariaceae. Interestingly, Rogers (2000) considered Thailand

to be an area containing a high percentage of unknown taxa.

A key to the known genera of Xylariaceae in Thailand is given. We have also selected a number of these genera or individual species for further discussion because of their interesting characteristics or rarity value. We also draw

attention to *Anthostomella* which was monographed by Lu & Hyde (2000) to which a number of new species for Thailand have since been added and to *Poronia* and *Podosordaria* which have been well covered by Somrithipol (2004).

#### Key to genera of Xylariaceae in Thailand \*

- |   |    |                      |
|---|----|----------------------|
| 1. Stromata bipartite, with outer membranous layer shed to expose ostiolar opening .....  | 2  |                      |
| 1. Unipartite .....   | 5  |                      |
| 2. Ascospores pale-coloured, ornamented, conspicuous with SEM, without germ slits; anamorph <i>Xylocadium</i> -like .....   |    | <i>Camillea</i>      |
| 2. Stromata widespread, applanate or applanopulvinate, erumpent through host substratum .....   | 3  |                      |
| 3. Stromata widespread and robust; ascospores brown to dark brown with a germ slit or two-celled with the larger cell brown and with a germ slit and the smaller cell hyaline; anamorph <i>Nodulisporium</i> or <i>Periconiella</i> -like .....   |    | <i>Biscogniauxia</i> |
| 3. Stromata usually less wide spreading and robust than <i>Biscogniauxia</i> .....  | 4  |                      |
| 4. Stromata applanate, solitary or confluent, ostioles below level of stromal surface, often punctuate mature surface white, grey, dull brown, dull black or with greenish tinge; ascospores unicellular, light to dark brown, ellipsoid, nearly equilateral, inequilateral, straight germ slit; anamorph <i>Libertella</i> -like with scolecosporous conidia .....   |    | <i>Jumillera</i>     |
| 4. Stromata applanate, solitary or occasionally confluent, stromal surface black when mature, ostioles often slightly sunken, punctuate; ascospores light brown, unicellular, ellipsoid-inequilateral with narrowly rounded ends, straight germ slit, often a space between perithecia mainly filled with hyphal tissue; anamorph <i>Geniculosporium</i> -like with denticulate conidial succession scars, dry scolecosporous conidia ..... |    | <i>Whalleya</i>      |
| 5. Stromata immersed in substratum, beneath a clypeus .....   | 6  |                      |
| 5. Stromata erumpent or superficial .....   | 8  |                      |
| 6. Ascospores usually with one large brown cell and one dwarf cell usually with a germ slit; anamorph <i>Geniculosporium</i> , <i>Nodulisporium</i> , <i>Libertella</i> -like....   |    | <i>Anthostomella</i> |
| 6. Germ slit lacking or indistinct .....  | 7  |                      |
| 7. Ascospores 1-septate, hyaline, long fusiform, without germ slits; anamorph unknown .....   |    | <i>Emarcea</i>       |
| 7. Ascospores aseptate, brown with broad equatorial pallid band and surrounded by distinct, usually wide mucilaginous sheath .....  |    | <i>Fasciatispora</i> |
| 8. Stromata mainly uniperitheciate .....  | 9  |                      |
| 8. Stroma multiperitheciate .....   | 11 |                      |

