Three novel species and a new record of *Daldinia* (Hypoxylaceae) from Thailand

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# Abstract

In an investigation of stromatic Xylariales in Thailand, several specimens of *Daldinia* were discovered. Three novel species (*D. flavogranulata*, *D. phadaengensis*, and *D. chiangdaoensis*) were recognized from a molecular phylogeny based on concatenated ITS, LSU, RPB2, and TUB2 sequence data, combined with morphological characters and secondary metabolite profiles based on high performance liquid chromatography coupled to diode array detection and mass spectrometry (HPLC-MS). The major components detected were cytochalasins (in *D. flavogranulata* and *D. chiangdaoensis*) and daldinin type azaphilones (in *D. phadaengensis*). In addition, *D. brachysperma*, which had hitherto only been reported from America, was found for the first time in Asia. Its phylogenetic affinities were studied, confirming previous suspicions from morphological comparisons that the species is closely related to *D. eschscholtzii* and *D. bambusicola*, both common in Thailand. *Daldinia flavogranulata*, one of the new taxa, was found to be closely related to the same taxa. The other two novel species, *D. phadaengensis* and *D. chiangdaoensis*, share characters with *D. korfii* and *D. kretzschmarioides*, respectively.

Keywords Ascomycota · Sordariomycetes · Chemotaxonomy · Three new species

# Introduction

The genus *Daldinia* was erected by Cesati and De Notaris (1863) in honor of the Swiss monk, Agostino Daldini. Today, it is one of largest genera in the Hypoxylaceae (Ascomycota, Xylariales). Traditionally, *Daldinia* species were recognized by the internal concentric zones below the perithecial layer in their stroma and by the presence of KOH-

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**Taxonomic novelties** *Daldinia chiangdaoensis* Sritikitkulchai, Wongkanoun, M. Stadler & Luangsaa-ard, *D. flavogranulata* Sritikitkulchai, Wongkanoun, M. Stadler & Luangsaa-ard, and *D. phadaengensis* Sritikitkulchai, Wongkanoun, M. Stadler & Luangsaa-ard.

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extractable pigments on and below their stromatal surface (Ju et al. 1997). The latest world monograph of the genus compiled morphological, ultra-structural, and chemotaxonomic data for more than a thousand specimens and cultures, and included a preliminary phylogeny based on ITS sequence data (Stadler et al. 2014). Daldinia species are extremely prolific secondary metabolite producers, and the metabolites of their stromata and cultures can be used as taxonomic markers, while others exert selective and prominent activities in biological systems (Helaly et al. 2018).

While the majority of Daldinia species are associated with dicots, some of them like D. bambusicola are associated with bamboo (monocot) in Thailand (Ju et al. 1997). Hsieh et al. (2005) reported that D. bambusicola is closely related to D. caldariorum based on TUB2 and ACTA1 sequences. In India, Daldinia graminis and D. sacchari are found on sugarcane (Dargan and Thind 1985). Narmani et al. (2018) revealed that D. sacchari is phylogenetically related to D. eschscholtzii, and even isolated two new cytochalasins, which are the characteristic stromatal metabolites of the D. eschscholtzii complex. Furthermore, several species of Daldinia produce stromata on fire-damaged woods, including D. vernicosa, D. loculata, D. caldariorum, D. gelatinoides, and D. loculatoides (Stadler et al. 2014). Stromata of some species of Daldinia (i.e., D. placentiformis, D. korfii, and D. kretzschmarioroides) appear morphologically similar to Hypoxylon as they are lacking internal concentric zones. However, the affinities of these species to Daldinia were confirmed by ITS and TUB2 sequences, and by the fact that stromata of D. korfii contain cytochalasins and concentricol B (Sir et al. 2016b). These compounds can be used as molecular markers for D. concentrica, D. eschscholtzii, and some members of the D. eschscholtzi group (Quang et al. 2002; Stadler et al. 2014). Morphologically, D. kretzschmarioroides is very closely linked to Hypoxylon, while multiple loci analyses and metabolomics profiles indicate a closer relationship with Daldinia (Wongkanoun et al. 2019). The phylogenetic affinities of Daldinia and allied genera were also recently confirmed using a multi-locus phylogeny in two independent studies by Wendt et al. (2018) and Daranagama et al. (2018). They used many type and authentic strains of the stromatic Xylariales, which led to a rearrangement of the genera, and provided a phylegetic backbone tree of these pyrenomycetes for the first time. Recently, some strains representing important lineages of the Hypoxylaceae have been selected for a phylogenomic study relying on high quality genomes and the first papers on comparative functional genomics (Wibberg et al. 2020) and on the occurrence of ITS polymorphisms (Stadler et al. 2020) have been published. Nevertheless, numerous species of the Hypoxylaceae remain to be recollected and cultured, and new taxa are steadily being discovered in particular from tropical countries.

In the course of taxonomic studies on stromatic Xylariales in Thailand, involving extensive field work, we have recently encountered three new species and a new record for the country. The present study is dedicated to their description and illustration, and we also provide evidence on their phylogenetic position and their chemotaxonomy.

Materials and methods

Survey and sample collection

Stromatic Xylariales were collected in selected forests, i.e., community forests, national parks, and reforestation areas (Pha Daeng Zinc Mine area) in Thailand. Macrophotographs were taken using a Canon 60D digital camera (Canon Inc. Tokyo, Japan). Fungal cultures were obtained using a multiple spore isolation method (Sir et al. 2016a). Germinated ascospores were transferred to new agar plates. Axenic cultures and vouchers were deposited in Thailand Bioresource Research Center (TBRC, BCC) and BIOTEC Bangkok Herbarium (BBH), respectively. Scanning electron microscopy (SEM) was carried out using a conventional procedure as described by Kuhnert et al. (2017).

Morphological characterizations and HPLC profiling

Morphological characters, such as stromatal size and shapes, perithecia, asci, and ascospores were examined in accordance with Stadler et al. (2014) using an Olympus ZX31 (Olympus Corporation, Tokyo, Japan) and a dissecting microscope Olympus SZ61 (Olympus). Fungal cultures were obtained on several media, i.e., oatmeal agar (Difco OA), potato dextrose agar (Difco PDA), and yeast malt glucose agar (1% malt extract, 0.4% glucose, and 0.4% yeast extract; agar 1%; YMGA). The morphological studies were carried out on 9 cm Petri dishes. Conidiogenous cells and conidiophore branching patterns of the anamorph were investigated as proposed by Ju and Rogers (1996). Furthermore, stromatal color, KOH-extractable pigments, and cultures are recorded using the color chart of Rayner (1970). For chemotaxonomic studies, stromatal secondary metabolites were extracted with acetone and analyzed using high performance liquid chromatography coupled with diode array and high resolution electrospray mass spectrometric detection (HPLC/DAD-HRESIMS) in a similar manner as described by Yuyama et al. (2018) and Kretz et al. (2019). Instrumental settings and conditions were the same as described in Kuhnert et al. (2017).

