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A stable phylogeny for Dactylosporaceae

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# A stable phylogeny for Dactylosporaceae

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## ABSTRACT

The apothecial ascomycete family Dactylosporaceae includes saprobes and lichenicolous fungi. In recent studies, the phylogenetic position of this family was unstable within the subphylum Pezizomycotina. The present study provides a stable phylogenetic placement for Dactylosporaceae within the class Eurotiomycetes and we introduce the new order: Dactylosporales Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, ord. nov. to accommodate this family. We also introduce two new species: *Dactylospora chiangraiensis* Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov. and *Dactylospora fusiformis* Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov. to this family and their relationships with other taxa are represented in a multigene phylogeny.

## RÉSUMÉ

*Une phylogénie stable pour les Dactylosporaceae.*

La famille des ascomycètes apothéciaires, les Dactylosporaceae, comprend des saprobes et des champignons lichénicoles. Dans des études récentes, la position phylogénétique de cette famille était instable dans le sous-phylum Pezizomycotina. La présente étude fournit un placement phylogénétique stable pour les Dactylosporaceae dans la classe des Eurotiomycètes. Nous décrivons un nouvel ordre : les Dactylosporales Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, ord. nov. pour accueillir cette famille. Nous introduisons également deux nouvelles espèces : *Dactylospora chiangraiensis* Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov. et *Dactylospora fusiformis* Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov. dans cette famille, et leurs relations avec d'autres taxons sont représentées dans une phylogénie multigénique.

**KEY WORDS**  
Apothecial ascomycetes,  
Eurotiomycetes,  
Pezizomycotina  
*incertae sedis*,  
polyphyletic,  
new order,  
new species.

**MOTS CLÉS**  
Ascomycètes  
apothéciaux,  
Eurotiomycètes,  
Pezizomycotina  
*incertae sedis*,  
polyphylétique,  
ordre nouveau,  
espèces nouvelles.

## INTRODUCTION

The family Dactylosporaceae was established by Bellemère & Hafellner (1982). Species of this family are mostly saprotrophic on bark, wood and liverworts and sometimes are lichenicolous (Pang *et al.* 2014; Jaklitsch *et al.* 2016). Currently, this family includes a single genus *Dactylospora* which has around 70 species (Jaklitsch *et al.* 2016; Index fungorum 2018). However, Pang *et al.* (2014) and Diederich (2015) suggested a close phylogenetic relationship of the asexual genus *Sclerococcum* with *Dactylospora*.

Various placements have been assigned for this family based on different molecular studies. This family was originally placed within Lecanoromycetes based on its morphology. Schoch *et al.* (2009), Diederich *et al.* (2013) and Pang *et al.* (2014) suggested its position should be within Eurotiomycetes. Miadlikowska *et al.* (2014) and Pino-Bodas *et al.* (2017) provide a phylogenetic analysis, suggesting the phylogenetic position for Dactylosporaceae should be in Lecanoromycetes. However, according to Jaklitsch *et al.* (2016) this family should be referred to the Pezizomycotina *incertae sedis*, while Wijayawardene *et al.* (2018) placed this family within Eurotiomycetes *incertae sedis*. Thus, the phylogenetic status of the family is presently unresolved.

The present study provides a multi-gene phylogenetic tree (ITS, LSU, SSU, TEF, RPB1 and RPB2) for Pezizomycotina, a stable phylogenetic position for the family Dactylosporaceae and introduces a new order: Dactylosporales Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, ord. nov. within the class Eurotiomycetes. Moreover, we introduce two new species to this family from Thailand and compare their morphology with similar taxa. Phylogenetic analysis inferred from LSU and ITS gene regions for Eurotiomycetes are provided to show the phylogenetic relationship of the new taxa.

## MATERIAL AND METHODS

### SAMPLE COLLECTION, SPECIMEN EXAMINATION AND DEPOSITION

*Dactylospora* specimens were collected from Chiang Rai Province, Thailand. A Motic SMZ-168 stereo microscope was used to observe the apothecial structures. Thin hand sections of apothecia were made with a razor blade and mounted in water. A Nikon ECLIPSE 80i compound microscope was used to observe the microscopic characters. Photomicrography was carried out with a Canon 450D digital camera fitted to the microscope. Measurements of paraphyses, ascii and ascospores were made from material mounted in water and the mean values were used in the descriptions. Measurements were made with the Taro soft (R) Image Frame Work v. 0.9.7 program and images used for figures were processed with Adobe Photoshop CS6 software (Adobe Systems Inc.). The type specimens are deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand and in the Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS). Facesoffungi

and Index Fungorum numbers are registered as described in Jayasiri *et al.* (2015) and Index Fungorum (2018).

