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. kretzschmarioroides*, 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-
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. loculatooides (Stadler et al. 2014).

Stromata of some species of Daldinia (i.e., D. placentiformis, D. korfii, and D. kretschnarioides) 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. eschscholtzii group (Quang et al. 2002; Stadler et al. 2014). Morphologically, D. kretschnarioides 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 phylogenetic 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 Kuhnhert 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 Kuhnhert 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; LORR), Liu et al. (1999; RP2–5F 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 100 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 (hLRs). 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 CBS 12260 obtained from GenBank were used as outgroups. The RAxML based phylogenetic tree is shown in Fig. 6.

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. caldarium 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. kretzschmarioides, D. kretzschmarioides, D. kretzschmarioides, D. phadangensis (BCC 89349, BCC 89350), and D. chiangdaensis (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. concenitra, D. dennisi, D. loculatoidea, D. macaronesica, and D. steglichii and the second one comprising D. petriniae, D. pyrenaica, D. subvernicosa, 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  |
|-------------------------------|--------------|----------|--------------------------|------------------------------------------------|---------|
| *Annulohypoxylon annulatum*    | CBS 140775   | Texas    | KY610418, KY610418, KY624263, KX376353 | Kuhnert et al. (2017; TUB2), Wendt et al. (2018; ITS, LSU, RPB2) | ET      |
| *A. moriforme*                 | CBS 123579   | Martinique | KX376321, KY610425, KY624289, KX271261 | Kuhnert et al. (2017; ITS, TUB2), Wendt et al. (2018; LSU, RPB2) |        |
| *A. nitens*                    | MFLUCC 12.0823 | Thailand | KJ934991, KJ934992, KJ934994, KJ934993 | Damanagama et al. (2015) |        |
| *A. stygium*                   | MUCL 54601   | French Guiana | KY610409, KY610475, KY624292, KX271263 | Wendt et al. (2018) |        |
| *A. truncatum*                 | CBS 140778   | Texas    | KY610419, KY610419, KY624277, KX376352 | Kuhnert et al. (2017; TUB2), Wendt et al. (2018; ITS, LSU, RPB2) | ET      |
| *Daldinia andina*              | CBS 114736   | Ecuador  | AM749918, KY610430, KY624239, KC977259 | Bitzer et al. (2008; ITS), *D. grandis*, Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2) | HT      |
| *D. bambusicola*               | CBS 122872   | Thailand | KY610385, KY610431, KY624241, AY951688 | Hsieh et al. (2005; TUB2), Wendt et al. (2018; ITS, LSU, RPB2) | HT      |
| *D. bambusicola*               | TBRC 8878    | Thailand | MH922869, MH922870, MK165431, MK165422 | Wongkanoun et al. (2019) |        |
| *D. bambusicola*               | TBRC 8879    | Thailand | MH922872, MH938543, MK165432, MK165423 | Wongkanoun et al. (2019) |        |
| *D. bambusicola*               | BCC27937     | Thailand | MN153861, MN153876, MN172217, N/a | This study |        |
| *D. bambusicola*               | BCC33678     | Thailand | MN153860, MN153877, MN172218, N/a | This study |        |
| *D. brachysperma*              | BCC33676     | Thailand | MN153854, MN153878, N/a, MN172205 | This study |        |
| *D. caldariorum*               | BCC88220     | Thailand | MN153850, MN153851, MN172208, MN172197 | This study |        |
| *D. chiangdaoensis*            | BCC88221     | Thailand | MN153851, MN153852, MN172209, MN172198 | This study |        |
| *D. concentrica*               | CBS 113277   | Germany  | KU683756, KU683756, KU684289, KU684128 | U'Ren et al. 2016 |        |
| *D. demissii*                  | CBS 114741   | Australia | JX658477, KY610435, KY624244, KC977262 | 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 |
|------------------------------|------------|------------|------------------------------------|------------------------------------------------|--------|
| *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) |        |
| *Daldinia eschscholtzii*     | TBRC 8876  | Thailand   | MH938532 MH938541 MK165429 MK165420 | Wongkanoun et al. (2019)                       |        |
| *Daldinia eschscholtzii*     | BCC27887   | Thailand   | MN153861 MN153878 MN172214 N/A     | This study                                     |        |
| *Daldinia flavogramulata*    | BCC89363   | Thailand   | MN153856 MN153873 MN172211 MN172200 | This study                                     |        |
| *Daldinia flavogramulata*    | BCC89365   | Thailand   | MN153857 MN153874 MN172212 MN172201 | This study                                     |        |
| *Daldinia flavogramulata*    | BCC89376   | Thailand   | MN153858 MN153875 MN172213 MN172202 | This study                                     |        |
| *Daldinia korfi*             | EBS 067    | Argentina  | KY204018 N/A N/A N/A              | Sir et al. (2016b)                             |        |
| *Daldinia korfi*             | EBS 473    | Argentina  | KY204020 N/A N/A N/A              | Sir et al. (2016b)                             |        |
| *Daldinia kretzschmarioroides* | TBRC 8875 | Thailand   | MH938531 MH938540 MK165425 MK165416 | Wongkanoun et al. (2019)                       | ET     |
| *Daldinia loculatoides*      | 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 phadengensis*      | BCC89349   | Thailand   | MN153852 MN153869 MN172206 MN172195 | This study                                     |        |
| *Daldinia phadengensis*      | 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 steglichii*        | MUCL 43512 | Papua New Guinea | KY610399 KY610479 KY624250 KX271269 | Wendt et al. (2018)                            |        |