DNA extraction, PCR, and sequencing

A method based on cetyltrimethyl ammonium bromide (CTAB) was used to extract total genomic DNA from the
mycelia according to Mackill and Bonman (1995). The internal transcribed spacer regions (ITS), and partial sequences of the large subunit of the rDNA (LSU), RNA polymerase II (RPB2), and beta tubulin (TUB2) were amplified, following the standard primers introduced by White et al. (1990; ITS1, ITS4 and ITS5), Vilgalys and Hester (1990; LR7), Bunyard et al. (1994; LROR), Liu et al. (1999; RPB2–5F and 7Cr), and O’Donnell and Cigelnik (1997; T1 and T22), according to the protocols of Otto et al. (2016) and Wendt et al. (2018). The polymerase chain reaction (PCR) products were purified and sequenced using the same primers as used for the PCR reaction. DNA sequences were checked and assembled using BioEdit v. 7.2.5 (Hall 2013). All newly generated sequences were submitted to GenBank (https://www.ncbi.nlm.nih.gov/) and listed in Table 1.

Phylogenetic analyses

All sequences were aligned in MUSCLE (Edgar 2004) and refined by direct examination. Multiple sequence alignments were analyzed with closely matched sequences and other reference taxa obtained from GenBank as shown in Table 1. Sequences were analyzed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian algorithm (MB). The MP analysis was performed in PAUP*4.0b10 (Swofford 2002), and all characters were equally weighted and gaps were treated as missing data. The most parsimonious trees were obtained from heuristic searches: 100 replicates of stepwise random addition and tree-bisection-reconnection (TBR) as branch swapping algorithm. Maximum parsimony bootstrap supports (MPBS) were estimated by 1000 replicates (10 replicates of stepwise random sequence addition). Tree length, consistency index (CI), retention index (RI), relative consistency index (RC), and homoplasy index (HI) were estimated. The ML tree and bootstrap analyses (MLBS) were conducted through the CIPRES Science Gateway V. 3.3 (Miller et al. 2010) using RAxML 8.2.4 (Stamatakis 2014) with the BFGS method to optimize GTR rate parameters. Bayesian posterior probabilities (BPP) of the branches were computed using MrBayes 3.0B4 (Huelsenbeck and Ronquist 2001) with the best-fit model (GTR + I + G) selected by AIC in Mr Modeltest 2.2 (Nylander 2004), tested with hierarchical likelihood ratios (hLRTs). Three million generations were run in four Markov chains and sampled every 100 generations with a burn-in value set at 3000 sampled trees. Sequence alignments were deposited at TreeBase (submission ID 25485: www.treebase.org). Sequences of Graphostoma platystomum CBS 270.87 and Xylaria hypoxylon CBS12260 obtained from GenBank were used as outgroups. The RAxML based phylogenetic tree is shown in Fig. 5.

