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FULL PAPER

Wood-inhabiting freshwater fungi from Thailand: *Ascothailandia grenadoidia* gen. et sp. nov., *Canalisporium grenadoidia* sp. nov. with a key to *Canalisporium* species (Sordariomycetes, Ascomycota)

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Abstract *Ascothailandia grenadoidia* gen. et sp. nov. is described and illustrated from submerged wood (*Wrightia tomentosa*) in a stream at Hala Bala Wildlife Sanctuary, southern Thailand. The new genus (teleomorph) is characterized by perithecoid, globose, dark brown, ostiolate ascomata, paraphysate, asci cylindrical, unitunicate with a prominent J-refractive apical ring and versicolurus, 3-euseptate ascospores. Ascospores germinated producing a *Canalisporium* (*C. grenadoidia* sp. nov.) anamorph. The morphological characterization of this new fungus is reported and compared with the genera *Ascotaiwania* and *Savoryella*. Phylogenetic analyses of the combined partial 18S, 28S ribosomal DNA and internal transcribed spacer, including 5.8S regions, of *Ascothailandia grenadoidia* and 10 *Canalisporium* species were undertaken and analyzed with maximum parsimony and Bayesian methods. The molecular data indicate that *A. grenadoidia* is closely related to *Canalisporium elegans* in the Sordariomycetes, Hypocreomycetidae, order *incertae sedis*. Both morphological and molecular characterization provides sufficient evidence to support the description of a new genus. A key to *Canalisporium* species is provided.

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Keywords Anamorphic fungi · Combined 18S and 28S rDNA · Hypocreomycetidae · ITS

Introduction

A long-term study (3 years) of the fungal colonization of nine tropical timbers (*Azadirachta indica* A. Juss var. *siamensis* Valeton, *Erythrophleum teysmannii* Craib, *Melaleuca cajuputi* Powell, *Shorea obtusa* Wall., *Shorea roxburghii* G. Don, *Shorea siamensis* Miq., *Wrightia tomentosa* Roem. & Schult., *Xylia xylocarpa* (Roxb.) W. Taub, and *Zollingeria dongnaiensis* Pierre) submerged in a stream at Hala Bala Wildlife Sanctuary, Narathiwat province, Thailand, has been in progress. During this study a fungus with morphological features (especially the asci and ascospores) similar to *Ascotaiwania* Sivan. & H.S. Chang and *Savoryella* E.B.G. Jones & R.A. Eaton was found on the test blocks of *Wrightia tomentosa*. *Ascotaiwania* and *Savoryella* species are frequently collected on trapped wood in streams and rivers, in both tropical and temperate zones (Jones and Eaton 1969; Sivanesan and Chang 1992; Jones and Hyde 1992; Chang et al. 1998; Sivichai et al. 2000a, b). The taxonomic position of *Ascotaiwania* and *Savoryella* has not been resolved, and they are referred to the Sordariales *incertae sedis* and Hypocreales *incertae sedis*, respectively (Cai et al. 2006). A phylogenetic study of these two genera was undertaken with the aim of resolving the identification of the new ascomycete. Nine species of *Canalisporium* Nawawi & Kuthub., saprobic on wood and plant debris, often in freshwater, have been described but have no known teleomorph (Nawawi and Kuthubutheen 1989; Goh et al. 1998; Goh and Hyde 2000; Cai et al. 2002; Ho and Hyde 2004). However, Cai et al. (2006) suggested a connection with the Tubeufiaceae, but

this was not supported by a molecular study. In this study, a new genus and species of *Ascothailandia grenadoidea* (anam. *Canalisporium grenadoidia*) are described and illustrated. In addition, the partial small subunit (SSU), large subunit (LSU) of ribosomal DNA (rDNA) and the internal transcribed spacer region (ITS) sequences of six *Canalisporium* species were compared to resolve their phylogenetic relationships.

Materials and methods

Morphological studies

Submerged wood test blocks (15 × 2.5 × 2.5 cm) of *Wrightia tomentosa* were collected from a stream at Hala Bala Wildlife Sanctuary, Narathiwat province, southern Thailand, washed of surface debris and incubated in a plastic box with damp tissue paper, then returned to the laboratory. Samples were periodically examined for 2 weeks. Single ascospore isolations were made on corn meal agar (CMA) with added antibiotics (penicillin G 0.5 g/l and streptomycin 0.5 g/l) and germinating spores transferred to potato dextrose agar (PDA). Axenic cultures were deposited at BIOTEC Culture Collection (BCC). Collections of *Canalisporium* species were made from various sites in Thailand and deposited in BCC. Dried specimens were deposited at BIOTEC Bangkok Herbarium (BBH).

Phylogenetic studies

The taxonomic placement of the selected freshwater lignicolous fungi was undertaken by comparing the partial 18S, 28S rDNA and ITS regions.

Culture selection

Selected fungal cultures used in this study are listed in Table 1 along with collection and isolation data. Fungi were grown on PDA for 4–16 weeks at 25°C. Actively growing mycelium was scraped off the surface of a culture and transferred to 2 ml of microcentrifuge tubes and the biomass lyophilized at −80°C for 24 h.

DNA extraction

Extraction buffer (1% CTAB, 0.7 M NaCl, 50 mM Tris-HCl, 10 mM EDTA, pH 8) was added to fungal samples. The samples were ground in a 2-ml microcentrifuge tube and the volume adjusted by adding 700 µl extraction buffer and mixed by inverting the tubes; they were incubated at 65°C for 1 h. Samples were centrifuged at 12,000×*g* for 10 min at 25°C. The aqueous supernatant was transferred into a new microcentrifuge tube with phenol-chloroform-isoamyl alcohol by mixing gently and by centrifugation at 12,000×*g* for 10 min at 25°C. The upper liquid phase was transferred to a new microcentrifuge tube containing 7.5 M of ammonium acetate. The DNA was precipitated by ethanol (−20°C overnight) by centrifugation at 12,000×*g* for 10 min at 15°C. The DNA pellet was washed with ice-cold 70% ethanol and dried at 25°C. The pellet was redissolved in 50 µl of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA pH 8.0).