### DNA EXTRACTION, PCR AND SEQUENCING

Genomic DNA was extracted directly from the apothecia using a Plant DNA Rapid Extraction Kit (Bio Teke corporation, Beijing, China). Polymerase chain reactions (PCR) for this study were carried out for the internal transcribed spacer (ITS), using ITS4 and ITS5 (White *et al.* 1990) primers and for the nuclear ribosomal large subunit (LSU) using LROR and LR5 (Vilgalys & Hester 1990) primers. The PCR mixtures (25 µL) contained ddH<sub>2</sub>O (11 µL), PCR Master Mix (QinKe Co., China) (11 µL; 2x), DNA template (1 µL), each primer (1 µL; 10 µM). PCR amplification conditions were consisted an initial denaturation step of 5 min at 94°C, 35 cycles consisted of denaturation at 94°C for 1 minute, annealing at 53°C for 50 seconds and elongation at 72°C for 3 minute and final extension step of 7 minutes at 72°C for all gene regions. The PCR products were viewed on 1% agarose electrophoresis gels, stained with ethidium bromide. PCR products were sent to a commercial sequencing provider, Qinke in Kunming, China.

### SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSIS

Newly generated sequences in this study were subjected to a standard BLAST search of GenBank for rough identification and are deposited in GenBank (Appendix 1).

Data set for phylogenetic analysis I includes sequences belonging to 5.8s, LSU and SSU gene regions from representative Pezizomycotina species and the out-group taxon *Taphrina deformans* (Berk.) Tul. and *Taphrina antarctica* Selbmann & Turchetti, were downloaded from GenBank (Appendix 1). Data set for phylogenetic analysis II includes sequences belonging to ITS, LSU, SSU, TEF, RPB1 and RPB2 gene regions from representative Pezizomycotina species and the out-group taxon *Taphrina deformans* (Berk.) Tul. and *Taphrina antarctica* Selbmann & Turchetti, were downloaded from GenBank (Appendix 1). Data set for phylogenetic analysis III includes sequences belonging to ITS, LSU and SSU gene regions from representative Eurotiomycetes species and the out-group taxon *Teloschistes flavicans* (Sw.) Norman, were downloaded from GenBank (Appendix 2).

For both phylogenetic analyses, the consensus sequences for each gene were aligned using MAFFT v. 6.864b (<http://mafft.cbrc.jp/alignment/server/> last consultation on 10 April 2019). The alignment was improved manually where necessary using Bioedit (Hall 2004). Ambiguously aligned regions were excluded and gaps were treated as missing data. The individual datasets were concatenated into a combined dataset using FaBox (1.41) (Villesen 2007). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Maximum likelihood phylogenetic analyses were performed in the CIPRES web portal (Miller *et al.* 2010) using RAxML-HPC2 Workflow on XSEDE (8.2.9) tool. The bootstrap analysis for each ML tree was performed with 1000 thorough bootstrap replicates with the same parameter settings using the GTR+I+G substitution model. The resultant trees were viewed

with FigTree v.1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>) last consultation on 10 April 2019). Posterior probabilities (PP) (Rannala & Yang 1996; Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Ambiguously aligned regions were excluded from the analysis; gaps were treated as missing data. Four simultaneous Markov chains were run for 5,000,000 generations for Phylogenetic analysis II and III and trees were sampled every 100th generation. MCMC heated chain was set with a “temperature” value of 0.15. The distribution of loglikelihood scores was examined to determine stationary phase for each search and to decide if extra runs were required to achieve convergence, using the program Tracer 1.5 (Rambaut & Drummond 2009). All sampled topologies beneath the asymptote (20%) were discarded as part of a burn-in procedure, while the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree. The resultant trees were viewed with FigTree v.1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>). Maximum Likelihood (ML) bootstrap values equal or greater than 60% are given as the first set of numbers above the nodes and Bayesian Posterior Probabilities (BYPP) equal or greater than 0.90 are given as the second set of numbers above the nodes (Figs 1–3).