Results and discussion

Molecular phylogeny

Sixty-one new sequences were generated and included into a combined ITS, LSU, RPB2, and TUB2 dataset to clarify the phylogenetic relationships of newly collected Thai specimens of Daldinia and distinguish them from other species and genera in the Hypoxylaceae (Table 1). PCR amplifications yielded approximately 840 bp, 1213 bp, 829 bp, and 1583 bp of ITS, LSU, RPB2, and TUB2 sequences. The dataset of the multi-locus DNA sequences included 67 taxa from the Hypoxylaceae based on Annulohypoxylon (5), Daldinia (35), Hypoxylon (12), Hypomontagnella (4), Jackrogersella (3), and Pyrenopolyporus (6). The combined dataset consisted of 4465 characters, of which 2600 were constant, 1434 parsimony informative, and 431 uninformative. In MP analysis, a CI of 0.357, a RI of 0.638, and a HI of 0.643 yielded three equally most parsimony trees. The phylogenetic tree included 5 major clades: a Daldinia clade subdivided into five branches (D I–D V) and one clade each representing Pyrenopolyporus (Py), Hypomontagnella (Hy), Annulohypoxylon, and Jackrogersella (AJ) and Hypoxylon (H) (Fig. 6). Clade D I, accommodating D. flavogranulata (BCC 89363, BCC 89365, and BCC 89376) and D. caldariorum appeared monophyletic and was supported with high bootstrap values. These data are in agreement with the morphological characters. Clade D II also group with a strong bootstrap support and comprised D. bambusicola and D. brachysperma. Clade D III included the D. eschscholtzii complex, where D. placentiformis and D. theissenii were grouping as a strongly supported monophyletic clade. The strongly supported clade D IV grouped with clades D II and D III as sister clades and consisted of D. koryi, D. kretzschmarioides, D. phadaengensis (BCC 89349, BCC 89350), and D. chiangdaoensis (BCC 88220, BCC 88221). In agreement with the morphological evidence, the four taxa were separated in a highly supported clade (100% BSMP, 100% BSML, and 1.00 BPP). Clade D V also formed a fully statistically supported, monophyletic clade (100% BSMP, 100% BSML, 1.00 BPP) appearing as sister clade to clades D II and D III. Within clade D V, two moderately supported subclades were observed; the first one consisting of D. andina, D. concentrica, D. dennisii, D. loculatoides, D. macaronesica, and D. steglichii and the second one comprising D. petriniae, D. pyrenaica, D. subverniciosa, and D. vernicosa. The fully supported clade Py contained Pyrenopolyporus species as sister clade to D V. Clade Hy included representatives of the recently erected genus Hypomontagnella (Lambert et al. 2019) represented
| Species                        | Strains     | Country      | GenBank accession numbers | Reference                          | Status   |
|-------------------------------|-------------|--------------|---------------------------|------------------------------------|----------|
|                               |             |              | ITS  | LSU   | RPB2 | TUB2 |                   |                       |          |
| *Annulohypoxylon annulatum*   | CBS 140775  | Texas        | KY610418 | KY610418 | KY624263 | KY376353 | Kuhnert et al. (2017; TUB2), Wendt et al. (2018; ITS, LSU, RPB2) | ET          |
|                               |             |              |             |        |        |      |                   |                       |          |
| *Annulohypoxylon moriforme*   | CBS 123579  | Martinique   | KX376321 | KY610425 | KY624289 | KY271261 | Kuhnert et al. (2017; ITS, TUB2), Wendt et al. (2018; LSU, RPB2) | ET          |
|                               |             |              |             |        |        |      |                   |                       |          |
| *Annulohypoxylon nitens*      | MFLUCC 12.0823 | Thailand    | KJ934991 | KJ934992 | KJ934994 | KJ934993 | Darnagama et al. (2015) | ET          |
| *Annulohypoxylon stygium*     | MUCL 54601  | French Guiana | KY610409 | KY610475 | KY624292 | KY271263 | Wendt et al. (2018) | ET          |
| *Annulohypoxylon truncatum*   | CBS 140778  | Texas        | KY610419 | KY610419 | KY624277 | KY376352 | Kuhnert et al. (2017; TUB2), Wendt et al. (2018; ITS, LSU, RPB2) | ET          |
| *Daldinia andina*             | CBS 114736  | Ecuador      | AM749918 | KY610430 | KY624239 | KJ972259 | Bitzer et al. (2008; ITS), D. grandis, Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2) | HT          |
|                               |             |              |             |        |        |      |                   |                       |          |
| *Daldinia bambusicola*        | CBS 122872  | Thailand     | KY610385 | KY610431 | KY624241 | KY951688 | Hsieh et al. (2005; TUB2), Wendt et al. (2018; ITS, LSU, RPB2) | HT          |
|                               |             |              |             |        |        |      |                   |                       |          |
| *Daldinia bambusicola*        | TBRC 8878   | Thailand     | MH922869 | MH922870 | MK165431 | MK165422 | Wongkanoun et al. (2019) | HT          |
| *Daldinia bambusicola*        | TBRC 8879   | Thailand     | MH922872 | MH938543 | MK165432 | MK165423 | Wongkanoun et al. (2019) | HT          |
| *Daldinia bambusicola*        | BCC 27937   | Thailand     | MN153861 | MN153876 | MN172217 | N/a     | This study |                       |
| *Daldinia bambusicola*        | BCC 33678   | Thailand     | MN153860 | MN153877 | MN172218 | N/a     | This study |                       |
| *Daldinia brachysperma*       | BCC 33676   | Thailand     | MN153854 | MN153871 | N/a     | MN172205 | This study |                       |
| *Daldinia caldariorum*        | MUCL 49211  | France       | AM749934 | KY610433 | KY624242 | KJ977282 | Bitzer et al. (2008; ITS), Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2) | HT          |
|                               |             |              |             |        |        |      |                   |                       |          |
| *Daldinia caldariorum*        | CBS 122874  | USA          | KU683756 | KU683756 | KU684298 | U684128  | U'Ren et al. 2016 |                       |
| *Daldinia chiangdaoensis*     | BCC 88220   | Thailand     | MN153850 | MN153867 | MN172208 | MN172197 | This study |                       |
| *Daldinia chiangdaoensis*     | BCC 88221   | Thailand     | MN153851 | MN153868 | MN172209 | MN172198 | This study |                       |
| *Daldinia concentrica*        | CBS 113277  | Germany      | KY610434 | KY624243 | KJ977274 |           | Triebel et al. (2005; ITS), Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2) | HT          |
| *Daldinia dennisii*           | CBS 114741  | Australia    | JX658477 | KY610435 | KY624244 | KJ977262 | Stadler et al. (2014; ITS), Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2) | HT          |
| Species                  | Strains  | Country | GenBank accession numbers    | Reference                                      | Status |
|-------------------------|----------|---------|------------------------------|------------------------------------------------|--------|
|                         |          |         | IT S  | LSU   | RPB2  | TUB2  |                     |        |
| **Daldinia eschscholtzii** | MUCL 45435 | Benin   | JX658484 | KY610437 | KY624246 | KC977266 | Stadler et al. (2014; ITS), Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2) |        |
|                         | TBRC 8876 | Thailand | MH938532 | MH938541 | MK165429 | MK165420 | Wongkanoun et al. (2019) |        |