| *Daldinia subvernosa*        | 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) | ET     |
| *Graphostroma platystomum*  | CBS 270.87 | France     | JX658535 DQ836906 KY624296 HG934108 | Stadler et al. (2014; ITS), Zhang et al. (2006; LSU), Koukol et al. |        |
| Species                     | Strains   | Country       | GenBank accession numbers | Reference                                                                 | Status       |
|----------------------------|-----------|---------------|----------------------------|---------------------------------------------------------------------------|--------------|
| Hypomontagnella monticulosa| MUCL 54604| French Guiana | KY610404, KY610487, KY624305, XX71273 | Wendt et al. (2018; TUB2), Wendt et al. (2018; LSU, RPB2)                  | ET           |
| Hypomontagnella monticulosa| BCC69203  | Thailand      | MN153864, MN153881, MN172219, MN172204 | This study                                                                |              |
| Hypomontagnella monticulosa| BCC69203  | Thailand      | MN153865, MN153882, MN172220, MN172203 | This study                                                                |              |
| Hypomontagnella submunticulosa| CBS 115280| France        | KC968923, KY610457, KY624226, KC977267 | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)           |              |
| Hypoxylon crocopeplum      | CBS 119004| France        | KC968907, KY610445, KY624255, KY977268 | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)           |              |
| Hypoxylon fragiforme       | MUCL 51264| Germany       | KC477229, KM186295, KM186296, XX71282 | 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, XX71271 | Wendt et al. (2018)                                                        | ET           |
| Hypoxylon haematostroma    | MUCL 53301| Martinique    | KC968911, KY610484, KY624301, KC977291 | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)           | ET           |
| Hypoxylon haematostroma    | BCC50533  | Thailand      | MN153866, MN153883, MN172221, N/A | This study                                                                |              |
| Hypoxylon investiens       | CBS 118183| Malaysia      | KC968925, KY610450, KY624259, KC977270 | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)           | ET           |
| Hypoxylon lateripigmentum  | MUCL 53304| Martinique    | KC968933, KY610486, KY624304, KC977290 | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)           |              |
| Hypoxylon lenormandii      | CBS 119003| Ecuador       | KC968943, KY610452, KY624261, KC977273 | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)           | HT           |
| Hypoxylon petriniae        | CBS 114746| France        | KY610405, KY610491, KY624279, XX71274 | Kuhnert et al. (2017; TUB2), Wendt et al. (2018; ITS, LSU, RPB2)           | HT           |
| Hypoxylon rickii           | MUCL 53309| Martinique    | KC968932, KY610416, KY624281, KC977288 | 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), Wendt et al. (2018; TUB2) | ET           |
| Hypoxylon samuelsii        | MUCL 51843| Guadeloupe    | KC968916, KY610466, KY624269, KC977286 | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; TUB2)                 | ET           |
| Species                  | Strains   | Country   | GenBank accession numbers | Reference                                      | Status      |
|-------------------------|-----------|-----------|---------------------------|------------------------------------------------|-------------|
|                         |           |           | ITS | LSU | RPB2 | TUB2 |               |               |
| **Jackrogersella cohaerens** | CBS 119126 | Germany   | KY610396 | KY610497 | KY624270 | KY624314 | Wendt et al. (2018) | (2018; LSU, RPB2) |
| **Jackrogersella minutella** | CBS 119015 | Portugal  | KY610381 | KY610424 | KY624235 | KX271240 | Kuhnert et al. (2017; TUB2), Wendt et al. (2018; ITS, LSU, RPB2) |
| **Jackrogersella multiformis** | CBS 119016 | Germany   | KC477234 | KY610473 | KY624290 | KX271262 | Kuhnert et al. (2014; ITS), Kuhnert et al. (2017; TUB2), Wendt et al. (2018; LSU, RPB2) |
| **Pyrenopolyporus hunteri** | MUCL 52673 | Ivory Coast | KY610421 | KY610472 | KY624309 | KU159530 | Kuhnert et al. (2017; TUB2), Wendt et al. (2018; ITS, LSU, RPB2) |
| **Pyrenopolyporus laminosus** | MUCL 53305 | Martinique | KC968934 | KY610485 | KY624303 | KC977292 | Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2) |
| **Pyrenopolyporus laminosus** | TBRC 8871 | Thailand  | MH938527 | MH938536 | MK165424 | MK165415 | Wongkanoun et al. (2019) |
| **Pyrenopolyporus nicaraguensis** | BCC89383 | Thailand  | MN153855 | MN153872 | MN172210 | MN172199 | This study |
| **Pyrenopolyporus symphyon** | CBS 117739 | Burkina Faso | AM749922 | KY610489 | KY624307 | KC977272 | Bizzer et al. (2008; ITS), Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2) |
| **Xylaria hypoxylon** | TBRC 8873 | Thailand  | MH938529 | MH938538 | MK165428 | MK165419 | Wongkanoun et al. (2019) |
|                         | CBS12260   | Sweden    | KY610407 | KY610495 | KY624231 | KX271279 | Sir et al. (2016a; TUB2), Wendt et al. (2018; ITS, LSU, RPB2) | ET |
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* Srikitkulchai, 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. Srikitkulchai 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–e 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 Ascospore by SEM. j Ascospore showing germ slit (white arrow). k 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)
Table 2 Comparison of morphological and chemotaxonomic characters of species with massive stromata and long tubular perithecia and *Daldinia* species that are similar to *flavogranulata*

