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A novel species and a new combination of *Daldinia* from Ban Hua Thung community forest in the northern part of Thailand

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Abstract

During a survey of Xylariales in northern Thailand, several specimens with affinities to the genus *Daldinia* were found and examined for morphological characters, secondary metabolites, and molecular phylogenetic traits. Aside from morphological and chemotaxonomic studies, a multi-locus phylogenetic analysis using internal transcribed spacers regions (ITS) and the large subunit (LSU) of the ribosomal DNA, the second largest subunit of the RNA polymerase (RPB2), and beta-tubulin (TUB2) genes was performed. Among the specimens was a new species and a new record of a species that had previously never been sequenced and studied for its anamorphic morphology. This species, previously described by Ju and Rogers as *Hypoxylon kretzschmarioides* based on a single record from Indonesia, showed secondary metabolite profiles reminiscent of those of the genus *Daldinia* and even clustered in the latter genus in the phylogenetic tree. Therefore, it is transferred to *Daldinia* as *D. kretzschmarioides* comb. nov. A second new species, *D. subvernicosa* sp. nov., was found to have a close relationship with *D. vernicosa* based on morphological and molecular evidence, but differs from *D. vernicosa* by long-stipitate asci with mostly subglobose ascospores, and the basal ascospores are often elongated.

Keywords Ascomycota · Phylogeny · Taxonomy · Xylariales · New species · New combination

Introduction

The genus *Daldinia* was described by Cesati and De Notaris (1863) and belongs to the Hypoxylaceae (Xylariales), since the recent rearrangement of the families of stromatic Xylariales by Wendt et al. (2018). The Hypoxylaceae is one of the largest families in this order and both the family and the genus *Daldinia* have been studied exhaustively for secondary metabolite production (Helaly et al. 2018). *Daldinia* was traditionally separated from *Hypoxylon* based on the presence of internal concentric zones in their stromata (Ju et al. 1997). However, *D. placentiformis* (Berk. and M.A. Curtis) Theiss. (1909) has for long been included in the genus *Hypoxylon*, to which it had belonged until Hsieh et al. (2005) provided evidence from molecular phylogenetic data that its affinities are indeed with *Daldinia*. This was later confirmed in the chemotaxonomic study by Bitzer et al. (2008). In the world monograph by Stadler et al. (2014), the genus was segregated by using a combination of morphological, chemotaxonomic, and molecular phylogenetic characters. While only ITS data had been used in the latter study, Wendt et al. (2018) have included several species and demonstrated by using a multi-locus phylogeny that *Daldinia* and allied species indeed represent an independent lineage in the Hypoxylaceae that is different from *Hypoxylon* as well as from the genus *Pyrenopolyporus*, which was resurrected and amended to accommodate some species with superficial similarities to *D. placentiformis*. A more comprehensive overview by Daranagama et al. (2018)

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Taxonomic novelties: *Daldinia subvernicosa* Srikitikulchai, Wongkanoun, M. Stadler & Luangsa–ard, sp. nov.; *D. kretzschmarioides* (Y.M. Ju & J. D. Rogers) Srikitikulchai, Wongkanoun, M. Stadler&Luangsa–ard, comb. nov

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includes a backbone phylogeny of important taxa in the Xylariaceae and other families of stromatic Xylariales and provided updated descriptions and illustrations for all taxa, thus serving as valuable reference. In Thailand and other Asian countries, the genus still needs more research.

During our ongoing surveys of Xylariales in northern Thailand, we have encountered two interesting *Daldinia* species, of which one represents a new taxon and the other shows affinities to another species that has so far only been found once in Indonesia. The present study is dedicated to the presentation of their morphological and chemotaxonomic features and their phylogenetic placement.

Materials and methods

Morphological characterization

Measurements of morphological characters, such as size and shapes of stromata, perithecia, asci, and ascospores, were examined according to Stadler et al. (2014). The cultures of the specimens were obtained from multiple spore isolation following Sir et al. (2016a). Preliminary classification was done by examining the conidiogenous cells and conidiophore branching pattern of the asexual morph according to Ju and Rogers (1996). Furthermore, the stromatal color, KOH extractable pigment, and cultures are recorded according to Rayner (1970). The cultures and the material vouchers were deposited in Thailand Bioresource Research Center (TBRC) and BIOTEC Bangkok Herbarium (BBH), respectively. Scanning electron microscopy (SEM) was carried out using a conventional procedure described by Kuhnert et al. (2017).

HPLC profiling

For chemotaxonomic studies, the natural products were extracted using the method by Yuyama et al. (2018), using high performance liquid chromatography coupled with diode array and electrospray mass spectrometric detection (HPLC/DAD-ESIMS). The instrumental settings and conditions were as described in Kuhnert et al. (2017).

DNA extraction, PCR, and phylogenetic analyses

The mycelium was extracted using cetyltrimethyl ammonium bromide (CTAB) following the method by Mackill and Bonman (1995). Four DNA loci including internal transcript spacer regions (ITS); large subunit of the rDNA (LSU); RNA polymerase II (RPB2); and beta tubulin (TUB2) were amplified by PCR, following the standard primers introduced by White et al. (1990; ITS1, ITS4, and ITS5), Vilgalys and Hester (1990; LR7 and LROR), Liu et al. (1999: RPB2–5F and 7Cr), and O'Donnell and Cigelnik (1997; T1 and T22) following the protocols of Otto et al. (2016) and Wendt et al. (2018). DNA sequences were checked and assembled using BioEdit v. 7.2.5 (Hall 2013). The new sequences were submitted to GenBank (Table 1). The molecular analyses were done following Wendt et al. (2018). All sequences were then aligned using MUSCLE (Edgar 2004) and alignments were refined by direct examination. Multiple sequence alignments were analyzed with the closely matched sequences obtained from GenBank (Table 1). Sequences were analyzed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian algorithm. Maximum parsimony analysis was performed in PAUP*4.0b10 Swofford (2002). The most parsimonious trees (MPTs) were obtained from the heuristic searches: 100 replicates of random stepwise addition of sequence, branch-swapping algorithm, tree-bisection-reconnection (TBR), and equal weight characters. Maximum parsimony bootstrap supports were estimated by 1000 replicates (stepwise addition of sequence, 10 replicates of random addition of taxa, TBR branching-swapping algorithm). Most parsimonious tree length, consistency index (CI), retention index (RI), relative consistency index (RC), and homoplasy index (HI) were estimated. The maximum likelihood and bootstrap analyses were conducted through the CIPRES web portal (Miller et al. 2010) using RAxML 8.2.4 (Stamatakis 2014) with the BFGS method to optimize GTR rate parameters. Finally, Bayesian posterior probabilities of the branches were performed 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) that was 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

Results and discussion

Taxonomy

Daldinia kretzschmarioides (Y.M. Ju & J.D. Rogers) Srikitikulchai, Wongkanoun, M. Stadler & Luangsa–ard, comb. nov. Fig. 1.