9. Stromata usually associated with a subiculum; ascospores one-celled, with a germ slit; hyaline appendages present in some taxa; anamorph <i>Geniculosporium</i> , <i>Dematophora</i> -like .....	<i>Rosellinia</i>
9. Stromata not associated with a subiculum .....	10
10. Stromata black and carbonaceous, usually surrounded by distinctive, lobed collar giving a stellate pattern; apical apparatus small, amyloid; anamorph <i>Acanthodochium</i> -like .....	<i>Astrocystis</i>
10. Pseudostromata leathery when fresh, white hyphal layer on surface bearing geniculate anamorph, large amyloid tapering apical apparatus with distinctive rim, on dead mangrove wood; anamorph not produced in culture .....	<i>Halorosellinia</i>
11. Stromata with a liquid-filled cavity when fresh; anamorph <i>Nodulisporium</i> -like; on wood.....	<i>Entonaema</i>
11. Stromata soft or hard when fresh and without a liquid filled cavity .....	12
12. Stromata soft to slightly woody at maturity, mostly light coloured inside and out; on dung; anamorph <i>Lindquistia</i> -like .....	13
12. Stromata waxy, woody to hard at maturity, bright-coloured to black; not on dung .....	14
13. Stromata flat-topped .....	<i>Poronia</i>
13. Stromata pulvinate .....	<i>Podosordaria</i>
14. Stromatal interior with well-defined concentric zones; anamorph <i>Nodulisporium</i> -like.....	<i>Daldinia</i>
14. Stromatal interior lacking well-defined concentric zones .....	15
15. Stromata erect usually several times higher than broad .....	16
15. Stromata pulvinate, globose, effused, always broader than high .....	18
16. Asci lacking an apical apparatus; stromata more or less clavate; perithecia immersed; anamorph <i>Nodulisporium</i> -like .....	<i>Rhopalostroma</i>
16. Asci usually with an amyloid apical apparatus .....	17
17. Stromata stipitate, flat-topped to pulvinate, often aggregated into a crust; anamorph <i>Geniculosporium</i> -like; on wood .....	<i>Kretzschmaria</i>
17. Stromata sessile to stipitate, various shapes but not aggregated to form a crust; anamorph <i>Geniculosporium</i> -like; associated with wood, seeds, fruits, leaves, insect nests and soil, flesh usually white.....	<i>Xylaria</i>
18. Stromata massive, more than 2 cm thick, hemispherical to grotesque, with a light coloured flesh; on wood .....	<i>Sarcoxydon</i>
18. Stromata rarely exceeding 2 cm thick, applano-pulvinate, pulvinate, or hemispherical, with dark or light coloured flesh; on wood .....	19
19. Stromata of various shapes, mostly coloured at maturity, usually with KOH soluble pigments; anamorph <i>Nodulisporium</i> -like in some species of <i>Annulohypoxylon</i> and in <i>Rostrohypoxylon</i> stromata carbonaceous .....	20
19. Stromata carbonaceous, pulvinate or effused .....	22
20. Stromata with ostiolar disc .....	<i>Annulohypoxylon</i>
20. Stromata without ostiolar disc .....	21