| Taxon                     | Metabolite (stroma) | KOH-extractable pigments | Ascospore perispore | Ascospore germ slit | Ascospore size (μm) |
|---------------------------|---------------------|---------------------------|---------------------|--------------------|---------------------|
| *Daldinia flavogranulata* | C, cytochalasins, BNT | Dark brown to blackish brown to reddish brown, uncellular, irregularly ellipsoid, with narrow rounded end (13–15) 15–18 (19) × 6–8 (−10) μm (x = 16.45 × 7.19 μm, n = 50), with straight to slightly curved germ slit covering full spore length on convex side, perispore dehiscent in 10% KOH, smooth. | Dehiscent | Spore length, dorsal | (10.3–14) 13–16 (17) × 4.8–6.2 μm |
| *Daldinia bambusicola*    | C, cytochalasins, BNT | Dark brown to blackish brown, uncellular, irregularly ellipsoid, with narrow rounded end (13–15) 15–18 (19) × 6–8 (−10) μm (x = 16.45 × 7.19 μm, n = 50), with straight to slightly curved germ slit covering full spore length on convex side, perispore dehiscent in 10% KOH, smooth. | Dehiscent | Spore length, dorsal | (10.3–14) 13–16 (17) × 4.8–6.2 μm |
| *Daldinia brachysperma*   | C, cytochalasins, BNT | Dark brown to blackish brown, uncellular, irregularly ellipsoid, with narrow rounded end (13–15) 15–18 (19) × 6–8 (−10) μm (x = 16.45 × 7.19 μm, n = 50), with straight to slightly curved germ slit covering full spore length on convex side, perispore dehiscent in 10% KOH, smooth. | Dehiscent | Spore length, dorsal | (10.3–14) 13–16 (17) × 4.8–6.2 μm |
| *Daldinia caldariorum*    | C, cytochalasins, BNT | Dark brown to blackish brown, uncellular, irregularly ellipsoid, with narrow rounded end (13–15) 15–18 (19) × 6–8 (−10) μm (x = 16.45 × 7.19 μm, n = 50), with straight to slightly curved germ slit covering full spore length on convex side, perispore dehiscent in 10% KOH, smooth. | Dehiscent | Spore length, dorsal | (10.3–14) 13–16 (17) × 4.8–6.2 μm |
| *Daldinia chiangdaoensis* | C, cytochalasins, BNT | Dark brown to blackish brown, uncellular, irregularly ellipsoid, with narrow rounded end (13–15) 15–18 (19) × 6–8 (−10) μm (x = 16.45 × 7.19 μm, n = 50), with straight to slightly curved germ slit covering full spore length on convex side, perispore dehiscent in 10% KOH, smooth. | Dehiscent | Spore length, dorsal | (10.3–14) 13–16 (17) × 4.8–6.2 μm |
| *Daldinia placentiformis* | C, cytochalasins, BNT | Dark brown to blackish brown, uncellular, irregularly ellipsoid, with narrow rounded end (13–15) 15–18 (19) × 6–8 (−10) μm (x = 16.45 × 7.19 μm, n = 50), with straight to slightly curved germ slit covering full spore length on convex side, perispore dehiscent in 10% KOH, smooth. | Dehiscent | Spore length, dorsal | (10.3–14) 13–16 (17) × 4.8–6.2 μm |

**Teleomorph.** *Stromata* superficial, hemispherical to spherical, 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, obvoid to lanceolate 1.14–1.43 mm high, 0.29–0.43 mm broad; ostioles papillate.