|                         | BCC27887 | Thailand | MN153861 | MN153878 | MN172214 | N/A | This study |        |
|                         | BCC28091 | Thailand | MN153862 | MN153879 | MN172215 | N/A | This study |        |
|                         | BCC62428 | Thailand | MN153863 | MN153880 | MN172216 | N/A | This study |        |
| **Daldinia flavogramulata** | BCC89363 | Thailand | MN153856 | MN153873 | MN172211 | MN172200 | This study | HT     |
|                         | BCC89365 | Thailand | MN153857 | MN153874 | MN172212 | MN172201 | This study |        |
|                         | BCC89376 | Thailand | MN153858 | MN153875 | MN172213 | MN172202 | This study |        |
| **Daldinia korfii**      | EBS 067  | Argentina | KY204018 | N/A | N/A | KY204014 | Sir et al. (2016b) |        |
|                         | EBS 473  | Argentina | KY204020 | N/A | N/A | KY204016 | Sir et al. (2016b) |        |
| **Daldinia kretzschmaroides** | TBRC 8875 | Thailand | MH938531 | MH938540 | MK165425 | MK165416 | Wongkanoun et al. (2019) | ET     |
| **Daldinia loculatooides**  | CBS 113279 | UK | AF176982 | KY610438 | KY624247 | KX271246 | Johannesson et al. (2000; ITS), Wendt et al. (2018; LSU, RPB2) | ET     |
| **Daldinia macaronesica**       | CBS 113040 | Spain | KY610398 | KY610477 | KY624294 | KX271266 | Wendt et al. (2018) | PT     |
| **Daldinia phadaengensis**      | BCC89349 | Thailand | MN153852 | MN153869 | MN172206 | MN172195 | This study | HT     |
|                         | BCC89350 | Thailand | MN153853 | MN153870 | MN172207 | MN172196 | This study |        |
| **Daldinia petriniae**        | MUCL 49214 | Austria | AM749937 | KY610439 | KY624248 | KC977261 | Bitzer et al. (2008; ITS), Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2) | ET     |
| **Daldinia placentiformis**    | MUCL 47603 | Mexico | AM749921 | KY610440 | KY624249 | KC977278 | Bitzer et al. (2008; ITS), Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2) |        |
| **Daldinia pyrenaica**        | MUCL 53969 | France | KY610413 | KY610413 | KY624274 | KY624312 | Wendt et al. (2018) |        |
| **Daldinia steiglii**         | MUCL 43512 | Papua New Guinea | KY610399 | KY610479 | KY624250 | KX271269 | Wendt et al. (2018) |        |
| **Daldinia subvernicosa**      | TBRC 8877 | Thailand | MH938533 | MH938542 | MK165430 | MK165421 | Wongkanoun et al. (2019) | HT     |
| **Daldinia theissenii**       | CBS 113044 | Argentina | KY610388 | KY610441 | KY624251 | KX271247 | Wendt et al. (2018) | PT     |
| **Daldinia vernicosa**        | CBS 119316 | Germany | KY610395 | KY610442 | KY624252 | KC977260 | Kuhnert et al. (2014; TUB2), Wendt et al. (2018; ITS, LSU, RPB2) |        |
| **Graphostroma platystomum**  | CBS 270.87 | France | JX658535 | DQ836906 | KY624296 | HG934108 | Stadler et al. (2014; ITS), Zhang et al. (2006; LSU), Koukol et al. | HT     |
| Species                     | Strains  | Country          | GenBank accession numbers                      | Reference                                                                 | Status   |
|-----------------------------|----------|------------------|------------------------------------------------|---------------------------------------------------------------------------|----------|
| Hypomontagnella monticulosa | MUCL 54604 | French Guiana    | KY610404 KY610487 KY624305 KX271273            | (2015; TUB2), Wendt et al. (2018; RPB2); Wendt et al. (2018)              | ET       |
| Hypomontagnella monticulosa | BCC58592  | Thailand         | MN153864 MN153881 MN172219 MN172204            | This study                                                               | ET       |
| Hypomontagnella monticulosa | BCC69203  | Thailand         | MN153865 MN153882 MN172220 MN172203            | This study                                                               | ET       |
| Hypomontagnella submonticulosa | CBS 115280 | France           | KC968923 KY610457 KY624226 KX977267            | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)          | ET       |
| Hypoxylon crocopeplum       | CBS 119004 | France           | KC968907 KY610445 KY624255 KX977268            | Wendt et al. (2018)                                                     | ET       |
| Hypoxylon fragiforme        | MUCL 51264 | Germany          | KC477229 KM186295 KM186296 KX271282            | Stadler et al. (2013; ITS), Daranagama et al. (2015; LSU, RPB2), Wendt et al. (2018; TUB2) | ET       |
| Hypoxylon fuscum            | CBS 113049 | France           | KY610401 KY610482 KY624299 KX271271            | Wendt et al. (2018)                                                     | ET       |
| Hypoxylon haematostroma     | MUCL 53301 | Martinique       | KC968911 KY610484 KY624301 KX977291            | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)          | ET       |
| Hypoxylon haematostroma     | BCC50533  | Thailand         | MN153866 MN153883 MN172221 N/A                 | This study                                                               | ET       |
| Hypoxylon investiens        | CBS 118183 | Malaysia         | KC968925 KY610450 KY624259 KX977270            | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)          | ET       |
| Hypoxylon lateripigmentum   | MUCL 53304 | Martinique       | KC968933 KY610486 KY624304 KX977290            | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)          | ET       |
| Hypoxylon lenormandii       | CBS 119003 | Ecuador          | KC968943 KY610452 KY624261 KX977273            | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LS, RPB2)           | ET       |
| Hypoxylon petriniae         | CBS 114746 | France           | KY610405 KY610491 KY624279 KX271274            | Kuhnert et al. (2017; TUB2), Wendt et al. (2018; ITS, LSU, RPB2)          | ET       |
| Hypoxylon rickii            | MUCL 53309 | Martinique       | KC968932 KY610416 KY624281 KX977288            | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)          | ET       |
| Hypoxylon rubiginosum       | MUCL 52887 | Germany          | KC477232 KY610469 KY624266 KY624311            | Stadler et al. (2013; ITS), Wendt et al. (2018; LSU, RPB2, TUB2)          | ET       |
| Hypoxylon samuelsii         | MUCL 51843 | Guadeloupe       | KC968916 KY610466 KY624269 KX977286            | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; TUB2)                | ET       |
| Species Strains | Species Country | GenBank accession numbers | Reference Status |
|-----------------|-----------------|--------------------------|------------------|
| Jackrogersella cohaerens | CBS 119126 Germany | KX60396, KY624270 | Wendt et al. (2018) |
| Jackrogersella minutella | CBS 119015 Portugal | KX60381, KY60424 | Kuhnert et al. (2017); Wendt et al. (2018) |
| Jackrogersella multiformis | CBS 119016 Germany | KX624235, KX271240 | Kuhnert et al. (2014); Wendt et al. (2018) |
| Pyrenopolyporus hunteri | MUCL 52573 Ivory Coast | KX60472, KX624300 | Wendt et al. (2018) |
| Pyrenopolyporus laminosus | TBCR 8871 Thailand | KX60485, KX624303 | Wendt et al. (2018) |
| Pyrenopolyporus nicaraguensis | CBS 117739 Burkina Faso | KX60407, KX624307 | Wendt et al. (2018) |
| Pyrenopolyporus symphyon | TBCR 8873 Thailand | KX60407, KX624307 | Wendt et al. (2018) |
| Xylaria hypoxylon | CBS 12360 Sweden | KX60407, KX624307 | Wendt et al. (2018) |
by *H. monticulosa* and *H. submonticulosa*. Clade AJ comprises species of *Annulohypoxylon* and *Jackrogersella*, while clade H includes species of *Hypoxylon*, which is in agreement with data of Wendt et al. (2018).