MB829270

Basionym: *Hypoxylon kretzschmarioides* Y.M. Ju & J.D. Rogers, Mycol. Mem. 20: 139 (1996)

Epitype (designated here): Thailand: Chiang Mai Province, Ban Hua Thung community forest, 19.42044′ N, 98.97140′ E, on dead angiosperm in the forest, 3 November 2016, P. Srikitikulchai, S. Wongkanoun, BBH 42276 (MBT383621)

Ex-epitype strain: TBRC 8875 (BBC); DNA sequences of ex-epitype strain: MH938531 (ITS), MH938540 (LSU), MK165425 (RBP2), MK165416 (TUB2)

Table 1 List of all taxa used in the current phylogeny study. ET indicates epitype strains, HT holotype, and PT paratype

Species	Strains	Country	GenBank accession numbers				Reference	Status
			ITS	LSU	RPB2	TUB2		
Annulohypoxylon annulatum	CBS 140775	Texas	KY610418	KY610418	KY624263	KX376353	Kuhnert et al. (2017; TUB2), Wendt et al. (2018; ITS, LSU, RPB2)	ET
Annulohypoxylon moriforme	CBS 123579	Martinique	KX376321	KY610425	KY624289	KX271261	Kuhnert et al. (2017; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)	
Annulohypoxylon nitens	MFLUCC 12.0823	Thailand	KJ934991	KJ934992	KJ934994	KJ934993	Daranagama et al. (2015)	
Annulohypoxylon stygium	MUCL 54601	French Guiana	KY610409	KY610475	KY624292	KX271263	Wendt et al. (2018)	
Annulohypoxylon 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
Daldinia bambusicola	CBS 122872	Thailand	KY610385	KY610431	KY624241	AY951688	Hsieh et al. (2005; TUB2), Wendt et al. (2018; ITS, LSU, RPB2)	HT
Daldinia bambusicola	TBRC 8878	Thailand	MH922869	MH922870	MK165431	MK165422	This study	
Daldinia bambusicola	TBRC 8879	Thailand	MH922872	MH938543	MK165432	MK165423	This study	
Daldinia caldariorum	MUCL 49211	France	AM749934	KY610433	KY624242	KC977282	Bitzer et al. (2008; ITS), Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2)	
Daldinia concentrica	CBS 113277	Germany	AY616683	KY610434	KY624243	KC977274	Triebel et al. (2005; ITS), Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2)	
Daldinia dennisii	CBS 114741	Australia	JX658477	KY610435	KY624244	KC977262	Stadler et al. (2014; ITS), Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU, RPB2)	HT
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 8872	Thailand	MH938528	MH938537	MK165426	MK165417	This study	
Daldinia eschscholtzii	TBRC 8874	Thailand	MH938530	MH938539	MK165427	MK165418	This study	
Daldinia eschscholtzii	TBRC 8876	Thailand	MH938532	MH938541	MK165429	MK165420	This study	
Daldinia korfii	EBS 067	Argentina	KY204018	N/a	N/a	KY204014	Sir et al. (2016b)	
Daldinia korfii	EBS 473	Argentina	KY204020	N/a	N/a	KY204016	Sir et al. (2016b)	
Daldinia kretzschmarioides	TBRC 8875	Thailand	MH938531	MH938540	MK165425	MK165416	This study	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 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)	PT
Daldinia subvernicosa	TBRC 8877	Thailand	MH938533	MH938542	MK165430	MK165421	This study	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		HT

Table 1 (continued)

Species	Strains	Country	GenBank accession numbers				Reference	Status
			ITS	LSU	RPB2	TUB2		
							Stadler et al. (2014; ITS), Zhang et al. (2006; LSU), Koukol et al. (2015; TUB2), Wendt et al. (2018; RPB2)	
Hypomontagnella monticulosa	MUCL 54604	French Guiana	KY610404	KY610487	KY624305	KX271273	Wendt et al. (2018)	ET
Hypomontagnella submonticulosa	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	KC977268	Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)	
Hypoxylon fragiforme	MUCL 51264	Germany	KC477229	KM186295	KM186296	KX271282	Stadler et al. (2013; ITS), Daranagama et al. (2015; LSU, RBP2), 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	KC977291	Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)	ET
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)	HT
Hypoxylon lenormandii	CBS 119003	Ecuador	KC968943	KY610452	KY624261	KC977273	Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)	
Hypoxylon petriniae	CBS 114746	France	KY610405	KY610491	KY624279	KX271274	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, RPU2, TUB2)	ET
Hypoxylon samuelsii	MUCL 51843	Guadeloupe	KC968916	KY610466	KY624269	KC977286	Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)	ET
Jackrogersella cohaerens	CBS 119126	Germany	KY610396	KY610497	KY624270	KY624314	Wendt et al. (2018)	
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)	ET
Pyrenopolyporus hunteri	MUCL 52673	Ivory Coast	KY610421	KY610472	KY624309	KU159530	Kuhnert et al. (2017; TUB2), Wendt et al. (2018; ITS, LSU, RPB2)	ET
Pyrenopolyporus laminosus	MUCL 53305	Martinique	KC968934	KY610485	KY624303	KC977292	Kuhnert et al. (2014; ITS, TUB2), Wendt et al. (2018; LSU, RPB2)	HT
Pyrenopolyporus laminosus	TBRC 8871	Thailand	MH938527	MH938536	MK165424	MK165415	This study	
Pyrenopolyporus nicaraguensis	CBS 117739	Burkina Faso	AM749922	KY610489	KY624307	KC977272	Bitzer et al. (2008; ITS), Kuhnert et al. (2014; TUB2), Wendt et al. (2018; LSU RPB2)	HT
Pyrenopolyporus symphyon	TBRC 8873	Thailand	MH938529	MH938538	MK165428	MK165419	This study	
Xylaria hypoxylon	CBS12260	Sweden	KY610407	KY610495	KY624231	KX271279	Sir et al. (2016a; TUB2), Wendt et al. (2018; ITS, LSU, RPB2)	HT