PCR amplification

Primers used for PCR amplification and for sequencing of SSU, LSU rDNA and ITS were NS1, NS3, NS4, NS6, JS1, JS8, ITS1, ITS4 and ITS5 (White et al. 1990; Bunyard et al. 1994; Landvik 1996). Amplification was performed in a 50-µl reaction mix: 10 mM of each dNTP (1 µl), 10 µM of each primer (1 µl), 10% of dilution buffer (5 µl), 25 mM of Mg (5 µl), 4 M of enhancer (5 µl) and 60–62% of sterile distilled water (30.8 µl), 0.2 µl of *Taq* DNA polymerase kit from FERMENTAS (Burlington, Canada) and 10–50 ng of genomic DNA template (1 µl) carried out using a PCR Model MJ Research DYAD ALD in 200-µl reaction tubes (95°C, 0.5 min; 52°C, 1 min; 72°C, 1.5 min; 35 cycles). PCR products (7 µl aliquots) were checked by electrophoresis in 1% agarose gels with 0.003% ethidium bromide in 0.5 × TBE buffer (0.044 M boric acid, 1.1 mM EDTA, 0.045 M Tris, pH 8) for purity.

DNA purification and sequencing

PCR products were purified using NucleoSpin® Extract Kit (Macherey-Nagel, Germany). The PCR products were sequenced by Macrogen Inc. in Korea with the same primers as in the PCR amplification. The sequences obtained were deposited in the National Center for Biotechnology Information (NCBI), and the accession numbers are listed in Table 1.

Phylogenetic analyses

Each sequence was checked for ambiguous bases and refined visually, assembled using BioEdit 6.0.7 (Hall 1999). The consensus sequences for each DNA region were multiple aligned by Clustal W 1.6 (Thompson et al. 1994) with all sequences derived from the GenBank database and the accession numbers that are included in the phylogenetic trees.

The alignment (P.I.N. 3801) included the most similar sequence identified through BLAST search. *Daldinia concentrica* (Bolton) Ces. & De Not. and *Xylaria hypoxylon*

Table 1 Fungal strains used in this study

Fungal species	Isolate	GenBank accession number			Substrate origin	Colleting site
		SSU	LSU	ITS		
Ascothailandia grenadoidia	SS03615	GQ390252	GQ390267	GQ390282	*Wrightia tomentosa*	Khlong Ai-kading, Hala Bala Wildlife Sanctuary, Narathiwat, Thailand
Canalisporium caribense	SS03839	GQ390253	GQ390268	GQ390283	Submerged natural wood	Khlong Ai-kading, Hala Bala Wildlife Sanctuary, Narathiwat, Thailand
Canalisporium caribense	SS03683	GQ390254	GQ390269	GQ390284	Submerged natural wood	Wang Kan Lueang Waterfall, Wang Kan Lueang Arboretum, Lop Buri, Thailand
Canalisporium elegans	SS00523	GQ390255	GQ390270	GQ390285	*Xylia dolabriformis*	Road marker at Km 29.2, Khao Yai National Park, Nakhon Ratchasima, Thailand
Canalisporium elegans	SS00895	GQ390256	GQ390271	GQ390286	*Stereospermum neuranthum*	Road marker at Km 29.2, Khao Yai National Park, Nakhon Ratchasima, Thailand
Canalisporium jinghongensis	SS03491	GQ390257	GQ390272	GQ390287	Submerged natural wood	Kaeng Krachan National Park, Prachuap Khiri Khan, Thailand
Canalisporium jinghongensis	SS03483	GQ390258	GQ390273	GQ390288	Submerged natural wood	Bo Khlueng Hot-spring, Ratchaburi, Thailand
Canalisporium pulchrum	SS03819	GQ390259	GQ390274	GQ390289	Submerged natural wood	Khao Pra—Bang Khram Wildlife Sanctuary, Krabi, Thailand
Canalisporium pulchrum	SS03823	GQ390260	GQ390275	GQ390290	Submerged natural wood	Khao Pra—Bang Khram Wildlife Sanctuary, Krabi, Thailand
Canalisporium pulchrum	SS00170	GQ390261	GQ390276	GQ390291	*Alstonia scholaris*	Road marker at Km 29.2, Khao Yai National Park, Nakhon Ratchasima, Thailand
Canalisporium pulchrum	SS03982	GQ390262	GQ390277	GQ390292	Submerged natural wood	Heo Narok Waterfall, Khao Yai National Park, Nakhon Nayok, Thailand
Canalisporium pulchrum	SS03773	GQ390263	GQ390278	GQ390293	Submerged natural leaf	Khlong Ai-kading, Hala Bala Wildlife Sanctuary, Narathiwat, Thailand
Canalisporium pallidum	SS00091	GQ390264	GQ390279	GQ390294	*Alstonia scholaris*	Road marker at Km 29.2, Khao Yai National Park, Nakhon Ratchasima, Thailand
Canalisporium pallidum	SS00498	GQ390265	GQ390280	GQ390295	*Xylia dolabriformis*	Road marker at Km 29.2, Khao Yai National Park, Nakhon Ratchasima, Thailand
Canalisporium exiguum	SS00809	GQ390266	GQ390281	GQ390296	Submerged natural wood	Khao Soi Dao Wildlife Sanctuary, Chanthaburi, Thailand

(L.) Grev. were chosen as the outgroup to root the phylogenetic tree for all analyses. The analyses of the combined nuclear ribosomal DNA dataset (18S and 28S rDNA) and ITS dataset were performed using PAUP*4.0b10 (Swofford 2002). Gaps were treated as missing data. The most parsimonious trees (MPTs) were searched using maximum parsimony (MP) in PAUP*4.0b10 with heuristic search algorithm with tree bisection-reconnection (TBR) branch swapping. One hundred replicates of random stepwise sequence addition were performed, and the shortest trees over all replicates kept and assumed to be the most parsimonious reconstructions to increase the chance of finding the best tree(s). The tree length (TL), consistency indices (CI) and retention indices (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for each tree generated. Trees were visualized with TreeView (Page 1996).