## RESULTS

### PHYLOGENETIC ANALYSES

In this study, the phylogenetic relationships of the family Dactylosporaceae within Pezizomycotina was investigated in the phylogenetic analysis I and II (Figs 1; 2). Phylogenetic analysis I based on 5.8s, LSU and SSU and the alignment of combined genes included 6656bp (LSU-1-1003, 5.8s-1004-1174, SSU-1175-2381). The analysis II based on LSU, ITS, SSU, TEF, RPB1 and RPB2 sequence data and the alignment of combined genes included 6656bp (LSU-1-1003, ITS-1004-1962, SSU-1963-2618, TEF-2619-3518, RPB1-3519-4565, RPB2-4566-6656). According to the results of the phylogenetic analysis I and II, the family Dactylosporaceae grouped within Eurotiomycetes (Figs 1; 2). Phylogenetic analysis III shows the phylogenetic relationships of the family Dactylosporaceae within Eurotiomycetes based on analysis of LSU and ITS sequence data (Fig. 3). The alignment of combined genes included 1740bp (LSU-1-944, ITS-945-1740). The topology of the trees from maximum likelihood analysis was similar to the tree from Bayesian analysis.

## TAXONOMY

### DACTYLOSPORALES Ekanayaka,

E.B.G. Jones, Q. Zhao & K.D. Hyde, ord. nov.

INDEX FUNGORUM NUMBER. — IF555303.

FACESOFFUNGI NUMBER. — FoF 04851.

TYPE GENUS. — *Dactylospora* Körb.

### DESCRIPTION

Saprobic or lichenicolous.

#### *Sexual morph*

Apothecial, superficial, sessile to stipitate.

**Hamathecium.** Cupulate.

**Hymenium.** Embedded in a thick gelatinous matrix.

**Paraphyses.** Sparingly branched, septate, apices slightly swollen.

**Asci.** Cylindrical to clavate, with an outer gelatinized layer, mostly amyloid.

**Ascospores.** Mostly 8 per ascus, septate, subglobose to broadly fusiform or ellipsoid, brown to greenish, non-amyloid.

#### *Asexual morph*

Sporodochial in the host thallus, conidia dark, consisting of two to several fused, subglobose cells.

### REMARK

Our phylogenetic study suggested the phylogenetic position for Dactylosporaceae within Eurotiomycetes and here we introduce the new order Dactylosporales Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, ord. nov. to accommodate it.

Family DACTYLOSPORACEAE Bellem. & Hafellner

*In* Cryptog. Mycol. 3: 79 (1982).

FACESOFFUNGI NUMBER. — FoF 04852

GENERA INCLUDED. — *Dactylospora*, possibly also *Sclerococcum*

### DESCRIPTION

Dactylosporaceae taxa are saprotrophic on bark and wood, liverworts or lichenicolous. This family includes two genera: *Dactylospora* and *Sclerococcum*. *Dactylospora* is a sexual genus and characterized by superficial to stalked blackish apothecia, excipulum composed of *textura angularis* to *globulosa* cells, hymenium consists of a thick gelatinous matrix, sparingly branched paraphyses apices slightly swollen and pigmented, cylindrical to clavate amyloid ascii with I– tholus covered by an I+ blue external gelatinous cap, subglobose to ellipsoid and one to several transverse septate ascospores, mostly 8 per ascus (Hafellner 1979, Bellemère & Hafellner 1982; Döbbeler & Buck 2017). *Sclerococcum* is an asexual genus and characterized by sporodochial conidiomata (Diederich *et al.* 2013, Miadlikowska *et al.* 2014). In this paper, we introduce two new species of *Dactylospora*.



Fig. 1. — Phylogram generated from maximum likelihood analysis of sequences of Pezizomycotina including *Dactylospora* based on 5.8s, LSU and SSU sequence data. Maximum likelihood bootstrap values  $\geq 60\%$  are given above the nodes. Strain/culture numbers are given after the taxon names. The tree was rooted with *Taphrina antarctica* Selbmann & Turchetti (CCFEE 5198) and *Taphrina deformans* (Berk.) Tul. (AFTOL ID 1234).