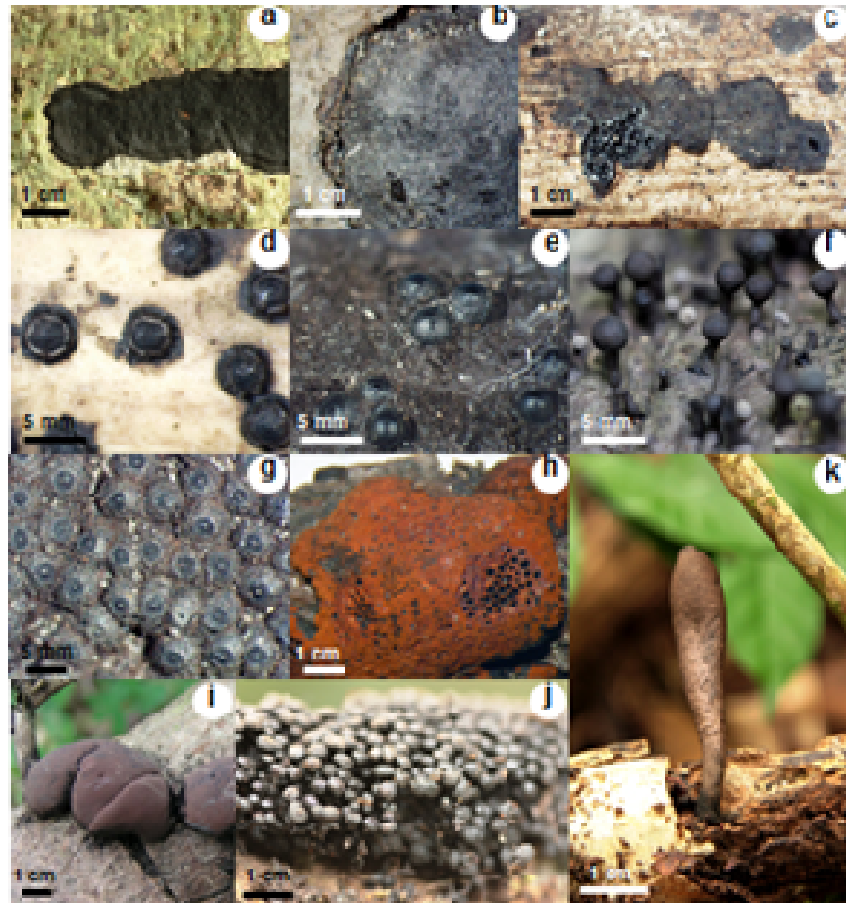
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|---|-------------------------|
| 21. Ascospores with germ slit usually on the convex (dorsal) side, perispore usually dehiscent in 10% KOH, usually with an amyloid apical apparatus .....   | <i>Hypoxylon</i>        |
| 21. Stromata effused pulvinate with stout ostiolar necks and deep cylindrical holes, carbonaceous, surface dull black but greenish olivaceous pigment extracted in 10% KOH; lacking apical apparatus; ascospores unicellular, brown cylindrical with broadly rounded ends, straight germ slit ..... | <i>Rostrophoxylon</i>   |
| 22. Stromata pulvinate; ascospores with a definite sheath; anamorph <i>Mirandina</i> -like, on bamboo culms.....  | <i>Kretzschmariella</i> |
| 22. Stromata mainly effused, usually not exceeding 2 mm thick, usually attached to the substratum by the entire base, usually not hollow with age; anamorph <i>Geniculosporium</i> -like, on wood .....   | <i>Nemania</i>          |

\**Muscodor albus* anam. gen. et sp. nov. Worapong, Strobel & W.H. Hess was erected for a sterile endophytic isolate from *Cinnamomum zeylandicum* and has molecular relatedness to the Xylariaceae (Worapong et al., 2001). This fungus appears to be widely distributed in tropical rainforests and has been recorded from Thailand (Sopalun et al., 2003). A second species *M. cinnamomi* Suwannarch, Bussaban, K.D. Hyde & Lumyong has now been described from Doi Suthep, Chiang Mai Province (Suwannarach et al., 2010). Although all known species and isolates remain sterile they represent a potential source of volatile compounds useful in biocontrol and on the basis of DNA studies are accepted as belonging to the Xylariaceae (Strobel, 2011).

#### Taxa of Xylariaceae (Figure 1)

*Astrocystis bambusae* P. Henn. and *A. mirabilis* Berk. & Broome are the two species which we have reported from Thailand and recently *A. eleiodoxae* Pinnoi, E.B.G. Jones & Hyde has been described from petioles of the peat swamp palm *Eleiodoxa conferta* in Sirindhorn Peat Swamp Forest, Narathiwat (Pinnoi et al., 2010). The genus is characterized by a skirt or volva on the stroma giving it a stellate appearance. It also possesses an *Acanthodochium* Samuels, J.D. Rogers & Nagasawa anamorph similar to those in *Collodiscula* Hino & Katumoto. Ju & Rogers (1990) concluded that *Astrocystis* is a *Rosellinia* and that the stellate stroma is probably related to

the bamboo substratum and therefore not particularly significant. Læssøe and Spooner (1994) disagreed pointing to clear differences in ascocarp structure, in ascus apical apparatus form. The apical apparatus in *Rosellinia* is typically massive and barrel-shaped but in *Astrocystis* it is small, parallel-sided or tapered and is similar to those found in species of *Nemania* and *Collodiscula*. They also stressed that the *Acanthodochium* anamorph separates the two genera. Whalley (1996) continued with separate status for *Astrocystis* again stressing anamorph form and we have since followed this separation as have Pinnoi et al. (2010). A detailed molecular study on *Xylaria* subgenus *Pseudoxylaria* based on sequences from  $\beta$ -tubulin, RPB2 and  $\alpha$ -actin sequences however has provided evidence for independent status for these two genera (Hsieh et al., 2010) and confirms our views and those of others that *Astrocystis* and *Rosellinia* are distinct genera. *Astrocystis* is not common in Thailand with *A. bambusae* recorded from Nan, Loei, and Kanchanaburi Provinces and *A. mirabilis* from Ratchaburi and Surat Thani Provinces. *Camillea* is generally considered to be mainly restricted to South and Central America with *C. tinctor* as the only representative widely distributed (Læssøe et al., 1989). As *C. tinctor* is virtually global in distribution a detailed comparison with specimens from Central America and the southern states of the United States is warranted