Tree topologies from different parsimony analyses were tested with the Kishino-Hasegawa (K-H) maximum likelihood test (Kishino and Hasegawa 1989) to find the most likelihood tree. Bootstrap support (BS) for the branches was based on 1,000 replicated with 10 replicates of random stepwise addition of sequence.

The model of substitution used for Bayesian analysis was chosen with Mrmodeltest 2.2 (Nylander 2004). Independent Bayesian phylogenetic analysis was performed in MrBayes3.0.b4 (Huelsenbeck and Ronquist 2001) using a uniform GTR + I + G (combined 18S + 28S rDNA dataset) and HKY + G model (ITS dataset) with a general time reversible (GTR) model for DNA distribution and gamma distribution rate variation across sites. The Metropolis-coupled Markov chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities. Four simultaneous Markov chains were run from a random starting tree for 2,000,000 generations and sampled every 100 generations. The first 2,000 generations were discarded as burn in the chain. A 50% majority rule consensus tree of all remaining trees, as well as the posterior

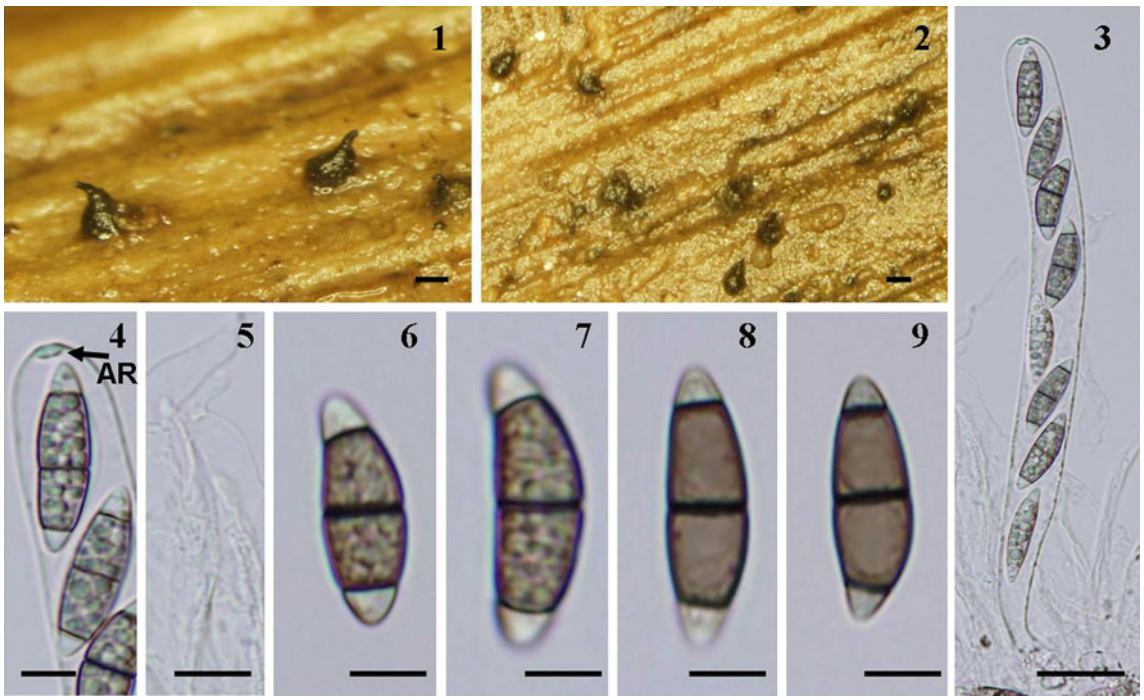

Fig. 1–9 Morphological characteristics of *Ascothailandia grenadoidia*. **1**, **2** Light microscope micrographs of ascomata with long neck on the test blocks. **3** Ascus and hyaline paraphyses. **4** Apical ring (*AR*). **5** Hyaline paraphyses. **6–9** Ascospores uniseriate to overlapped biseriate squeezed from ascus. *Bars* **1**, **2** 100 μm; **3–5** 20 μm; **6–9** 5 μm

probabilities (PP), was calculated. Parsimony bootstrap values greater than 50% and Bayesian posterior probabilities greater than 0.95 are given above and below each clade, respectively.

Results

Species descriptions

Ascothailandia Sri-indrasutdhi, Boonyuen, Sivichai & E.B.G. Jones, gen. nov.

MycoBank no.: MB 515145

Ascomata immersa, semi-immersa vel superficialia, perithecioidia, globosa vel subglobosa, brunnea usque atra, solitaria, dispersa, collo longo, ostiolata; Ostiolum ut plurinum centrale sed ascomata horizontalia hospitalis pagina, deinde ad longo vel brevissimus, prerumque brunnea vel atra, periphysis; Paraphyses hyphoideae, numerosae, contractae distaliter, non in gelatinosus matricis inclusae; Asci 8-spora, cylindricus longo, pedunculus, unitunica, apice truncato, massa affinisis (ca. 4–8 μm diam.), annulusio apicalis, J-refractivi, cylindricus, persistensa; Ascospora uniseriata vel uniseriata imbricata, fusiformis, rectus vel curvata, 3-euseptata et versicolurus.