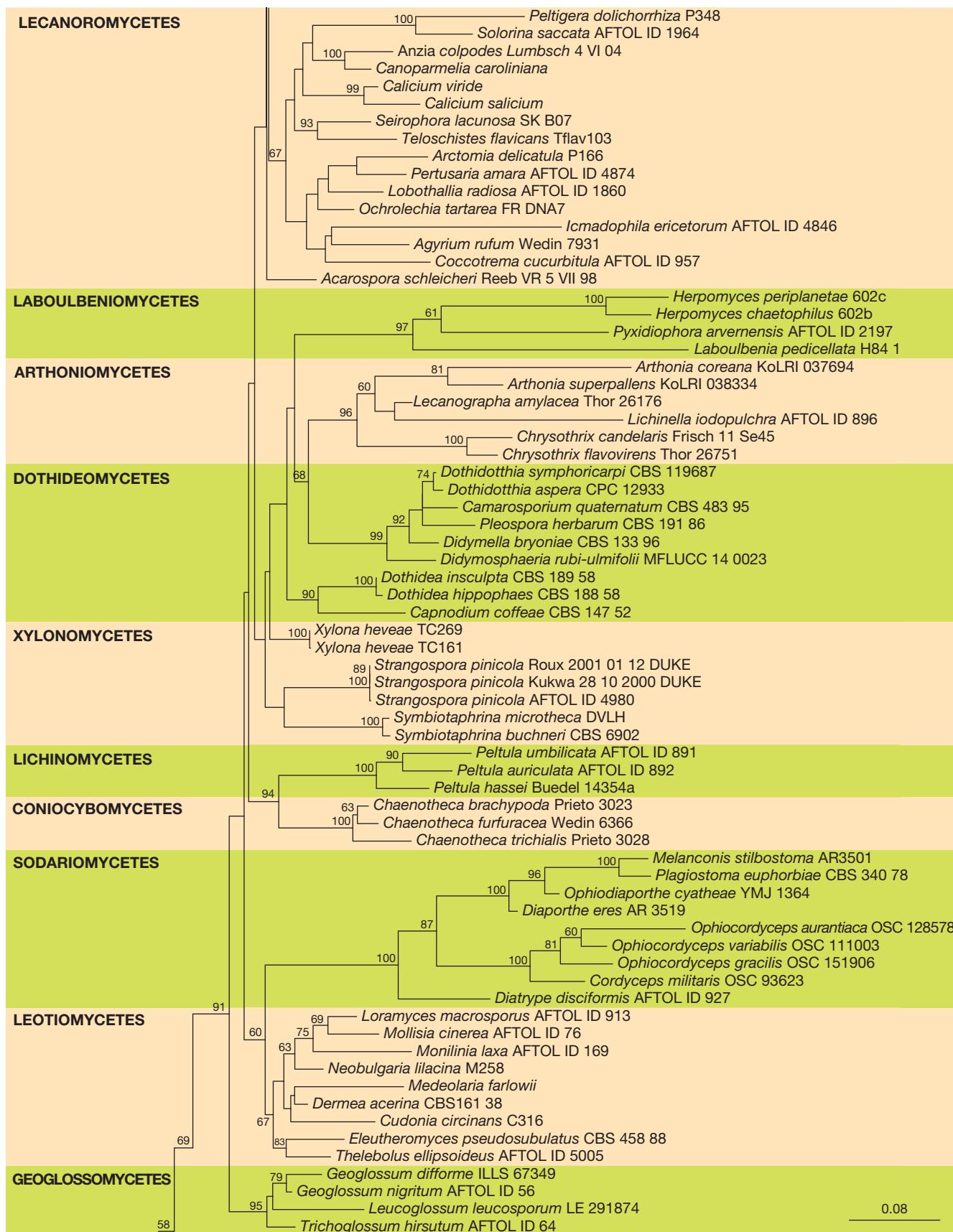


FIG. 1. — Continuation.