**Figure 1** Stromatal characteristics of some xylariaceous fungi from Thailand. (a) *Biscogniauxia capnodes* (SUT212) (Bar = 1 cm), (b) *Camillea tinctor* (SUT099) (Bar = 1 cm), (c) *Nemanina* sp. (SUT258) (Bar = 1 cm), (d) *Astrocystis mirabilis* (SUT055) (Bar = 5 mm), (e) *Rosellinia procera* (SUT102) (Bar = 5 mm), (f) *Rhopalostroma leakei* (PK148) (Bar = 5 mm), (g) *Annulohypoxylon purpureonitens* (H125) (Bar = 5 mm), (h) *Hypoxylon haematostroma* (H114) (Bar = 1 cm), (i) *Daldinia eschscholzii* (SUT039) (Bar = 1 cm), (j) *Kretzschmaria clavus* (PK270) (Bar = 2 mm) and (k) *Xylaria cubensis* (PK046) (Bar = 1 cm). Samples are deposited at SUT Herbarium, Suranaree University of Technology, Nakhon Ratchasima and SWU herbarium, Srinakharinwirot University Bangkok, Thailand, incorporating collections from national park and forest of Thailand i.e. Khao Kra Yang Plantation, Phitsanulok province, Phu Kheio Wildlife Sanctuary, Chaiyaphum province.

to confirm its true status. This might prove to be interesting since comparison of ITS1-5.8S-ITS2 sequence data for Thai isolates exhibited some variation and although clustering with *C. tinctor* sequences from GenBank database, they did exhibit differences. (Suwannasai, 2005). Vasiljeva et al. (2012) noted that “one might reasonably expect that a separate taxon occurs in Southeast Asia”. Further studies on *C. tinctor* from different geographical regions is ongoing. *Camillea selangorensis* M.A. Whalley, E.B.G. Jones & A.J.S. Whalley and *C. malaysianensis* M.A. Whalley

have subsequently been described from Malaysia (Whalley et al., 1996; 1999) and reported from Thailand (Whalley et al., 1999) and *C. malaysianensis* M.A. Whalley var. *macrospora* Lar.N. Vasiljeva, S.L. Stephenson & K.D. Hyde has recently been described from northern Thailand (Vasiljeva et al., 2012). *Rhopalostroma* is characterized by its stipitate more or less clavate stromata and asci which lack an amyloid apical apparatus. Apart from *R. africanum* Wakef. and *R. angolense* (Welw. & Curr.) Sacc. which reside in