Type species: *Ascothailandia grenadoidia* Sri-indrasutdhi, Boonyuen, Sivichai & E.B.G. Jones.

Ascomata immersed, semi-immersed or superficial, perithecioid, globose or sub-globose, dark brown to black, solitary, scattered, long neck, ostiolate. Ostiole mostly central but if ascomata are lying horizontal to the host surface, then at one end and curving upwards, long or short, usually brown or black, periphysate. Paraphyses hyphalike, numerous, tapering distally, not embedded in a gelatinous matrix. Asci 8 spored, long cylindrical, pedunculate, unitunicate, apically truncate, with a relative massive (ca. 4–8 μm diameter), with an apical ring J-refractive, cylindrical, persistent. Ascospores uniseriate or overlapping uniseriate, fusiform, straight or curved, 3-euseptate and versicolurus.

Etymology: from *asco* in reference to Ascomycota and *Thailandia* in reference to Thailand country of origin.

Ascothailandia grenadoidia Sri-indrasutdhi, Boonyuen, Sivichai & E.B.G. Jones, sp. nov. Fig. 1–9

MycoBank no.: MB 515146

Ascomata immersa vel semi-immersa, dispersa, pyriformia, brunnea usque atra, 110–200 μm alta ($\bar{x} = 160, n = 6$), 100–150 μm in diam ($\bar{x} = 123, n = 6$), coriacea, ostiolata, papillata; paraphyses sparsae, hyalinae, numerosae, contractae distaliter, non in gelatinosus matricis inclusae; Asci 10–12 × 70–86 μm ($\bar{x} = 11.5 \times 78.6$ μm, $n = 6$), 8-spori, collum cylindricus, unitunicati, pedicellusri breves, apice truncato, J-refractivus; Ascospora 16–22 × 4–8 μm ($\bar{x} = 19.7 \times 5.9$, $n = 25$), uniseriatus, ovoideae vel fusiformes,

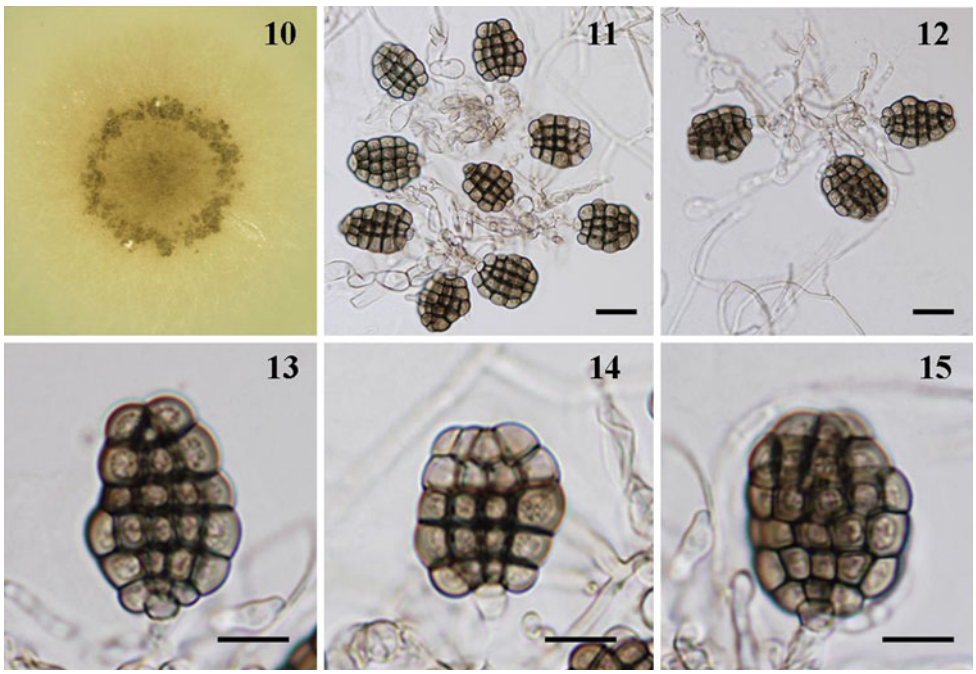

Fig. 10–15 *Canalisporium grenadoidia* on corn meal agar. **10–12** Squash mount of a portion of the sporodochium conidia. **13–15** Pale-olive to brown and globose to sub-globose conidia. *Bars* **11**, **12** 20 μm; **13–15** 10 μm

rectae vel curvae, hyalinae, 3-euseptatae, paries levis, guttula in quoqueae cellula, circumvallatus muco tenuis.

Holotypus: In lignum submerses emortuisque *Wrightia tomentosa* 26/08/2005 S. Sivichai, BBH26383.

Ascomata immersed or semi-immersed, scattered, pyriform, dark brown to black, 110–200 μm high ($\bar{x} = 160$, $n = 6$), 100–150 μm in diam ($\bar{x} = 123$, $n = 6$), coriaceous, ostiolate, papilate. Paraphyses sparse, hyaline, numerous, tapering distally, not embedded in a gelatinous matrix. Asci 10–12 × 70–86 μm ($\bar{x} = 11.5 \times 78.6$ μm, $n = 6$), 8-spored, cylindrical, unitunicate, short pedicellate, apically truncate, with a refractive, J-apical ring. Ascospores 16–22 × 4–8 μm ($\bar{x} = 19.7 \times 5.9$, $n = 25$), uniseriated ovoid to fusoid, straight to curved, hyaline, 3-euseptate, smooth-walled, with a large guttule in each cell, surrounded by a thin layer of mucilage (Fig. 1–9).