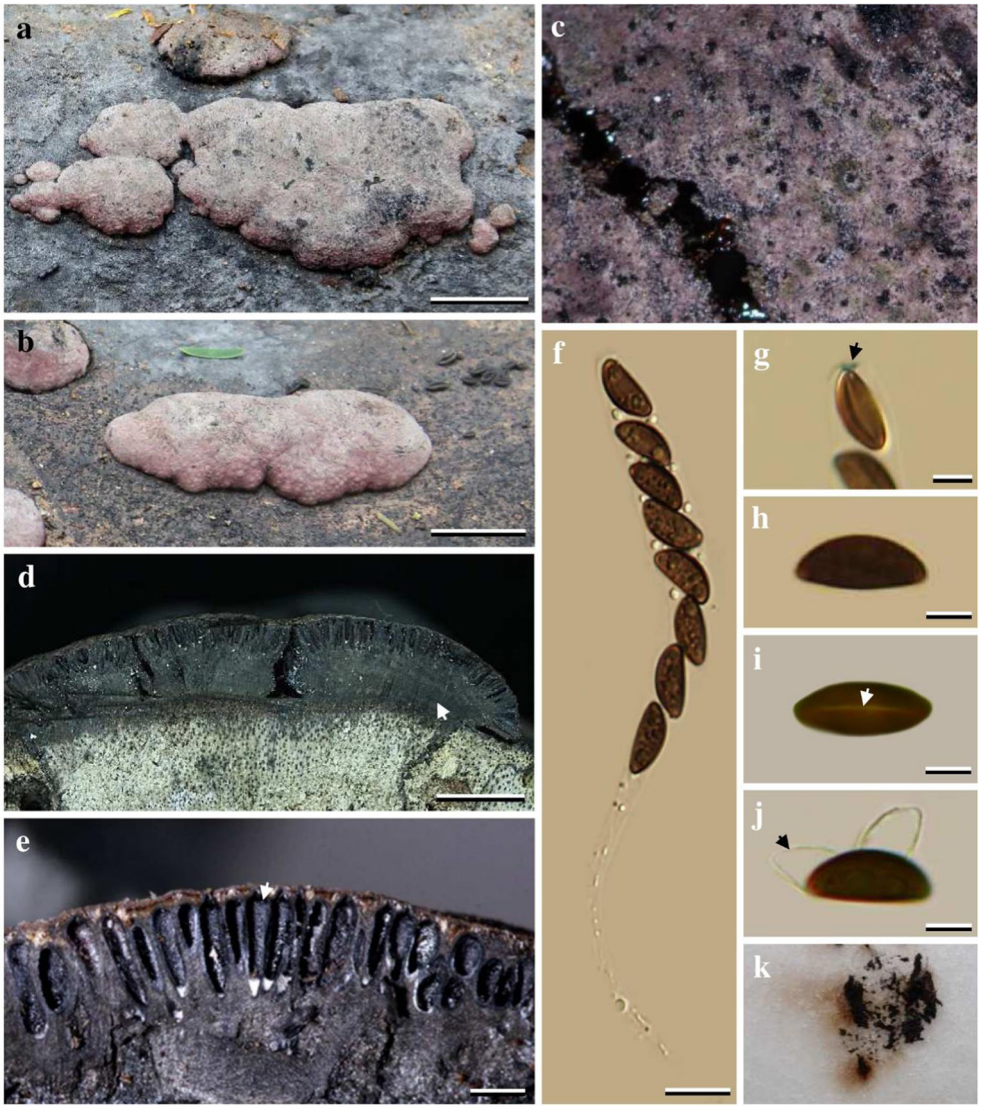


Fig. 1 *Daldinia kretzschmarioides* (BBH 42281). **a–b** Stromata in wood; **c** stromatal surface with ostioles; **d** cross section of stroma showing perithecia and the tissue below the perithecial layer (white arrow); **e** perithecia (white arrow); **f** ascus; **g** apical apparatus bluing in Melzer's reagent (black arrow); **h** ascospore; **i** ascospore showing germ slit; **j** ascospore in KOH showing dehiscent perispore (black arrow); **k** pigments in 10% KOH. Scale is indicated by bars (**a** 20 mm. **b** 10 mm. **d** 5 mm. **e** 1 mm. **f** 10 μm, **g–j** 5 μm)

Teleomorph. *Stroma* superficial, small to widely effused, pulvinate or peltate, the base broadly attached to the substrate, conspicuous or inconspicuous perithecial mounds, 25–29 mm long × 9.45–13 (27) mm broad × 2–3 mm thick; surface mouse gray (118) to pale mouse gray (117) brownish yellow or red-orange granules forming a thin crust above perithecia, with 10% KOH producing dark vinaceous (82) extractable pigments, the tissue between perithecia gray or blackish brown, the tissue below perithecia layer gray, 1.2–2.4 mm thick. *Perithecia* monostichous, lanceolate, 0.14–0.28 mm broad × 1.40–1.42 mm high; ostioles black, umbilicate. *Asci* cylindrical, spore bearing part 60–63 μm long × 8 μm; apical apparatus bluing in Melzer's reagent, discoid, 0.5–1 × 2.5–3 μm. *Ascospores* dark brown to blackish brown, unicellular, ellipsoid, (4) 5–6 × 13–15 (16) μm (mean = 5.13 × 13.83 μm, $n = 30$) with straight to slightly oblique germ slit much less than spore length on convex size, perispore dehiscent in 10% KOH, smooth.

Anamorph in culture. Conidiophores with virgariella-like to (much more frequently) nodulisporium-like branching patterns as defined in Ju and Rogers (1996). Main axis hyaline and cell walls rough or smooth dark brown to blackish brown. Conidiogenous cells cylindrical, hyaline, finely roughened, 10–17 × 3–4 μm. Conidia hyaline, smooth, ellipsoid 5−7 × 3−4 μm.

Culture characteristics. Colonies on OA reaching the edge of a 9 cm Petri dish in 1 week, at first whitish becoming velvety to felty, azonate with entire margin, grayish yellow–green (68), olivaceous (48) and dark herbage green (69) to dull green (70) after 2 weeks incubation (Fig. 2e)**.** Colonies on YMGA covering Petri dish in 1 week at first whitish becoming smoke gray, dark herbage green (69), and dull green (70) velvety to felty, azonate with entire margin.