Africa, the remaining 9 species recorded so far are from India, Thailand, Philippines and China. *Rhopalostroma gracile* D. Hawksworth & Whalley was the first to be reported from Thailand and found to produce a *Nodulisporium* anamorph (Hawksworth & Whalley, 1985). Later *R. kanyae* Whalley & Thienhirun and *R. lekae* Whalley, Thienhirun, M.A. Whalley & Sihanonth were described from Thailand (Whalley et al., 1998). Stadler et al. (2010) have reported on fresh collections of *R. angolense* including its *Nodulisporium* or *Virgariella*-like anamorph and chemical and molecular data confirmed its position as a member of the hypoxylid Xylariaceae with links to *Daldinia*, *Phylacia* Le'v. and in particular *Thamnomycetes* and also to *Entonaema* and *Ruwenzoria* with the production of mellein type isocoumarins. *Rhopalostroma* characteristically has small and fragile stromata and appears to be more frequent on bark and not decorticated wood often occurring in damp habitats. We suggest that its delicate and often tiny stromata have resulted in it being overlooked and under collected. *Entonaema* species are characterized by pulvinate to hemispherical to globose stromata which are often folded, lobed giving a convoluted appearance and are hollow with the cavity or cavities filled with liquid when fresh. The flesh is gelatinous when wet but becoming very hard when dry. Rogers monographed the genus recognizing 5 species (Rogers, 1981) and later confirmed the addition of a sixth species following a collection of fresh material (Rogers et al., 1996). *Entonaema liquescens* A. Møller and *E. mesenterica* A. Møller are the only two species widely distributed. *Entonaema splendens* (Berk & Curtis) Lloyd was the first *Entonaema* to be reported from Thailand (Jadamara, 1996) but based on the description and illustration given we suspect that it is in fact *E. globosum* Heim but unfortunately we have not been able to locate the Thai material. However *E. liquescens* was subsequently collected at Wang Takrai,

Nakornnayok Province and *E. siamensis* P. Sihanonth, S. Thienhirun & A.J.S. Whalley from Mae Rim, Chiang Mai Province and described as new (Sihanonth et al., 1998). Extensive collecting of the Xylariaceae over a period of 20 years suggests that this genus is either rare or has a very short life span. Rogers (1981) suggested that it is probably not collected as frequently as other pyrenomycetes since the stromata dry out quickly or decay following ascospore production and that stromata are only in prime condition for 4-6 weeks in the southern United States. Experience in Thailand indicates that the stromata are in prime condition, and therefore recognizable, for only a short period and this may explain its infrequent collection here. It is worth noting that we have not had confirmation of any further collections of *Entonaema* from Thailand since the publication of *E. siamensis* in 1998. *Hypoxylon comedens* Ces. was placed in his *Applanata* section of *Hypoxylon* by Miller in his monograph of the genus (Miller, 1961). Following their revision of *Camillea* and related taxa. Læssøe et al. (1989) concluded that its position was untenable and Ju & Rogers in their revision of *Hypoxylon* excluded *H. comedens* from *Hypoxylon* S.Str. (Ju & Rogers, 1996). To date, no home has been found for this taxon which almost certainly warrants individual generic status. *Hypoxylon comedens* was originally described from Borneo and a number of collections have been made in Thailand from Nam Nao National Park (5 collections), Petchaboon Province, Kaeng Krachan National Park (1 collection), Petchaburi Province, and Doi Inthanon National Park (1 collection), Chiang Mai Province. Stromata are orbicular to elongate, discrete, plano-convex with abrupt margin, black and very hard and carbonaceous. Ostioles are slightly sunken and the perithecia are large occupying the entire stroma with bases resting on the wood. Ascospores are oblong with rounded ends and light brown in colour (Miller, 1961). The ascospores are smooth by SEM and possibly bear

a germ slit and are clearly unlike *Camillea* species (Læssøe et al., 1989). San Martín and Rogers (1993) reported on a fungus which was similar to *H. comedens* and exhibited some similar characteristics as found in *Biscogniauxia* especially the absence of a basal entostroma, applanate and erumpent stroma, ostiole pits and light brown and smooth spores. They further stated that "It seems most prudent, however, to await the availability of fresh material so that further observations and culturable data can be brought to bear on the taxonomic statues of this fungus (San Martín & Rogers, 1993). *Rostrohypoxylon terebratum* J. Fourn & M. Stadler is certainly an interesting and unusual member of the Xylariaceae. It possesses strikingly shaped ostiolar necks which resemble the neck of a stubby bottle with a broadly rounded apex. The stroma is carbonaceous but yields a dilute greenish olivaceous pigment with 10% KOH and the surface has cylindrical holes or pits irregularly scattered between the perithecial mounds which are reported to be usually filled with olivaceous-yellow material (Fournier et al., 2010). The anamorph is described as having simple conidiophores similar to *Sporothrix*-like or *Virgariella*-like types as recognized by Ju and Rogers (1996). Fournier et al. (2010) noted that it has only been found on dead bark of *Lithocarpus* with just the two collections from Chiang Mai Province. *Jumillera* & *Whalleya* (Ju et al., 1997) are two genera closely related to, and previously included in, the *Applanata* section of *Hypoxylon* (Miller, 1961). Following their revision of the genus *Hypoxylon* (Ju & Rogers, 1996) their position in the genus was untenable. They both possess characteristics in common with *Biscogniauxia* but were separated as two separate genera on the basis of their more restricted stromata which is less robust than in *Biscogniauxia* and on the form of their anamorph (Rogers et al., 1997). Although both have been reported from Thailand they do not appear to be common. Certainly *Whalleya microplaca* (Theiss.)