In summary, the phylogeny allowed for a clear separation of the taxa that are described below as new, even though the topology of the phylogenetic tree was not in accordance with the grouping of *Daldinia* as proposed by Stadler et al. (2014) based on ITS sequences, chemotaxonomy, and morphology. This may be due to different modes of taxon selection and the variability of ITS.

**Taxonomy**

*Daldinia chiangdaoensis* Srikitikulchai, Wongkanoun, M. Stadler & Luangsa-ard, sp. nov. Fig. 1, MB 833760

**Etymology.** “chiangdaoensis” referring to the locality where the type specimen was collected.

**Holotype:** Thailand: Chiang Mai Province, Chiang Dao, Ban Hua Thung community forest, 19.420°N, 98.971°E, hill evergreen forest, on decaying dicot wood, 13 December 2017, P. Srikitikulchai 6 S. Wongkanoun (BBH 47512).

Ex-holotype strain: BCC 88220. DNA sequences of ex-holotype strain: MN153850 (ITS), MN153867 (LSU), MN172208 (RPB2), MN172197 (TUB2).

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**Fig. 1** *Daldinia chiangdaoensis* (BBH 47512). a–c Stromatal habit. d Stromatal surface and ostioles with pigments in 10% KOH. e Longitudinal section of stroma showing perithecia and the tissue below the perithecial layer. f Perithecia (white arrow). g Ascus. h Ascus and ascospore showing germ slit (white arrow). i Ascal apical apparatus, bluing in Melzer’s reagent (black arrow). j Ascospore by SEM. k Ascospore showing germ slit (white arrow). l Ascospore in KOH showing dehiscent perispore (black arrow). Scale is indicated by bars (e 2 mm, f 0.5 mm, g–h 10 μm, i 2 μm, j–l 5 μm)
Fig. 2 *Daldinia phadaengensis* (BBH 47511). a, c Stromatal habit. b Stromatal surface with ostioles with pigments in 10% KOH. d Longitudinal section of stroma showing perithecia and the tissue below the perithecial layer. e Perithecia. f Cells of the tissue below the perithecial layer in distilled water under light microscope. g Perithecium in distilled water under light microscope. h–i Ascospores by SEM. j Ascospore showing germ slit. k–l Ascospores in KOH showing dehiscent perispore (black arrow). Scale is indicated by bars (a 10 mm, c 5 mm, d 1 mm, e 0.5 mm, g 0.1 mm, h–l 5 μm)
Comparison of morphological and chemotaxonomic characters of species with massive stromata and long tubular perithecia and Hypoxylaceae species that are similar to *Daldinia*

| Taxon Ascospore germ slit | KOH-extractable pigments | Metabolite (stroma) |
|---------------------------|--------------------------|---------------------|
| *Daldinia bambusicola* (holotype) Dehiscent Spore-length on convex side 6–7 × 3–4 | Dark Livid | BNT and cytochalasins. |
| *Daldinia brachysperma* | Without BNT and cytochalasins. | N.A. |
| *Daldinia caldariorum* Dehiscent Spore-length on convex side (13–15) × 4.5–5.5 | Livid purple | BNT, cytochalasins. |
| *Daldinia flavogranulata* *Daldinia korfii* Dehiscent Spore-length on convex side (10.3–11) × (4.8–5.2) | Brown vinaceous to dark | BNT, concentricol B, and cytochalasin. |
| *Daldinia phadaengensis* *Daldinia cf. placentiformis* Dehiscent Spore length, dorsal 14–16 × 6.5 | Isabelline | N/A |
| *Hypoxylon kretzschmarioides* Dehiscent Spore length, dorsal (12–16) × 5 | Brown vinaceous to dark | BNT, naphtthols, naphthoquinones |
| *Pyrenopolyporus laminosus* Indehiscent Spore length, dorsal 11–13.5 × 4.2–5.2 | Olivaceous | BNT, naphtthols, and unknown |
| *Pyrenopolyporus nicaraguensis* Indehiscent Spore length, dorsal (11–15) × 5–6 | Dark | BNT, naphtthols, and unknown |

**Teleomorph.** *Stromata* superficial, with conspicuous perithecial outlines, (11–16–20 mm long, 9–11 mm broad, 4–5 mm thick; surface Olivaceous (48) to Dull Green (70), with 10% KOH - extractable pigments Vinaceous Gray (116) or Fuscous Black (104); dark brown to reddish brown granules forming a thin crust above perithecial layer; the tissue between perithecia orange brown or gray; the tissue below the perithecial layer without internal concentric zones, gray or black, 2.1–3.2 mm thick. *Perithecia* monostichous, obovoid to lanceolate 1.14–1.43 mm high, 0.29–0.43 mm broad; ostioles papillate.

Anamorph on OA. *Conidiophores* with virgariella-like to (much more frequently) nodulisporium-like branching patterns as defined in Ju and Rogers (1996), erect, main axis hyaline to pale green and smooth to roughened. *Conidiogenous cells* cylindrical, hyaline, finely roughened, 11–13 (–27) × 3–4 μm (𝑥 = 19.60 × 4.3 μm, n = 5). *Conidia* hyaline to pale green, smooth, ellipsoid, 7–8 × 3–4 μm (𝑥 = 7.6 × 3.6 μm, n = 10).

**Culture characteristics.** Colonies on OA reaching the edge of the Petri dish in 3 weeks, at first whitish, becoming velvety to felty, Grayish Lavender (98); reverse Dark Purple (36) and Herbage Green (71), azonate with distinct margins (Fig. 5b1). Colonies on YMGA, reaching the edge of the Petri dish in 3 weeks, azonate, aerial mycelium at first whitish becoming velvety to felty, smoke, Rosy Vinaceous (58); reverse Olivaceous (48) (Fig. 5b2). Colonies on PDA, reaching the edge of the Petri dish 9 cm in 3 weeks, aerial mycelium at first whitish, becoming Rosy Vinaceous (58); reverse Olivaceous (48) (Fig. 5b3).

**Anamorph on OA.** *Conidiophores* with virgariella-like to (much more frequently) nodulisporium-like branching patterns as defined in Ju and Rogers (1996), erect, main axis hyaline to pale green and smooth to roughened. *Conidiogenous cells* cylindrical, hyaline, finely roughened, 11–13 (–27) × 3–4 μm (𝑥 = 19.60 × 4.3 μm, n = 5). *Conidia* hyaline to pale green, smooth, ellipsoid, 7–8 × 3–4 μm (𝑥 = 7.6 × 3.6 μm, n = 10).

**Anamorph on YMGA.** *Conidiophores* with the same branching pattern and dimensions of conidiogeneous cells and conidia as on OA.