Holotype: Thailand, Narathiwat, Hala Bala Wildlife Sanctuary, on submerged wood of *Wrightia tomentosa* Roem & Schult., August 26, 2005, collected and isolated by S. Sivichai, BBH26383 (single ascospore isolate ex holotype SS03615 = BCC20507).

Etymology: from *grenade* and *-oidia*, in reference to the similarity of the conidia of the anamorph in culture to a grenade outline.

rDNA sequence ex holotype: GQ390252 (18S), GQ390267 (28S), GQ390282 (ITS)

Anamorph: *Canalisporium grenadoidia*.

Canalisporium grenadoidia Sri-indrasutdhi, Boonyuen, Sivichai & E.B.G. Jones, sp. nov. Fig. 10–15

MycoBank no.: MB 515143

Colonia in CMA, crescens tarde, pallide brunnea usque obscure brunnea, effusa vel punctiformis, densa, cum parvus aerius mycelia, hypha alba versus obscurus brunnea, 2–2.5 μm lata, septata, ramosus; Conidia obscure olivaceus usque brunnea, solitaria, acrogena, globosa versus subglobosa vel ovalis, holoblastica, parieto denso, leviter curvatus, 3–6 longitudinalis septate, et 4–6 transversalis septate, atris, crassis praedita, brunnea usque nigrobrunnea, suffultus ad pallide brunnea parvus cellula basali, Conidiis 17–26, apicalis series cum 3–4 cella, nigro lumen canalis 1–2 μm.

Holotypus: On CMA 18/10/2005 by S. Sivichai, BBH26384.

Colonies in CMA, slow growing, pale brown to dark brown, effuse or punctiform, compact, with little aerial mycelium, hyphae white to pale brown, 2–2.5 μm wide, septate, branched; Conidia are olive green to brown, solitary, acrogenous, globose to sub-globose or oval, holoblastic, thick-walled, slightly curved, with 3–6 longitudinal septa, and 4–6 transverse septa, some constricted at the septa, brown to dark brown at the septa, supported by a pale brown small basal cell, the number of cells per conidium varies from 17 to 26, apical row with 3–4 cells, cell lumen connected by canals obscured by dark pigment, 1–2 μm.

Holotype: In culture of CMA, October 18, 2005 by S. Sivichai, BBH26384.

Etymology: from *grenade* and *-oidia*, in reference to the similarity of the conidia of the anamorph in culture to a grenade outline.

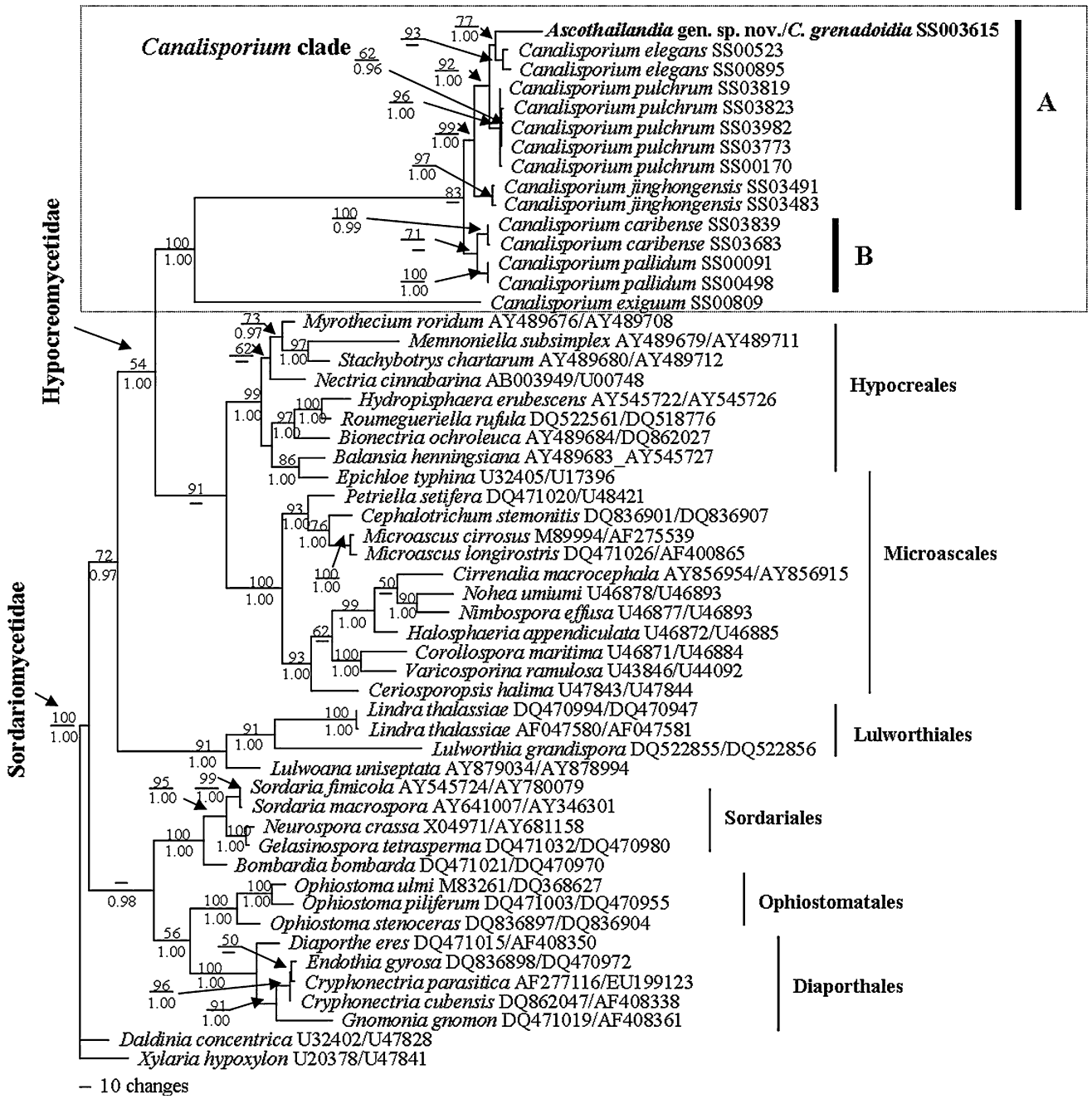

Fig. 16 Most parsimonious tree obtained from combined (18S + 28S) rDNA dataset. The tree rooted with *Xylaria hypoxylon* and *Daldinia concentrica* from the order Xylariales. Bootstrap values higher than 50% from maximum parsimony analysis are given above nodes, and Bayesian posterior probabilities more than 0.95 are indicated as below nodes