Secondary metabolites. BNT, Cytochalasins

Notes. The specimen showed very similar characteristics to the holotype of the monotypic species *Hypoxylon*

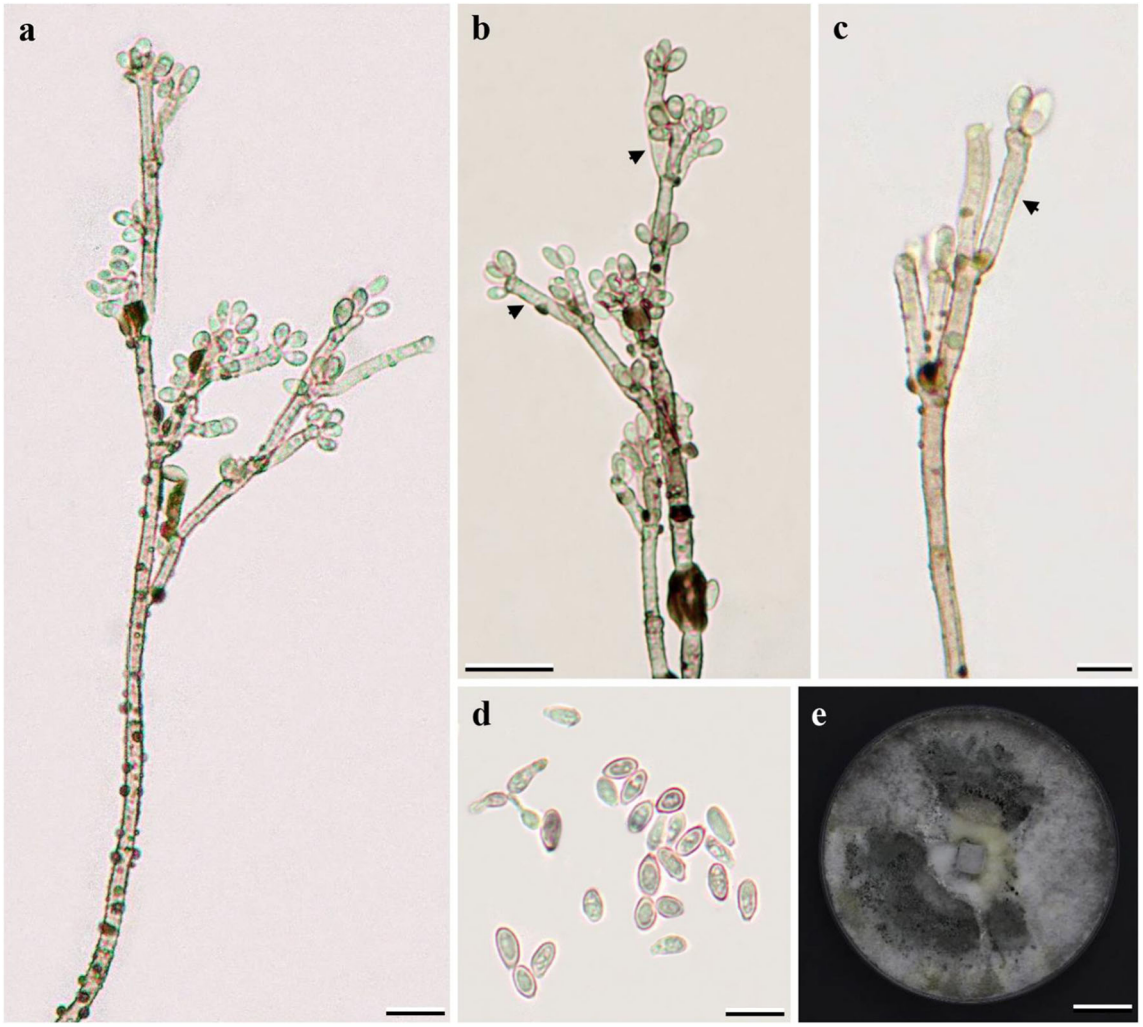


Fig. 2 *Daldinia kretzschmarioides* (TBRC 8875). **a** asexual morph showing conidiophores with virgariella-like to nodulisporium-like branching patterns; **b** nodulisporium-like branching patterns, conidiogenous cells (arrows); **c** conidiogenous cell (arrow); **d** conidia; **e** culture on OA medium after 2 weeks. Scale is indicated by bars (**a**–**b** 20 μm. **c**–**d** 10 μm. **e** 2 cm)

kretzschmarioides, which originates from Indonesia and has never been cultured or subjected to DNA sequencing. As already mentioned by Wendt et al. (2018), Ju et al. (1997) have described in the protologue that the perispore of the ascospores of this specimen was indehiscent, but a re-examination of the type specimen in NY (J. Fournier and M.S., unpublished) had revealed that the perispore is actually dehiscent. All other salient morphological characters of the Thai specimen that we propose as epitype of *H. kretzschmarioides* are in agreement with the holotype. Therefore, we regard the current specimen as conspecific to *H. kretzschmarioides*. Since the results of the molecular phylogeny leave no doubt that the phylogenetic affinities of the fungus are with the genus *Daldinia*, we have moved *H. kretzschmarioides* to the latter genus.

There are two other *Daldinia* species with similar stromatal morphology, lacking internal concentric zones: *Daldinia kretzschmarioides* is morphologically similar to the circumtropically distributed *D. placentiformis* but differs in its ascospore size range as well as in having olivaceous stromatal pigments, owing to the presence of daldinone A as predominant stromatal metabolite. The Argentine species, *Daldinia korfii* (cf. Sir et al. 2016b) is also similar but differs in its ascospore size range. HPLC profiling showed that both the holotype and the selected epitype specimen contained BNT and cytochalasins (Table 2). The BNT peak was more prominent in the epitype, which explains the stronger purple color as compared to the holotype specimen in NY, which had been collected several years previously. The major cytochalasins in the stromata of the Thai specimens were recently identified as the new phenochalasins C and D (Figs. 3 and 4) and found to exhibit significant anti-biofilm effects in *Staphylococcus aureus* (Yuyama et al. 2018).

Table 2 Comparison of morphological and chemotaxonomic characters of Hypoxylaceae species with massive stroma and long tubular perithecia and *Daldinia* species that are similar to *D. subvernicosa* sp. nov.