Asci cylindrical, spore bearing part (62–) 75–87 × 12–15 μm, 8 spored; apical apparatus bluing in Melzer’s reagent, discoid, (0.6–) 1 × 1.7–2.2 μm (x = 0.96 × 1.93 μm, n = 10). *Ascospores* dark brown to blackish brown, unicellular, irregularly ellipsoid, with narrow rounded end (13–15) 15–18 (19) × 6–8 (−10) μm (x = 16.45 × 7.19 μm, n = 50), with straight to slightly curved germ slit covering full spore length on convex side, perispore dehiscent in 10% KOH, smooth.

**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.
Secondary metabolites. 1,1′-Binaphthalene-4,4′,5,5′-tetrol (BNT, 1), cytochalasans (Supplementary Fig. S1).

Notes. There are three species that are most similar to D. chiangdaensis in producing massive, azonate tissue below the perithecial layer and oboviod 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′-tetrol (BNT, 1) Dalldinia 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. chiangdaensis is larger than that of D. kretzschmarioides [(13–15)18–19–(5–6)–8–10] vs 13–15 (–16) × (4–5) 5–6 µm. Phylogenetic relationships revealed that DNA sequences of D. chiangdaensis 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.

Dalldinia 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 Pulpish 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, obovoid to lanceolate 0.71–0.85 mm high, 0.28–0.35 mm broad; ostiolo umbilicate to slightly raised discoid.

Asci 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 (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 felly, 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. Dalldinia phadaengensis is morphologically similar to D. chiangdaensis, 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 Dalldinia species. Table 2 provides a synopsis of the morphological characters and secondary metabolites of this group of Dalldinia species and the related genus Pyrenopolyporus. Dalldinia placentiformis, another morphologically similar species, which has so far not been found in Thailand, has olivaceous pigments, owing to the presence of daldinone A (Bitzer et al. 2008). Daldinin A derivatives were originally isolated from a species referred to as “D. concentrica” by Hashimoto (1994), which was revised as D. childiae by Stadler et al. (2014). They are chemically similar to the lenormandins and fragirubrins that are known from Hypoxylon species (Kuhner 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. childiae 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

Fig. 4 Daldinia brachysperma (BBH 25493). a–b Stroma. c Stromatal 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, obvoid, 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 dehiscent 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. 5e1). 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. 5e2). 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. 5e3).

**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, wooly; 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, obvoid 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 (\(\bar{x} = 14.00 \times 3.60 \mu m, n = 10\)). *Conidia* hyaline to pale yellow, smooth, ellipsoid, 4–5 × 2–3 μm (\(\bar{x} = 4.48 \times 2.64 \mu m, n = 25\)).

**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...
Kretz et al. (2019) as well as representative cytochalasans from Stromata associated with bamboo ..............................................

Therefore, either artificial stromata production or re-collection of the scarce stromatal material representing the type specimens.remain to be isolated and identified, which was not possible from secondary metabolites have been detected in the stromata of these species by chemotaxonomic methodology, but these metabolites remain to be isolated and identified, which was not possible from the scarce stromatal material representing the type specimens. Therefore, either artificial stromata production or re-collection of the fungi in the field will be necessary in the future to accomplish this task. Daldinia as well as other genera of the stromatic Xylariales in Thailand (e.g., Pyrenopolyporus and in particular the large genus Hypoxylon) need further studies. Apart from molecular systematics and chemotaxonomy, this also concerns the generation of data based on innovative technologies such as genomics, proteomics, and metabolomic data in order to explore the full biotechnological potential of these fungi.

**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. subvernicosa*

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. phaadagensis*

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. kretzschmariodes*

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

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**Author contributions** SW did the isolation of compounds, morphological and molecular analyses as well as writing of the manuscript. MS, NB and JYL edited the manuscript, PS and KB (Rangsit University) contributed to the experimental designs. BC did the DNA extractions and PCR amplifications. KB did the chemical analysis of the stromata.

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Compliance with ethical standards

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

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