We also compare the *Canalisporium grenadoidia* with currently known species of *Canalisporium* in the shape and size of the conidia (Table 3).

Molecular phylogeny of combined 18S and 28S rDNA dataset

The combined 18S rDNA and 28S rDNA dataset including the new genus (one taxon) and *Canalisporium* strains (14 taxa) from the BIOTEC Culture Collection (BCC) were aligned along with 37 representative taxa from the Class Sordariomycetes and three subclasses: Hypocreomycetidae (Microascales and Hypocreales), Sordariomycetidae (Diaporthales, Ophiostomatales and Sordariales) and the Lulworthiales with their accession numbers (Fig. 16). Two representative taxa of the order Xylariales (*Daldinia concentrica* and *Xylaria hypoxylon*) were used as an outgroup. The maximum parsimony analysis, with gaps treated as

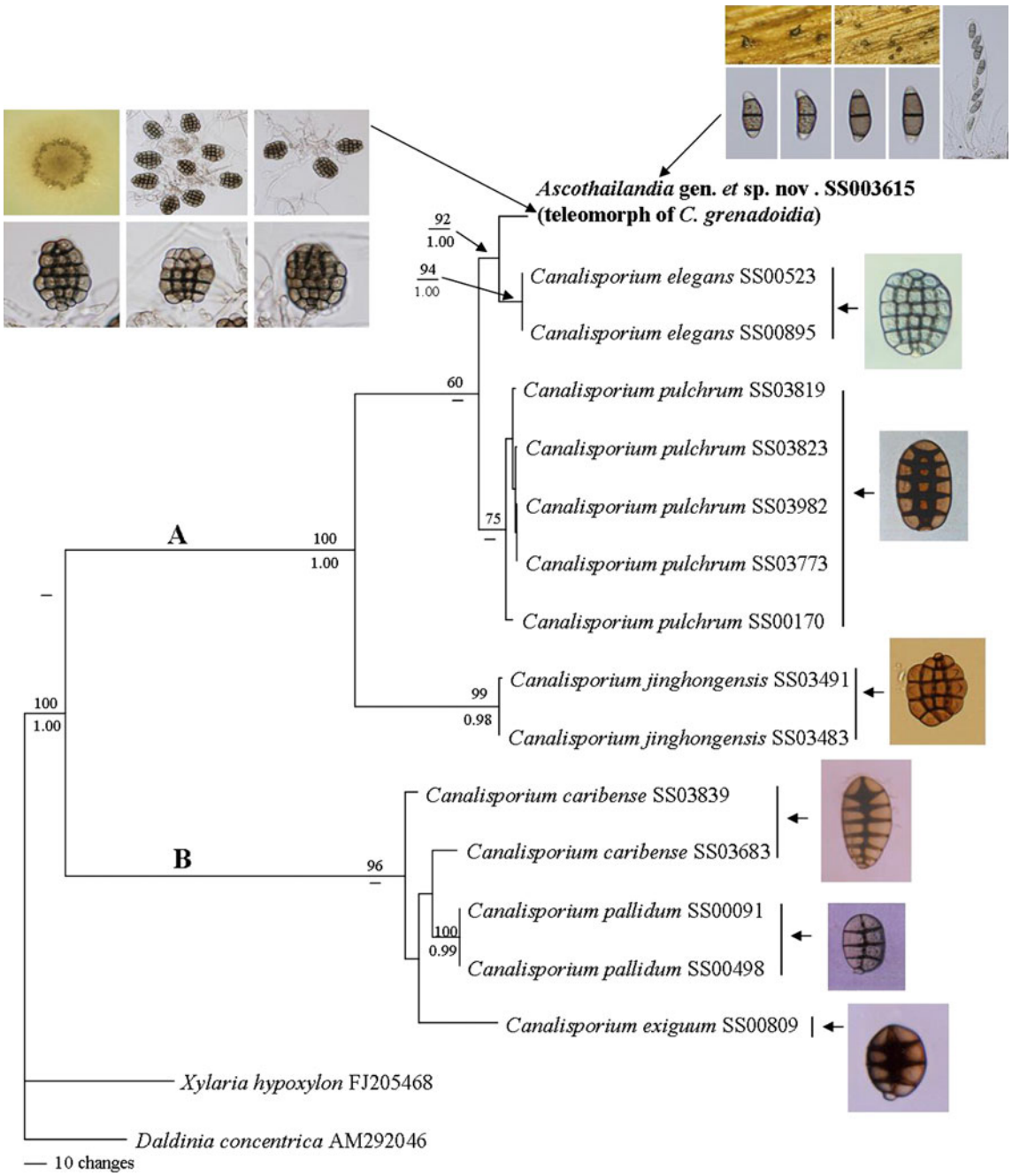

Fig. 17 Phylogeny obtained from ITS rDNA dataset. Bootstrap values more than 50% are shown above the branches, and Bayesian posterior probabilities more than 0.95 are indicated as below nodes