Taxon	Ascospore perispore	Ascospore germ slit	Ascospore size (μm)	KOH-extractable pigments	Metabolite (stroma)
Daldinia kretzschmarioides	Dehiscent	Much less than spore length	13–15(–16) × (4–) 5–6	Dark vinaceous*	BNT, cytochalasins
Hypoxylon kretzschmarioides (holotype)	Dehiscent	Spore length, dorsal	(12–) 13–16 × 5–6	Dilute purple or absent	BNT, cytochalasins
Hypoxylon begae	Dehiscent	Short, dorsal	21–29 × 12–14.5	Dense isabelline	BNT, napththols, and unknown metabolite
Pyrenopolyporus nicaraguensis	Indehiscent	Spore length, dorsal	(11–) 12–15(–16) × 5–6.5	Dense purple or absent	BNT, napththols, naphthoquinones
Pyrenopolyporus laminosus	Indehiscent	Spore length, dorsal	11–13.5 × 4.2–4.5	Dilute purple	BNT, napththols, naphthoquinones
Daldinia placentiformis	Dehiscent	Spore length, dorsal	14.5–16 × 6.5–7	Olivaceous	BNT, napththols, naphthoquinones
Daldinia korfii	Dehiscent	Straight germ slit spore-length on convex side	(10.3–) 11.0−14.0 (–16.0) × (4.8–) 5.2− 6.2 (–7.0)	Brown vinaceous to dark vinaceous	BNT, concentricol B and Cytochalasin
Daldinia vernicosa	Indehiscent	Straight to slightly shorter than spore length	11.5–14.5(–15) × 6.5–8(–9)	Dark livid, livid violet	BNT
Daldinia loculata	Indehiscent	Straight	11–14(–15) × 6–8	Dense purple	BNT
Daldinia subvernicosa	Indehiscent	Straight to slightly shorter than spore length	12–15 × (5–) 8–10	Mouse gray*	BNT

Daldinia subvernicosa Srikitikulchai, Wongkanoun, M. Stadler & Luangsa–ard, sp. nov. Fig. 5.

MB828032

Etymology. In reference to the morphological similarities to *Daldinia vernicosa*

Holotype: Thailand: Chiang Mai Province, Ban Hua Thung community forest, 19.42044′ N, 98.97140′ E, on dead angiosperm in the forest, 3 November 2016, P. Srikitikulchai and S. Wongkanoun, BBH 42281

Ex-holotype strain: TBRC 8877 (BBC). DNA sequences of ex-holotype strain: MH938533 (ITS), MH938542 (LSU), MK165430 (RPB2), MK165421 (TUB2)

Teleomorph. *Stroma* hemispherical to depressed-spherical, widely attached to the substrate, very rarely substipitate, smooth or with inconspicuous perithecial outline, 2.90–5 cm × 1.68–3.40 cm; surface fuscous black (104), with 10% KOH extractable pigments mouse gray (118), dark brown to dark black immediately beneath the surface; tissue between perithecia blackish brown, woody; tissue below the perithecia layer composed of alternating zones, darker zones blackish brown, 0.1 mm thick, lighter zones, white, 1 mm thick. *Perithecia* subglobose 1 mm diam; ostioles umbilicate, lower than the stromatal surface. *Asci* unitunicate, cylindrical, 220–236 long, with long stipe, 135–143 μm, the spore bearing part 85–93 × 13–15 μm, 8-spored, without visible apical apparatus, not bluing in Melzer's reagent. *Ascospores* unicellular, dark brown to blackish brown, (5)–8–10 × 12–15 μm (mean = 9.25 × 13.44 μm, $n = 100$), rectangular, subglobose, often oriented transverse to the ascal axis, the basal ascospore often ellipsoid, oblong to elongate, with conspicuous germ slit spore length, without dehiscing layer in 10% KOH.

Culture characteristics. Colonies on OA covering the edge of 9 cm. Petri dish in 7 days, at first whitish, floccose, azonate, becoming smoke gray (105) and isabelline (65) (Fig. 3j). After 3 weeks incubation, the fungus produced synnemata on the agar medium but did not sporulate. Colonies on YMGA covering the edge of a 9 cm Petri dish in 6–7 days, at first whitish, becoming smoke gray (105), velvety to felty, azonate with entire margin.

Secondary metabolite. BNT (binaphthalene tetrol).

Notes. The closest relative of the new species is clearly *D. vernicosa*, which has eventually been regarded as cosmopolitan by Child (1932) and Ju et al. (1997, as *D. fissa*) but was only encountered in the more comprehensive study by Stadler et al. (2014) among the specimens originating from the temperate Northern hemisphere. A peculiar feature of *D. vernicosa* is that this species readily produces not only a very characteristic virgariella-like anamorph in culture but often forms stromata, in particular on Oatmeal agar (Ju et al.

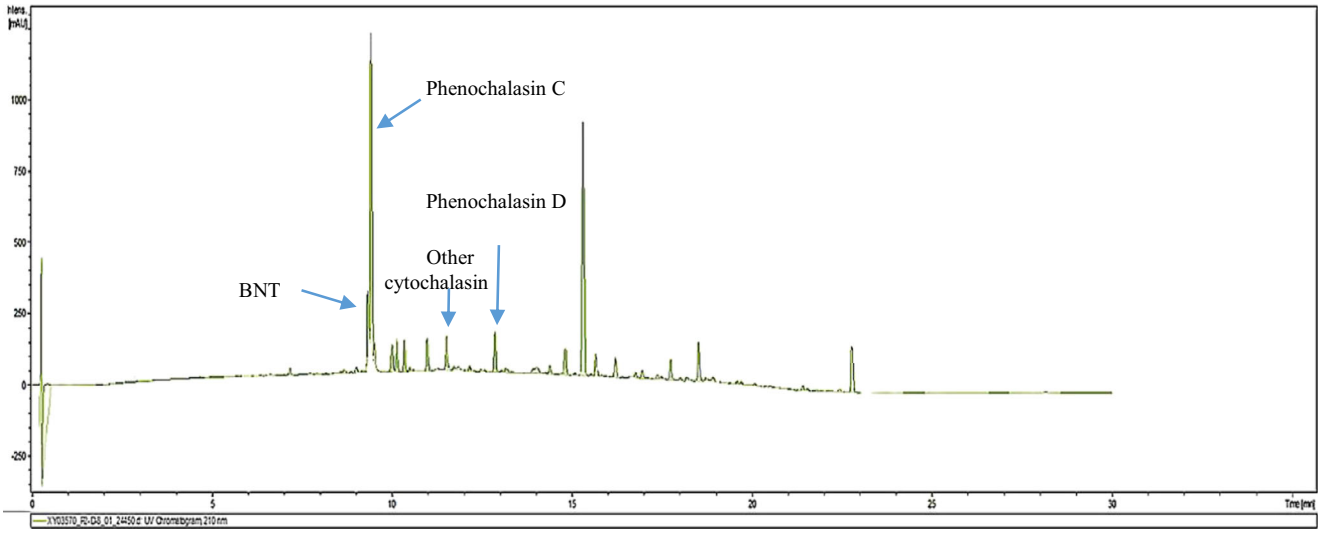


Fig. 3 Stromatal HPLC–UV profiles of *Daldinia kretzschmarioides* (epitype) (BNT–binaphthalene tetrol; phenochalasin C; phenochalasin D; cytochalasin)