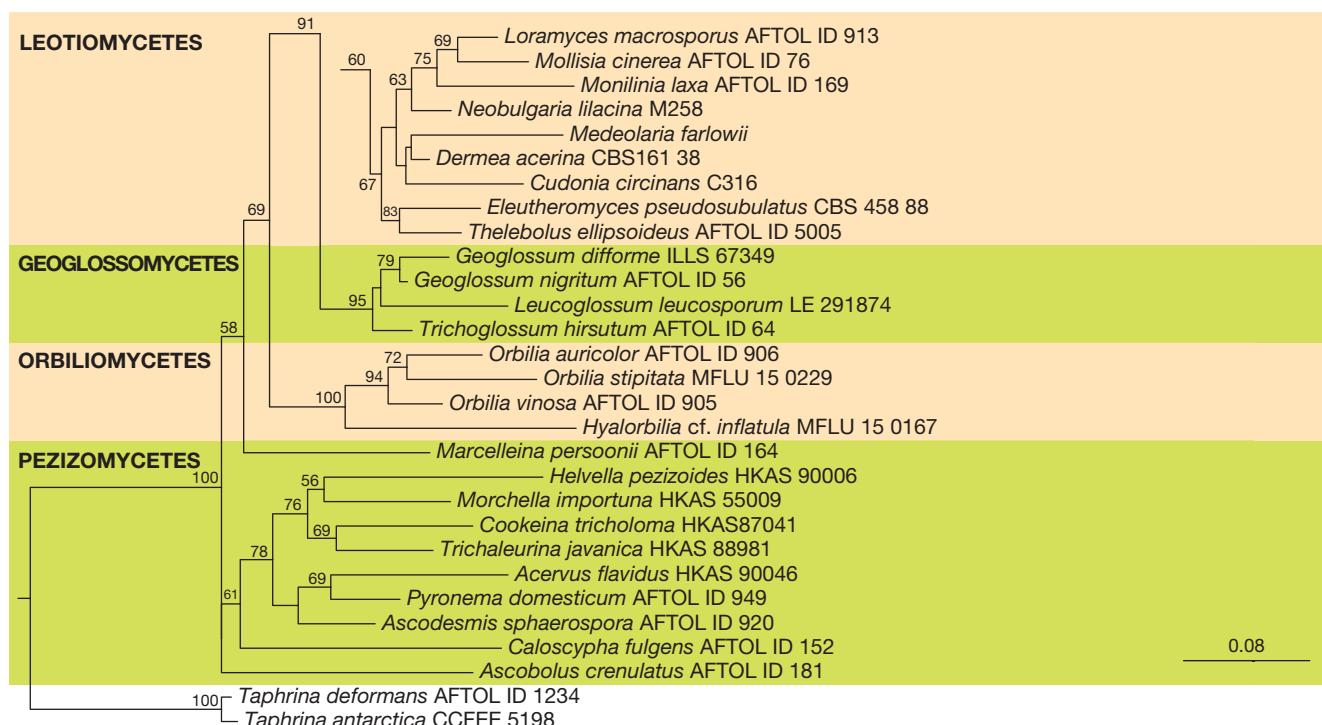


FIG. 1. — Continuation.

Genus *Dactylospora* Körb.

*Dactylospora chiangraiensis* Ekanayaka,  
E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov.  
(Fig. 4)

Black apothecia with maroon to brown margins, ectal excipulum of *textura angularis*, paraphyses with slightly swollen apices, unitunicate, cylindrical, short pedicellate, inoperculate asci with an amyloid gelatinous cap and smooth one-septate ascospores.

INDEX FUNGORUM NUMBER. — IF555304.

FACESOFFUNGI NUMBER. — FoF 04853.

HOLOTYPE. — MFLU 16-0570.

TYPE LOCALITY. — Thailand

ETYMOLOGY. — With reference to the province where the holotype was collected.

MATERIAL EXAMINED. — Thailand. Tham Pla Temple M.14 (Bann Tham Pla Temple), Pongpha, Mae Sai District, Chiang Rai Province, 25th November 2014, A. H. Ekanayaka (HD 003) (holo-, MFLU [MFLU 16-0570]; iso-, HKAS). Sequence data: ITS-MH718440, LSU-MH718433

## DESCRIPTION

Saprobic on unidentified wood, stems and twigs.

## Sexual morph

Apothecia. 450-500 × 1000-1500 µm ( $\bar{x} = 469.7 \times 1242.5$  µm, n = 10) superficial, sessile, arising singly or in small groups.

**Hamathecium.** Cupulate, outer surface black.

**Margins.** Raised, maroon to brown, rough surface.

**Disc.** Flat to slightly concave, black, rough granulated surface.

**Ectal excipulum.** 55-65 µm wide at margins, comprising cells of *textura angularis*, outer cell layer is dark brown to light brown, inner cells are hyaline to brownish.

**Medullary excipulum.** 15-25 µm wide at margins, composed of brown intertwined hyphae, embedded in a gelatinous matrix.

**Pseudo epithecium.** Composed of hyaline to brown amorphous matter surrounding the paraphysis tips.

**Paraphyses.** 1.8-2.3 µm ( $\bar{x} = 2.1$  µm, n = 10) wide at the middle, 3.5-5 µm ( $\bar{x} = 4.3$  µm, n = 10) wide at the tips, numerous, filamentous, septate, branched, slightly swollen and branched at the apices extending beyond the asci and apices are glued together to form the epithecium.

**Asci.** 65-75 × 12-15 µm ( $\bar{x} = 69.7 \times 13.6$  µm, n = 30), 8-spored, unitunicate, cylindrical, short pedicellate, inoperculate with an amyloid gelatinous cap.