missing data, yielded eight trees (TL = 2,316, CI = 0.506; RI = 0.494; RC = 0.422; HI = 0.494). The final aligned dataset comprised 2,301 characters, out of which 592 were parsimony informative, 233 parsimony uninformative and 1,476 constant characters. The difference between the eight trees is in the branch swapping pattern in the clade Lulworthiales and Diaporthales (results not shown). Bootstrap values, greater than 50%, are shown on the upper nodes, whereas Bayesian posterior probabilities greater than 0.95 are indicated on lower nodes. Based on the Kishino-Hasegawa (K-H) maximum likelihood test, one of the eight trees (the best likelihood tree) is shown in Fig. 16. Phylogenies based on the same dataset sequences calculated on the K-H maximum likelihood test under different loci of small ribosomal DNA and large ribosomal DNA were almost identical to those obtained from individual datasets,

Table 2 Characters that distinguish *Ascotaiwania*, *Savoryella* and *Ascothailandia* (modified from Chang et al. 1998)

	Ascotaiwania	*Savoryella*	*Ascothailandia*
Ascomata	200–240 μm diam., generally large	100–250 μm diam., smaller than *Ascotaiwania* and larger than *Ascothailandia*	100–150 μm diam., smaller than *Ascotaiwania* and *Savoryella*
Asci	90–308 × 8.5–21 μm, cylindrical, longer and narrow. Spores uniseriate to biseriate, short pedicellate	80–225 × 10–35 μm, cylindrical to clavate. Spores generally biseriate	70–86 × 10–12 μm, cylindrical. Spores uniseriate or overlapping uniseriate, long pedicellate
Paraphyses	Filiform, septate, narrow, up to 2 μm	Septate, wide, <8 μm constricted at the septa	Non septate, filiform 1–2 μm, hypha-like
Apical ring	Present (non-amyloid apical ring)	Absent	Present
Ascospores	3 to 7-septate, generally more than 3 septa, end cell pointed (42–55 × 8–13 μm), mid brown central cells and smaller hyaline to sub-hyaline end cells	3-Septate, end cell rounded (20–47 × 6–18 μm), mid brown to black central cells and hyaline to sub-hyaline end cells	Generally 3-septa (16–22 × 4–8 μm), mid brown to black central cells and hyaline to sub-hyaline end cells
Nutritional mode	Saprobic	Saprobic	Saprobic
Habitat	On terrestrial dead wood, lignicolous, freshwater; also palm rachis	On freshwater, brackish and marine	On submerged wood in rainforest stream
Anamorph stage	*Monotosporella* state	None known	*Canalisporium grenadoidia*

Table 3 Comparison of *Canalisporium* species

Species	Pigmentation	Length (μm)	Width (μm)	Lateral thickness (μm)	Accentuation of septa	Columns of longitudinal septa	Rows of transverse septa	Cells at the apex	Cells at the base
C. caribense	Moderate to dark	24–51	15–29	9–12.5	Yes	1	3–6	2	Single
C. elegans	Moderate	32–58	25–38	10–13	Moderately	4–5	5–8	1–5	Single
C. exiguum	Moderate to dark	18–25	13–15	5–8	Yes	1	2–3	2	Single
C. grenadoidia	Pale	22–38	16–28	16–22	Moderately	4–6	4–5	1–4	Single
C. jinghongensis	Pale	25–33	20–28	7.5–11.5	Moderately	4–5	2–4	1–4	Single
C. kenyense	Dark	34–56	24–34	14–18	Yes	2	4–5	1	Triple
C. pallidum	Pale	25–39	15–20	8–10	No	Mostly 1	4–5	1–2	Single
C. panamense	Dark	50–70	46–60	7 up	Moderately	6–8	6–8	–	Single
C. pulchrum	Moderate to dark	25–63	20–32	12–17	Yes	2	3–9	1–3	Single
C. variabile	Pale	22–35	15–23	10–10.5	No	Mostly 2	2–4	1	Single

except for the position of the Lulworthiales in the 18S rDNA and 28S rDNA dataset with minor branch swapping (results not shown).

The new ascomycete is well placed in the *Canalisporium* clade with strong support (1.00 PP and 100% BS). This clade comprised two subclades: Subclade A composed of *Ascothailandia*, *C. elegans* Nawawi & Kuthub., *C. jinghongensis* L. Cai, K.D. Hyde & McKenzie and *C. pulchrum* (Hol.-Jech. & Mercado) Nawawi & Kuthub. with strong support (1.00 PP and 99% BS), Subclade B consisted of *C. caribense* (Hol.-Jech. & Mercado) Nawawi & Kuthub. (type species) and *C. pallidum* Goh, W.H. Ho & K.D. Hyde with low support, while another species, *C. exiguum* Goh & K.D. Hyde, grouped as a sister taxon to Subclade A and Subclade B.

The relationship between *A. grenadoidia* (teleomorph of *C. grenadoidia*) and the other *Canalisporium* species showed that this new taxon has close phylogenetic affinities with *C. elegans* (SS00523, SS00895) with a bootstrap support of 77% and posterior probabilities of 1.00, while *C. exiguum* formed a basal clade to the other *Canalisporium* species. Therefore, *A. grenadoidia* is proposed as a new ascomycete with its anamorph (*C. grenadoidia*), supported by both morphological and molecular data.

Molecular phylogeny of internal transcribed spacer dataset

A dataset consisting of 17 taxa including the new ascomycete, *Canalisporium* species and outgroup taxa

(*D. concentrica* and *X. hypoxylon*) is presented in Fig. 17. This dataset contained 748 characters, 218 parsimony-informative, 415 constant and 115 parsimony-uninformative. The maximum parsimony analysis yielded three most parsimonious trees (MPTs) 443 steps long (with CI = 0.905, RI = 0.915, RC = 0.828, HI = 0.095). The overall topologies for all three MPTs are the same, and only differ in the minor swapping position of *C. caribense* (SS003839). One of the three MPTs inferred with the best topology from K-H test is shown in Fig. 17.