1999; Stadler et al. 2014). *Daldinia loculata* (Lév.) Sacc. is closely related with *D. subvernicosa* but differs in stromatal and ascospore morphology. *Daldinia loculatoides* Wollweber & M. Stadler also has affinities to the new taxon, but has more regular ascospores and brown internal concentric zones. In addition, the species is only known from the temperate Northern hemisphere. *Daldinia singularis* Y.M. Ju, Lar.N. Vassiljeva & J.D. Rogers also is lacking a well-developed apical apparatus but differs by having smaller ascospores and a different anamorphic structure. *Daldinia bakeri* Lloyd (1919) is highly similar to *D. subvernicosa* in the shape and color of stroma while the ascospore length but has much larger ascospores than *D. subvernicosa*. The molecular phylogeny (Fig. 6) also confirmed the status of *D. subvernicosa* as a new species.

Molecular phylogeny

Twenty-seven new sequences were generated from the amplification of ITS, LSU, RPB2, and TUB2 regions (Table 1). These gene regions were selected to clarify the phylogenetic relationships of *Daldinia* and how they differ from other species and genera in the Hypoxylaceae. PCR amplifications yielded approx. 500 bp of ITS rDNA, 1000 bp of the LSU rDNA, approx. 800 bp of the RPB2, and approx. 1000 bp of the TUB2 region that were selected to clarify the phylogenetic relationships of several genera belonging to the Hypoxylaceae. The phylogenetic relationships were estimated using maximum parsimony (MP) and maximum likelihood (ML) analyses. The dataset of the multi-loci DNA sequences including 51

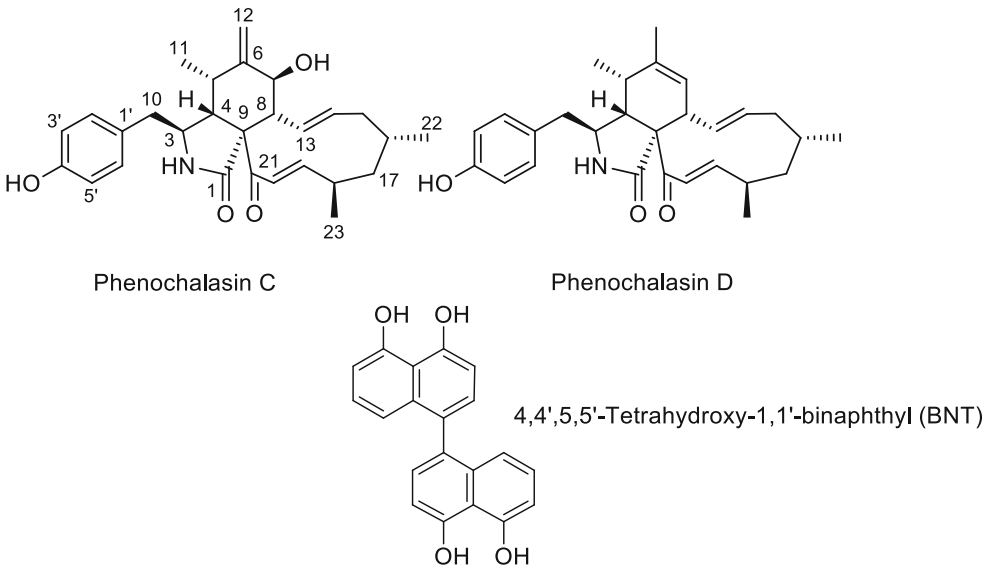


Fig. 4 Chemical structures of the major stromatal metabolites of *Daldinia kretzschmaroides*