**Anamorph on PDA not observed even after up to 3 months.**

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**Table 2** Comparison of morphological and chemotaxonomic characters of *Hypoxylaceae* species with massive stromata and long tubular perithecia and *Daldinia* species that are similar to *Daldinia*

| Taxon Ascospore germ slit | KOH-extractable pigments | Metabolite (stroma) |
|---------------------------|--------------------------|---------------------|
| *Daldinia bambusicola* (holotype) Dehiscent Spore-length on convex side 6–7 × 3–4 | Dark Livid | BNT and cytochalasins. |
| *Daldinia brachysperma* | Without BNT and cytochalasins. | N.A. |
| *Daldinia caldariorum* Dehiscent Spore-length on convex side (13–15) × 4.5–5.5 | Livid purple | BNT, cytochalasins. |
| *Daldinia flavogranulata* *Daldinia korfii* Dehiscent Spore-length on convex side (10.3–11) × (4.8–5.2) | Brown vinaceous to dark | BNT, concentricol B, and cytochalasin. |
| *Daldinia phadaengensis* *Daldinia cf. placentiformis* Dehiscent Spore length, dorsal 14–16 × 6.5 | Isabelline | N/A |
| *Hypoxylon kretzschmarioides* Dehiscent Spore length, dorsal (12–16) × 5 | Brown vinaceous to dark | BNT, naphtthols, naphthoquinones |
| *Pyrenopolyporus laminosus* Indehiscent Spore length, dorsal 11–13.5 × 4.2–5.2 | Olivaceous | BNT, naphtthols, and unknown |
| *Pyrenopolyporus nicaraguensis* Indehiscent Spore length, dorsal (11–15) × 5–6 | Dark | BNT, naphtthols, and unknown |

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**Fig. 3** *Daldinia flavogranulata* (BBH 47510). a–e Stromatal habit. d Longitudinal section of stroma showing the tissue below the perithecial layer producing internal concentric zones. f Perithecia (white arrow). g–h Ascus with ascospores. i Ascal apical apparatus, bluing in Melzer’s reagent (black arrow). j Ascospores showing germ slit (white arrow). k–m Ascospore by SEM. Scale is indicated by bars (e 2 mm, f 0.5 mm, g–h 10 μm, i 5 μm, j–m 2 μm
Secondary metabolites. 1,1′-Binaphthalene-4,4′,5,5′-tetrrol (BNT, 1), cytochalasans (Supplementary Fig. S1).

Notes. There are three species that are most similar to *D. chiangdaoensis* in producing massive, azonate tissue below the perithecial layer and obvioid perithecia as the following details: *D. placentiformis*, *D. korfii*, and *D. kretzschmarioides*. The former species differs in its ascospores size ranges, 14.5–16 × 6.5–7 μm. 1,1′-Binaphthalene-4,4′,5,5′-tetrrol (BNT, 1) *Daldinia kretzschmarioides* differs in the production of a green olivaceous pigment and a brown KOH-extractable pigment from the outer stroma. The ascospore size range of *D. chiangdaoensis* is larger than that of *D. kretzschmarioides* [13–15–18 (−19) × (5–)6–8 (−10) vs 13–15 (−16) × (4–)5–6 μm]. Phylogenetic relationships revealed that DNA sequences of *D. chiangdaoensis* clustered together with *D. kretzschmarioides* supported by high bootstrap values (Fig. 6). Morphologically, *D. korfii* (Sir et al. 2016b) differs by its ascospores size ranges, (10.3–)11–14 (−16) × (4.8–)5.2–6.2 (−7). Our molecular data also confirmed a clear separation with strong statistical support as shown in Fig. 6.

*Daldinia phadaengensis* Srikitkulchai, Wongkanoun, M. Stadler & Luangsa-ard, sp. nov. Fig. 2 MB 833761

Etymology. “phadaengensis” referring to the locality where the type specimen was collected.

Holotype: Thailand: Tak Province, Pha Daeng, Pha Daeng Zinc Mine, 16.665′ N, 98.649′ E, reforestation forest, on decaying dicot wood, 6 September 2018, P. Srikitikulchai & S. Wongkanoun (BBH 47511).

Ex-holotype strain: BCC 89349. DNA sequences of ex-holotype strain: MN153852 (ITS), MN153869 (LSU), MN172206 (RPB2), MN172195 (TUB2).

Teleomorph. Stromata superficial, spreading flat over the substrate, pulvinate, with inconspicuous perithecial outlines, 15–18 (−25) mm long, 9–13 (−16) mm broad, 1.4–2 mm thick; surface Vinaceous Gray (116) to Pale Puriplish Gray (117), with 10% KOH producing Isabelline (65) and Cinnamon (62) extractable pigments; dark brown or blackish brown granules forming a thin crust above perithecial layer; the tissue between perithecia gray or blackish brown; the tissue below perithecial layer without internal concentric zones, gray, 0.57–0.85 mm thick. Perithecia monostichous, obvoid to lanceolate 0.71–0.85 mm high, 0.28–0.35 mm broad; ostioles umbilicate to slightly raised discoid.

Ascii cylindrical; apical apparatus not observed. Ascospores dark brown to blackish brown, unicellular, irregularly ellipsoid, with narrow rounded ends, (11–)14–16 (−18) × 5–6 μm (x 5.45 × 14.05 μm, n = 50) with straight to slightly oblique germ slit covering ca. 2/3 length of the spore on convex side, perispore dehiscent in 10% KOH, smooth.

Culture characteristics. Colonies on OA reaching the edge of the Petri dish 9 cm in 2 weeks, zonate, at first whitish becoming Smoke Gray (106), with distinct margins; reverse Herbage Green (18) (Fig. 5a1). Colonies on YMGA, reaching the edge of the Petri dish 9 cm in a week, azonate, aerial mycelium initially whitish, becoming velvety to felfty, Olivaceous (48); reverse Brick (59) and Cinnamon (52) (Fig. 5a2). Colonies on PDA, reaching the edge of the Petri dish 9 cm in 1 week, aerial mycelium initially whitish, becoming Olivaceous (48), Dark Herbage Green (69) and yellow green (71); reverse Gray Olivaceous (107) to Smoke Gray (106) (Fig. 5a3).

Anamorph on OA. Conidiophores with virgariella-like to (much more frequently) nodulisporium-like branching patterns as defined in Ju and Rogers (1996), erect, main axis hyaline to pale green and smooth to roughened. Conidiogenous cells cylindrical, hyaline, finely roughened, 15–18 (−20) μm × 3 (x = 16.8 × 3 μm, n = 10). Conidia hyaline to pale yellow, smooth, ellipsoid, 6–7 × 3–4 μm (x = 6.2 × 3.04 μm, n = 25).

Anamorph on YMGA similar to that on OA.

Cultures on PDA not producing anamorphic structures in 3 months.

Secondary metabolites. BNT (1); daldinins A1 (2) and A4 (3) (Hashimoto 1994).