**Ascospore.** 12-15 × 4-4.5 µm ( $\bar{x} = 14.2 \times 4.3$  µm, n = 40), ellipsoid to fusoid, hyaline at immature stage and brown at maturity, one-septate, smooth walled, guttulate.

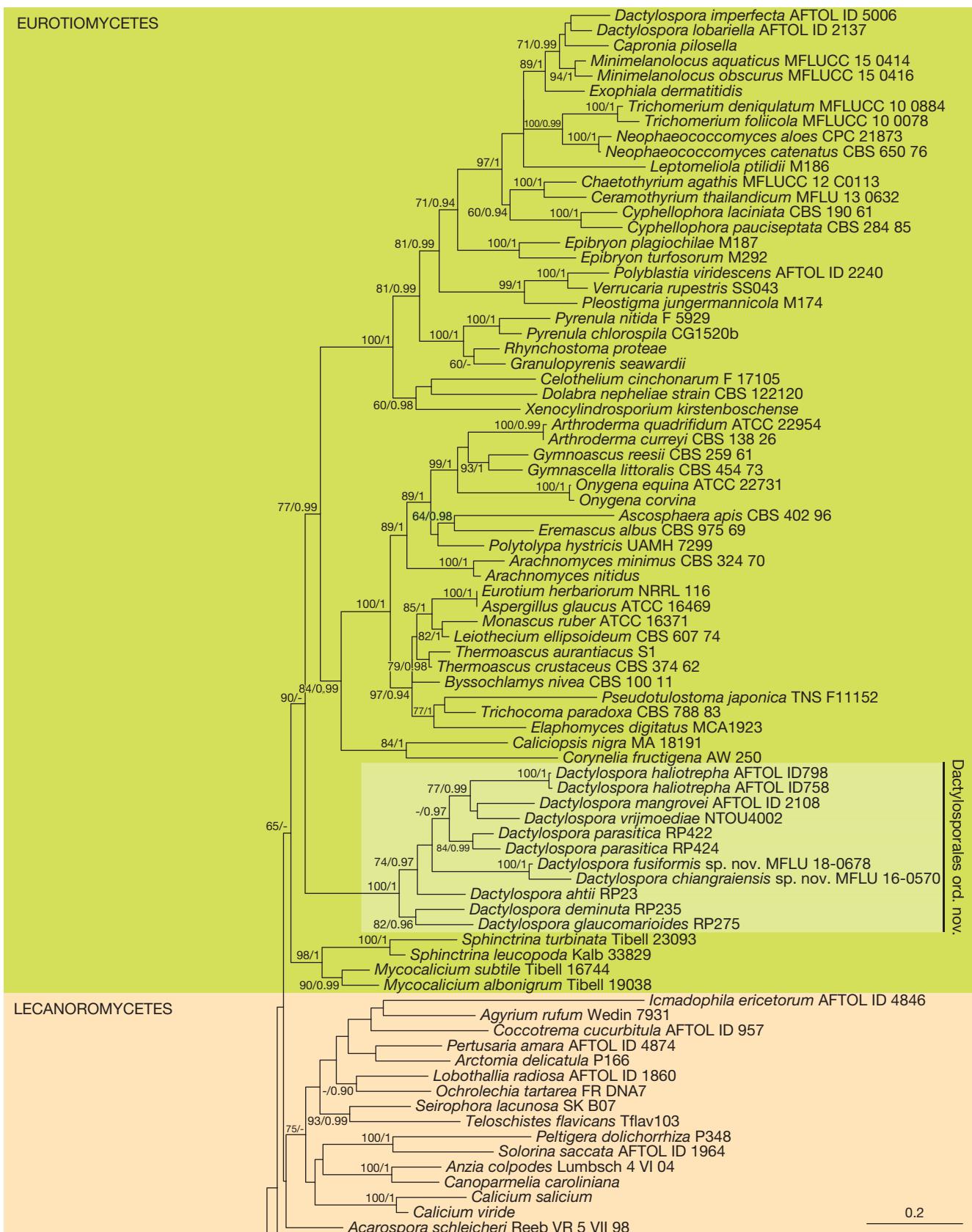


Fig. 2. — Phylogram generated from maximum likelihood analysis of sequences of Pezizomycotina including *Dactylospora* based on ITS, LSU, SSU, TEF, RPB1 and RPB2 sequence data. Maximum likelihood bootstrap values  $\geq 60\%$  and Bayesian Posterior Probabilities  $\geq 0.90$  are given above the nodes. Strain/culture numbers are given after the taxon names. The tree was rooted with *Taphrina antarctica* (CCFEE 5198) and *Taphrina deformans* (AFTOL ID 1234).