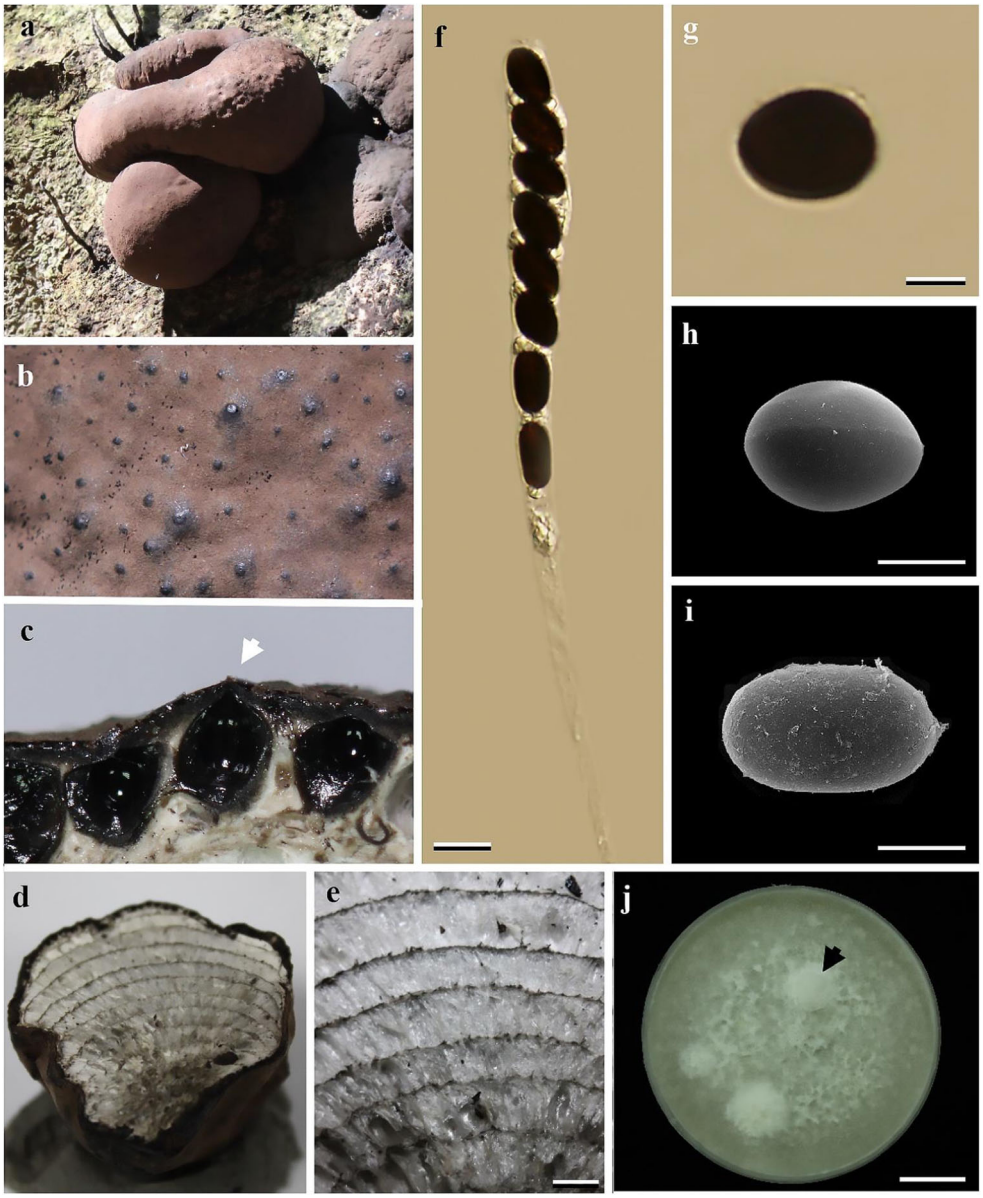
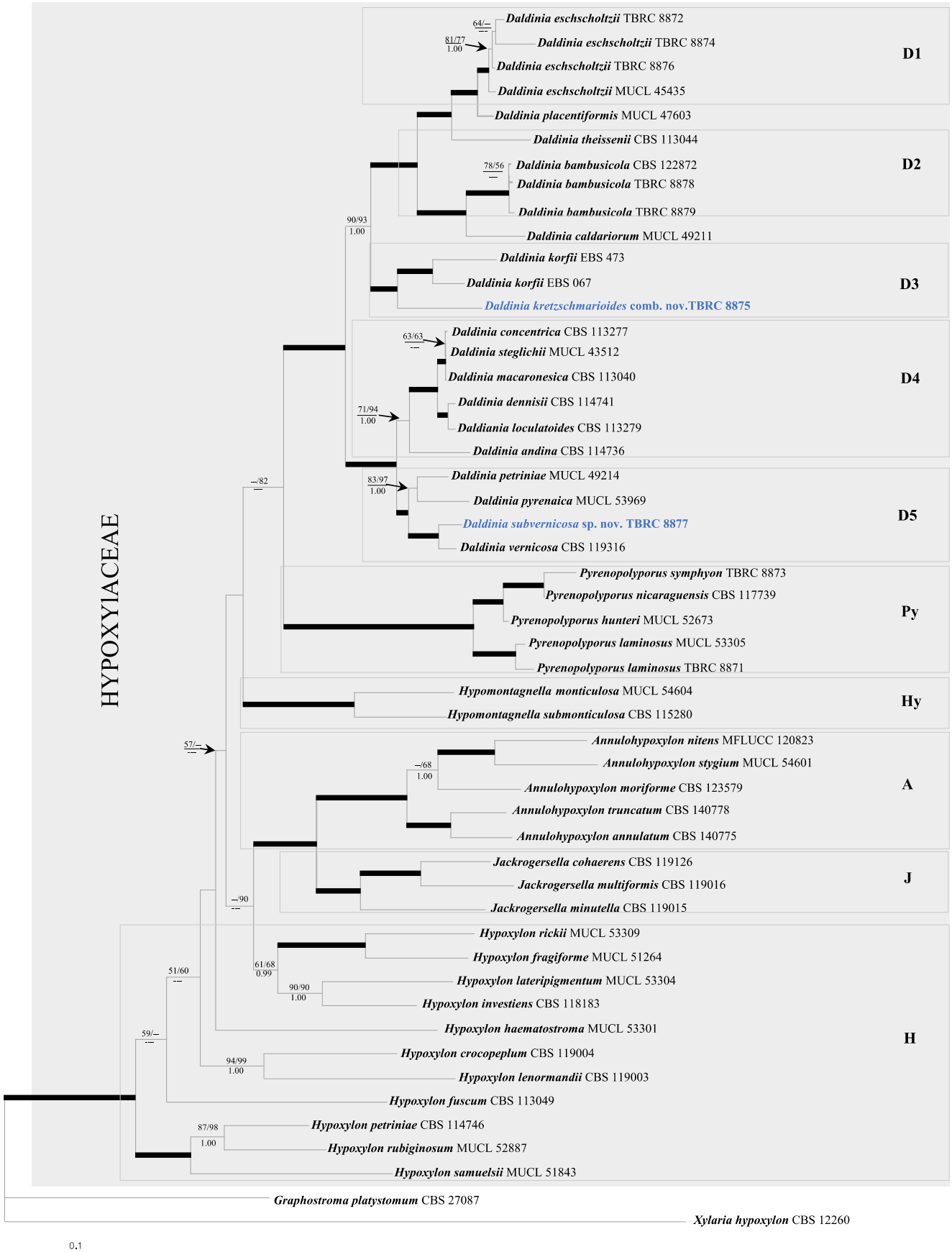