Maximum parsimonious phylogenies from the ITS dataset showed that the branches leading to the *Canalisporium* species are reasonably stable with respect to the position of *A. grenadoidia* (teleomorph of *C. grenadoidia*) and essentially similar to those derived from the combined 18S + 28S rDNA and each individual dataset (18S and 28S rDNA). An exception was *C. exiguum* swapping position with the *C. pallidum* and *C. caribense* subclade with low support (less than 50% BS and 0.95 PP). The new taxon grouped with *C. elegans* with high statistical bootstrap support (92%) and Bayesian values (1.00).

Discussion

The genera *Ascotaiwania*, *Savoryella* and *Ascothailandia* share many common morphological features (Table 2), especially the nature of ascomata, paraphyses and versicolurus ascospores. However, morphologically they can be separated by two characters: *Ascotaiwania* has a non-amyloid apical ring and ascospores with more than 3 septa, *Savoryella* lacks an apical ring and ascospores are 3-septate, while *Ascothailandia* has an apical ring and 3-septate ascospores. Furthermore, molecular data show they are not monophyletic (Boonyuen et al., personal communication).

However, this paper focuses on the new freshwater ascomycete (*Ascothailandia*) and its *Canalisporium* anamorph, the first time the genus has been linked to a teleomorph. Molecular sequences show that the *Canalisporium* spp. and the new ascomycete form a well-supported monophyletic clade in the Hypocreomycetidae, with the Hypocreales and Microascales as sister clades. The long branches of the *Canalisporium* clade and weak bootstrap support suggest little affinity with the Hypocreales and Microascales. *Ascothailandia* and *Canalisporium* share few morphological characters with the Microascales and Hypocreales, especially the versicolurus 3-septate ascospores and multicellular, pigmented conidia.

Goh et al. (1998) emended the generic concept of *Canalisporium* from that of Nawawi and Kuthubutheen (1989), in the habitat of the taxa, conidial septation, order and arrangement of septa in the conidial body and the presence of septal canals, basal cells, conidial secession, conidiogenesis, morphology of the mycelium in pure culture and fossilized conidia features.

Canalisporium grenadoidia differs from all other *Canalisporium* species in shape and size of the conidia (Table 3). Currently, there are nine *Canalisporium*: *C. caribense* (type species), *C. elegans*, *C. pulchrum* (Nawawi and Kuthubutheen 1989); *C. exiguum*, *C. kenyense* Goh, W.H. Ho & K.D. Hyde, *C. pallidum* (Goh et al. 1998); *C. variabile* Goh & K.D. Hyde (Goh and Hyde 2000); *C. jinghongensis* (Cai et al. 2003) and *C. panamense* A. Ferrer & Shearer (Ferrer and Shearer 2005). *Canalisporium grenadoidia* is described as the anamorph of *Ascothailandia grenadoidia*.

Within the *Canalisporium* clade, two subclades are observed: subclade A. *Canalisporium* spp. with many rows of cells and subclade B., with these forming only two rows. Whether these differences are sufficient to designate them as separate genera remains to be resolved. No previous sequences have been available for *Canalisporium*, although Cai et al. (2003) drew attention to the similarities of the conidia to those of *Dictyosporium* Corda. Clearly, *Canalisporium* species are not the anamorphs of members of the Tubeufiaceae as suggested by Cai et al. (2006).

Key to species of *Canalisporium* (after Goh et al. 1998)

1a. Conidiophore absent……..……..*C. panamense*
1b. Conidiophore present……..…….2
2a. Conidia with the three smaller cells at the base and have a single cell at the apex…….. *C. kenyense*
2b. Conidia with a single cell at the base and one (rarely), two or more cells at the apex……...…3
3a. Conidia with a single column of longitudinal septa, scattered, pale olivaceous with clearly visible septa and canals, septa thin and not banded…….……*C. pallidum*
3b. Conidia with a single, double, or 4–5 column(s) of longitudinal septa, pale brown to dark brown, septa usually thick and darkly banded, canals obscured or not readily visible……...……..……4
4a. Conidia with a single column of longitudinal septa……..……...……5
4b. Conidia with two or more columns of longitudinal septa……..……..……6
5a. Conidia 24–51 × 15–29 × (8–)10–16 µm, with 3–6(–7) rows of transverse septa…….…….. *C. caribense*
5b. Conidia 18–25 × 13–15 × 5–8 µm, with 2–3(–4) rows of transverse septa…….. *C. exiguum*
6a. Conidia regularly with 2 columns of longitudinal septa……7

6b. Conidia irregularly with 4–5 columns of longitudinal septa……8

7a. Conidia with 2–4 rows, 1 cell at the apex, 22–35 × 15–23 × 10–10.5 μm, 2.5–5 wide……..…….. *C. variabile*

7b. Conidia with 2–4 rows, 1–4 cells at the apex, 25–33 × 20–28 × 7.5–11.5 μm, up to 25 μm long and 1.5–2 μm wide ……..……..…..…… *C. jinghongenses*

8a. Conidia with 3–9 rows, 1–3 cells at the apex, 25–63 × (16–)20–32 × 12–17 μm……..… *C. pulchrum*

8b. Conidia with 5–8 rows, 1–5 cells at the apex, 32–58 × 25–38 × 10–13 μm…….…..…… *C. elegans*

8c. Conidia with 4–6 rows, 3–4 cells at the apex, 27.5–37.5 × 24–27.5 × 17.5–22.5 μm……...…… *C. grenadoidia*

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