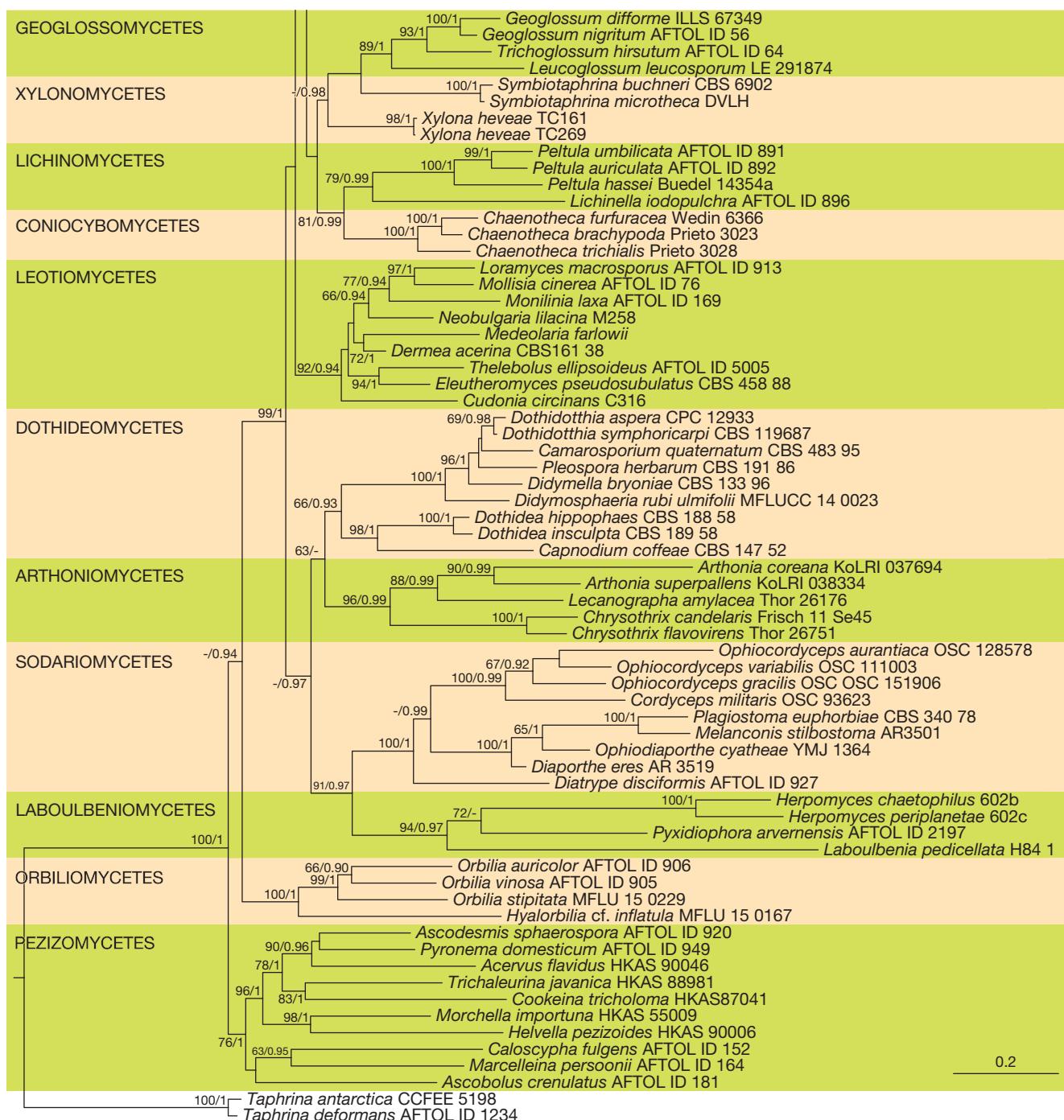


FIG. 2. — Continuation.

*Asexual morph*  
Undetermined.

#### REMARKS

*Dactylospora chiangraiensis* sp. nov. is characterized by black apothecia with maroon to brown margins, paraphyses with slightly swollen apices and smooth one-septate ascospores. Our species is phylogenetically close to *D. stygia* (Berk. & M.A. Curtis) Hafellner. However,

*D. chiangraiensis* sp. nov. differs from *D. stygia* by having maroon to brown raised margins with rough granulated surface in disc, margins and outer surface of apothecia (Berkeley 1875; Baral & Marson 2005).