J.D.Rogers., Y-M. Ju & San Martín is more frequent in Malaysia and has a host preference for *Sassafras* (Whalley & Whalley, 2007).

### Endophytic Xylariaceae

The Xylariaceae are regular and often dominant members of the endophytic communities of tropical plants (Petrini et al., 1995). There have been numerous studies on endophytes from Thai plants and these invariably show the presence of family members. Lumyong et al. (2004) have subsequently been regularly publishing new records. Okane et al. (2008) undertook a major study of endophytic Xylariaceae in Thailand in which 405 strains of Xylariaceae (273 endophytic and 132 saprobic strains) were studied to reveal the diversity and taxonomy of endophytes and the relationships between those endophytes and saprobic Xylariaceae in Thailand that had been recorded according to fruit-body formation on decayed plant materials. This study from Khao Yai National Park revealed a dominance by *Xylaria* species and based on analysis of 28S rDNA D1/D2 sequences revealed 21 xylariaceous species inhabiting tropical foliage at the Khao Yai site. Species of *Hypoxylon* and related fungi were much less common. Our own studies from various sites and host plants in Thailand support these findings with *Xylaria* being the dominant genus in most cases (Mekkamol et al., 1997; Chareprasert et al., 2006; 2010; 2012). Interesting Mekkamol et al. (1997) found *Daldinia eschscholtzii* to be dominant in teak leaves sampled early in the rainy season with *Xylaria* later becoming more frequent. It was also shown that inoculation of pre-sterilized twigs with pure cultures isolated from teleomorphic stromata on decaying plant material collected from the sites where endophytic isolates had been obtained resulted in the formation of mature stromata of *D. eschscholtzii* in 12 to 14 weeks whereas species of *Xylaria* took over 24 weeks or longer (Mekkamol et al., 1997; Mekkamol, 1998). *Xylaria grammica* (Mont.) Mont. and *X. cubensis* (Mont.)

Fr. were identified from mature stromata following inoculation with endophytic isolates. They suggested that this was a result of the higher inoculum potential of the *Daldinia* ascospores following the earlier development of mature stromata of that fungus and its ability to produce large numbers of spores. *Xylaria cubensis* is a frequent endophytic isolate (Okane et al., 2008).

### Secondary metabolites

The Xylariaceae are a rich and major resource for novel chemical compounds and since our early studies on different genera (Anderson et al., 1982;

1983; 1984a, b; 1985; 1988; Edwards & Whalley, 1979; Edwards et al., 1982, 1991a, b; 1989a, b; Whalley & Edwards, 1987; 1995; Poyser et al., 1986) considerable use has been made of secondary metabolites, often in combination with molecular data, and these data have proved fundamental in the recognition of inter and intra generic relationships (e.g. Stadler et al., 2001a, b; 2004; 2008b) and in the recognition of new species (Stadler et al., 2008a; Fournier et al., 2010). Many of the compounds isolated over this period are summarized by Stadler & Hellwig (2005).

### Conclusions

1. The family Xylariaceae is very well represented in Thailand with almost one third of all known genera being recorded.
2. *Rhopalostroma* is a mainly Asian inhabitant and three new species out of the 11 known species are from Thailand.
3. *Rostrohypoxylon* and *Emarcea* are new genera described from Thailand.
4. Xylariaceous genera are commonly isolated as endophytes from a range of Thai plants.
5. Thailand is considered to be a country where there are many unknown species of the Xylariaceae.

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