Fig. 5 *Daldinia subvernicosa* (BBH 42276). **a** stromatal habit; **b** stromatal surface with ostioles; **c** perithecia; **d** cross section of stroma showing alternating zones; **e** concentric zones; **f** ascus; **g** ascospore in distilled water**; h**–**i** ascospore by SEM; **j** colony on OA medium for 1 week. Scale is indicated by bars (**e** 0.5 mm. **f** 10 μm. **g**–**i** 5 μm. **j** 2 mm)

taxa in the Hypoxylaceae comprising 5 taxa in *Annulohypoxylon*, 23 taxa of *Daldinia*, 11 taxa of *Hypoxylon*, 2 taxa of *Hypomontagnella*, 3 taxa of *Jackrogersella*, and 5 taxa of *Pyrenopolyporus* with *Graphostoma platystomum* and *Xylaria hypoxylon* used as the out groups. The combined dataset consists of 4451 characters, of which 2578 were constant, 1380 parsimony informative, and 493 un-informative. The best tree generated through maximum parsimony analysis yielded only one most parsimonious tree. The molecular analyses revealed that DNA sequences are placed in the Hypoxylaceae. The phylogenetic tree including 4 major clades comprising the top clade is *Daldinia* clade and the *Pyrenopolyporus* clade, *Hypomontagnella*, *Annulohypoxylon*, *Jackrogersella*, and the lower clade as *Hypoxylon*, respectively (Fig. 6). The upper clade forms a monophyletic clade consisting of *D. eschscholtzii*, *D. placentiformis*, and *D. theissenii* placed in clade **D1** as a cryptic clade. However, we need more samples of *D. eschscholtzii* to fill the taxonomic database. Clade **D2** formed a monophyletic group with high support consisting of *D. bambusicola* and *D. caldariorum*. Our two samples (TBRC 8878, TBRC 8879) were placed in this clade with closest affinities to *D. bambusicola*, forming a sister clade to clade **D1**. Besides, the clade **D3** forms a distinct clade within the genus *Daldinia* with strong statistical support (100% BSMP, 100% BSML, and 1.00 BYPP), with **D1** and **D2** as sister clades and consists of *D. korfii* and *D. kretzschmarioides* comb. nov. In agreement with the morphological characteristics, and the two taxa are separated with high statistical support. **Clade D4**, consisting of *D. andina*, *D. concentrica*, *D. dennisii*,


HYPOXYLACEAE
Daldinia eschscholtzii TBRC 8872
Daldinia eschscholtzii TBRC 8874
Daldinia eschscholtzii TBRC 8876
Daldinia eschscholtzii MUCL 45435
Daldinia placentiformis MUCL 47603
Daldinia theissenii CBS 113044
Daldinia bambusicola CBS 122872
Daldinia bambusicola TBRC 8878
Daldinia bambusicola TBRC 8879
Daldinia caldariorum MUCL 49211
Daldinia korfii EBS 473
Daldinia korfii EBS 067
Daldinia kretzschmarioides comb. nov.TBRC 8875
Daldinia concentrica CBS 113277
Daldinia steglichii MUCL 43512
Daldinia macaronesica CBS 113040
Daldinia dennisii CBS 114741
Daldiania loculatoides CBS 113279
Daldinia andina CBS 114736
Daldinia petriniae MUCL 49214
Daldinia pyrenaica MUCL 53969
Daldinia subvernicosa sp. nov. TBRC 8877
Daldinia vernicosa CBS 119316
Pyrenopolyporus symphyon TBRC 8873
Pyrenopolyporus nicaraguensis CBS 117739
Pyrenopolyporus hunteri MUCL 52673
Pyrenopolyporus laminosus MUCL 53305
Pyrenopolyporus laminosus TBRC 8871
Hypomontagnella monticulosa MUCL 54604
Hypomontagnella submonticulosa CBS 115280
Annulohypoxylon nitens MFLUCC 120823
Annulohypoxylon stygium MUCL 54601
Annulohypoxylon moriforme CBS 123579
Annulohypoxylon truncatum CBS 140778
Annulohypoxylon annulatum CBS 140775
Jackrogersella cohaerens CBS 119126
Jackrogersella multiformis CBS 119016
Jackrogersella minutella CBS 119015
Hypoxylon rickii MUCL 53309
Hypoxylon fragiforme MUCL 51264
Hypoxylon lateripigmentum MUCL 53304
Hypoxylon investiens CBS 118183
Hypoxylon haematostroma MUCL 53301
Hypoxylon crocopeplum CBS 119004
Hypoxylon lenormandii CBS 119003
Hypoxylon fuscum CBS 113049
Hypoxylon petriniae CBS 114746
Hypoxylon rubiginosum MUCL 52887
Hypoxylon samuelsii MUCL 51843
Graphostroma platystomum CBS 27087
Xylaria hypoxylon CBS 12260
D1
D2
D3
D4
D5
Py
Hy
A
J
H
64/–
81/77
1.00
78/56
90/93
1.00
63/63
71/94
1.00
83/97
1.00
–/82
57/–
–/68
1.00
–/90
61/68
0.99
51/60
90/90
1.00
59/–
94/99
1.00
87/98
1.00
0.1

◀ **Fig. 6** Phylogeny of the Hypoxylaceae. The phylogenetic relationships are depicted as RA × ML tree was generated base on genetic multiples loci alignment of ribosomal (ITS and LSU) and proteinogenic (TUB2 and RPB2) sequence information**.** In maximum parsimony analysis, a CI of 0.372, a RI of 0.572, and a HI of 0.628 yielded only one parsimony tree with a length of 8802 changes. The phylogenetic relationships inferred from RA × ML had a likelihood of − 43934.190 and likelihood of the Bayesian tree was − 43244.290. Support values were calculated via maximum parsimony (MP), maximum likelihood (ML), and Bayesian analysis; and are indicated above (MP/ML) and below (B) the respective branches, if the bootstrap support (BS) values exceeded 50% from 1000 replicates or the posterior probability (PP) value from 3,000,000 MCMC chains (sampling frequency 1000, 10% burn-in) was 0.95 or higher. Branches of significant support (BS ≥ 95% and PP ≥ 0.98) are thickened

D. loculatoides, *D. macaronesica*, and *D. steglichii*, also formed a sister clade with clade **D5**. The **clade D5** was comprised of two subclades, one of which included *D. petriniae* and *D. pyrenaica*, while the other contained *D. subvernicosa* sp. nov. and *D. vernicosa*. The **clade PY** consisted of *Pyrenopolyporus* species and in agreement with Wendt et al. (2018) was a sister clade to **D4**. The **clade HY**, contained representatives of the recently erected genus *Hypomontagnella* (Lambert et al. 2019) with *H. monticulosa* and *H. submonticulosa.* The clade **A** included species of *Annulohypoxylon* and **clade J**, the members of *Jackrogersella*, appeared as a sister clade with clade **A**. The clade **H** comprised the species of *Hypoxylon*. The latter findings are in accordance with Wendt et al. (2018).

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