Notes. *Daldinia phadaengensis* is morphologically similar to *D. chiangdaoensis*, *D. korfii*, and *D. kretzschmarioides* in lacking internal concentric zones below the perithecial layer. The new species is distinguishable from the aforementioned species by morphology as well as by comparison of the molecular phylogenetic data. Strikingly, *D. phadaengensis* also differs from the other species by having yellowish orange KOH-extractable stromatal pigments and the tissue below the perithecial layer, and has the thinnest tissue below the perithecial layer (1.4–2 mm) of all known *Daldinia* species. Table 2 provides a synopsis of the morphological characters and secondary metabolites of this group of *Daldinia* species and the related genus *Pyrenomycoporus*. *Daldinia placentiformis*, another morphologically similar species, which has so far not been found in Thailand, has olivaceous pigments, owing to the presence of dalidine A (Bitzer et al. 2008). Dalidine A derivatives were originally isolated from a species referred to as “*D. concentrica*” by Hashimoto (1994), which was revised as *D. chilidiae* by Stadler et al. (2014). They are chemically similar to the lenormandins and fragirubrins that are known from *Hypoxylon* species (Kuhnert et al. 2015; Surup et al. 2018). However, this is the first time they have been identified as a major metabolites in a species that does not belong to the *D. chilidiae* group as defined by Stadler et al. (2014). Several peaks corresponding to cytochalasans were also observed but could not be further elucidated without preparative isolation, which was not possible due to scarcity of material. A major unknown compound (UCP) was also detected, whose molecular formula could not yet be identified.
**Daldinia flavogranulata** Srikitikulchai, Wongkanoun, M. Stadler & Luangsa-ard, sp. nov. Fig. 3 MB 833762

**Etymology.** “flavogranulata” refers to the yellow granules forming a thin layer above the perithecia.

**Holotype:** Thailand: Tak Province, Pha Daeng, Pha Daeng Zinc Mine, 16.665′ N, 98.649′ E, reforestation forest, on bamboo trunk (Bambusoideae) in fire damaged area, 6 September 2018, P. Srikitikulchai & S. Wongkanoun (BBH 47510).

Ex-holotype strain: BCC 89363. DNA sequences of ex-holotype strain: MN153856 (ITS), MN153873 (LSU), MN172211 (RPB2), MN172200 (TUB2).

**Teleomorph.** Stromata superficial, hemispherical, pulvinate or peltate the base broadly attached to the substrate, with conspicuous perithecial outlines, 3.6–4 cm long, 2.8–3 cm wide, 0.9–1 cm thick; surface Vinaceous Gray (116) or Purplish Gray (128), with 10% KOH producing Livid Vinaceous (83) or Brown Vinaceous (84) extractable pigments; yellow granules form a thin layer above the perithecia; the tissue between perithecia blackish brown or white; the tissue below the perithecial layer Olivaceous Buff (89) and Greenish Olivaceous (90), composed of alternating zones, darker zone dark brown to

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**Fig. 4 Daldinia brachysperma (BBH 25493).**

- **a-b** Stroma.
- **c** Stomatal surface and negative pigment test in 10% KOH.
- **d** Longitudinal section of stroma showing the tissue below the perithecial layer with internal concentric zones.
- **e** Perithecia.
- **f** Tissue below perithecial layer under light microscope.
- **g** Ascospore by SEM.
- **h** Ascospore showing germ slit (black arrow).
- **i-j** Ascospores by scanning electron microscopy.
- **k** Ascospore. Scale is indicated by bars (a, b 5 mm, e 0.5 mm, d 2 mm, h 5 μm, g, i-k 2 μm)
blackish brown 0.14–0.28 mm thick, lighter zones white, 0.42–0.57 mm thick. *Perithecia* monostichous, obovoid, lanceolate 0.87–1 mm × 0.21–0.28 mm; ostioles papillate. *Asci* cylindrical, 256–260 μm total length, the spore-bearing part, 100–108 × 8 μm; apical apparatus rectangular in outline, bluing in Melzer’s reagent, 0.5–1 high, 2–2.5 μm wide. *Ascospores* dark brown to blackish brown, unicellular, irregularly ellipsoid (9–) 10–11 (–12) × 4–5 μm (X = 10.44 × 4.64 μm, n = 25) with straight to slightly curved germ slit covering 2/3 length of the spore on convex side, without dehiscing perispore in 10% KOH.

**Culture characteristics.** Colonies on OA, reaching the edge of the Petri dish in 2 weeks, zonate, at first Dark Green (21), Dark Bluish Green (24); reverse Herbage Green (17) (Fig. 5c1). Colonies on YMGA, reaching the edge of the Petri dish in 2 weeks, aerial mycelium at first whitish becoming smoke, Herbage Green (17) and Green (20); reverse Dark Green (21) and Yellow Green (18) (Fig. 5c2). Colonies on PDA, reaching the edge of the Petri dish in 3 weeks, aerial mycelium at first whitish becoming Green (50), Dark Green (21), Herbage Green (17); reverse Green (50) (Fig. 5c3).

**Anamorph** on OA. *Conidiophores* with virgilliota-like to (much more frequently) nodulisporium-like branching patterns as defined in Ju and Rogers (1996), erect, main axis green olivaceous and smooth to roughened. *Conidiogenous cells* cylindrical, hyaline, finely roughened, 14–15 × 4–5 μm. *Conidia* hyaline, smooth, ellipsoid 4–5 × 2–3 μm.

Cultures on YMGA and PDA not producing anamorphic structures in 3 months.

**Secondary metabolites.** (BNT, 1) cytochalasans (Supplementary Fig. 2).

**Additional materials examined.** Thailand: Chiang Mai Province, Chiang Dao, Ban Huang community forest, 19.420’ N, 98.971’ E, hill evergreen forest; on dead monocot (Bambusae), 13 December 2017, P. Srikitkulchhai & S. Wongkanoun (BBH 42283); Tak Province, Pha Daeng, 16.667’ N, 98.657’ E, 520 m above sea level elevation, on bamboo trunk (Bambusoideae) in fire damaged area, 6 September 2018, P. Srikitkulchhai & S. Wongkanoun (BCC 89358, BCC 89365, BCC 89367, BCC 89376).

**Notes.** Daldinia flavogranulata closely resembles *D. bambusicola*, also on Bambusoideae, and has similar ascospore morphology and size range, 8.5–11 × 4–5 μm. However, *Daldinia flavogranulata* differs in producing yellowish orange granules in a thin layer above the perithecia. Furthermore, *D. caldariorum* resembles *D. flavogranulata* in shape and size of ascospores but differs in lacking the yellowish orange granules.

*Daldinia brachysperma* F. San Martín, Y.M. Ju, & J.D. Rogers, Mycotaxon 61: 255. 1994 Fig. 4.

**Material studied.** Thailand: Chiang Mai Province, Mae Taeng, Huai Nam Dang National Park, 16.665’ N, 98.649’ E, hill evergreen forest, on decaying wood, 25 September 2010, P. Srikitkulchhai, (BBH 25493).

Culture: BCC 33676. DNA sequences of the Thai strain: MN153854 (ITS), MN153871 (LSU), MN172205 (TUB2).

**Teleomorph.** *Stromata* superficial, stromatal surface smooth to slightly wrinkled, peltate, 2–5 mm high, fertile part 3–5 mm high, 6–8 mm wide, with narrow, smooth to slightly wrinkled stipe attached to substrate, with inconspicuous perithecial outlines, surface Fuscous Black (104) and Grayish Sepia (106), dull reddish brown granules immediately beneath stromatal surface, without apparent KOH-extractable pigments; the tissue between perithecia grayish brown, pithy, woody; the tissue below the perithecial layer composed of internal concentric zones, darker zones blackish brown, 0.2 mm thick, lighter zones white, 0.4–0.8 mm thick. *Perithecia* monostichous, obovoid to slightly lanceolate, 0.6–0.8 mm high × 0.3 mm broad; ostioles slightly papillate, inconspicuous.

*Asci* fragmentary, without visible apical apparatus, not bluing in Melzer’s reagent. *Ascospores* dark brown to blackish brown, unicellular, irregularly ellipsoid, with narrowly rounded to almost acute ends, 6–7 × 3–4 (X = 6.88 × 3.48 μm, n = 25), with straight to slightly oblique germ slit germ slit covering ca. 2/3 length of the spore on convex side, perispore dehiscent in 10% KOH, smooth under light microscope, but revealing conspicuous ornamentations by SEM; epispore smooth.

**Culture characteristics.** Colonies on OA, reaching the edge of the Petri dish 9 cm in 1 week, azonate, at first whitish becoming floccose, Chestnut (40), Green (20), Herbage Green (17) and producing Dull Green (70) pigments, with distinct margins; reverse Pale Vinaceous (85) to Vinaceous Buff (86) (Fig. 5c1). Colonies on YMGA, reaching the edge of the Petri dish 9 cm in 1 week, azonate, aerial mycelium at first whitish, becoming velvety to felty, Dull Green (70), Dark Herbage Green (79) or Yellow Green (71); reverse Pale Vinaceous (85) to Vinaceous Buff (86) (Fig. 5c2). Colonies on PDA,
reaching the edge of the Petri dish 9 cm in 1 week, azonate, at first whitish, becoming floccose, Olivaceous (4); reverse Grayish Gray (110) to Olivaceous Black (108) (Fig. 5c3).

Anamorph on OA. Conidiophores with nodulisporium-like branching patterns as defined in Ju and Rogers (1996), erect, main axis hyaline to pale green and smooth to roughened. Conidiogenous cells cylindrical, hyaline, finely roughened, 10–15 (–18) × 3–4 μm (̄x = 14.00 × 3.60 μm, n = 10). Conidia hyaline to pale yellow, smooth, ellipsoid, 4–5 × 2–3 μm (̄x = 4.48 × 2.64 μm, n = 25).

Anamorph on YMGA and PDA similar to that on OA.

Secondary metabolites. BNT (1) in traces and a multitude of peaks corresponding to cytochalasans that could not further elucidated without preparative isolation, which was not possible due to scarcity of material. Additionally, two unidentifiable peaks (UCB1, UCB2) not corresponding to cytochalasans were detected.

Notes. The Thai specimen of D. brachysperma corresponds well with the descriptions made in Ju et al. (1997) and Stadler et al. (2014). This species is distinctive for its stromatal morphology and the characteristic short ascospores. The HPLC profile matched the data reported by Stadler et al. (2014). The phylogenetic position and the characteristics of the anamorph are reported here for the first time, and this confirmed the affinities of this species to the D. eschscholtzii group as postulated by Stadler et al. (2014) (Figs. 6, 7, and 8).

Conclusion
The present study focused on the taxonomy of Daldinia in Thailand, from which only four species (D. bambusicola, D. eschscholtzii, D. kretzschmarioides, D. subvernicosa) had been recorded. Here, we describe three additional novel taxa and a new...
Dichotomous key of *Daldinia* in Thailand

1a Stromata associated with bamboo .............................................. 2

1b Stromata not associated with bamboo ........................................ 3

2a Stromata not found in fire-damaged area; ascospores dark unicellular, ellipsoid, brown to blackish brown, 8–9 (–10) × 4–5 μm .................................................. *D. bambusicola*

2b Stromata found in fire-damaged area; ascospores dark brown to blackish brown, unicellular, ellipsoid–inequilateral (9–) 10–11 (–12) × 4–5 μm .................................................. *D. flavogranulata*

3a Stromata with internal concentric zones below the perithecial layer.................................................. 4

3b Stromata without internal concentric zones below the perithecial layer ........................................... 6

4a Stromata with short stout stipe; ascospores dark brown to blackish brown, unicellular, ellipsoid–inequilateral, with narrowly rounded to almost acute ends, 6–7 × 3–4 μm .................................................. *D. brachysperma*

4b Stromata without a stipe .................................................. 5

5a KOH-extractable pigment immediately mouse gray; ascospores dark brown to blackish brown, rectangular, subglobose, often oriented transverse to the ascal axis, the basal ascospore often ellipsoid, oblong to elongate (5–) 8–10 × 12–15 μm .................................................. *D. subvernicaosa*

5b KOH-extractable pigments mouse gray, appearing with delay (several minutes); ascospores 11–12 (–13) × (5–) 6–7 μm .................................................. *D. eschscholtzii*

6a KOH-extractable pigment cinnamon; scarce tissue below perithecial layer; ascospores dark brown to blackish brown, ellipsoid–inequilateral, with narrow rounded ends, (11–) 14–16 (–18) × 5–6 μm .................................................. *D. phadaengensis*

6b KOH-extractable pigment vinaceous; massive tissue below perithecial layer ........................................... 7

7a KOH-extractable pigment mouse gray; ascospores ellipsoid, (4–) 5–6 × 13–15 (–16) .................................................. *D. kretzschmarioides*

7b KOH-extractable pigment vinaceous gray; ascospores inequilateral with narrowly rounded end (13–) 15–18 (–19) × (5–) 6–8 (–10) .................................................. *D. chiangdaoensis*
Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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