*Dactylospora parellaria* (Nyl.) Arnold, 1877, *D. borealis* Holien & Ihlen, 2004, *D. rhyparizae* Arnold, 1874, *D. parasitica* (Flörke) Arnold, 1887, *D. attendenda* (Nyl.) Arnold, 1895, *D. diminuta* (Th. Fr.) Triebel, 1989, *D. urceolata* (Th. Fr.) Arnold, 1874, *D. frigida* Hafellner,

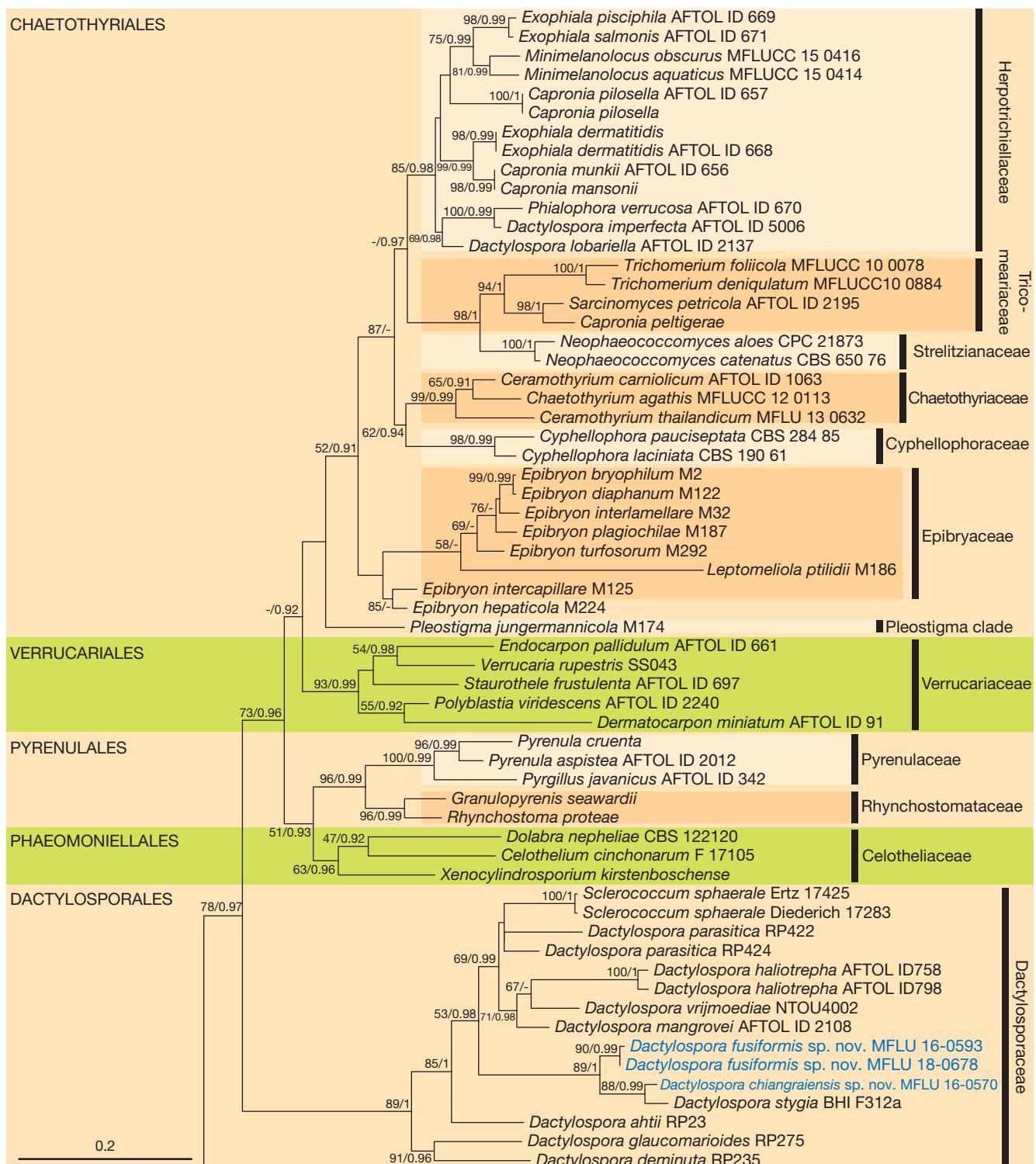


Fig. 3. — Phylogram generated from maximum likelihood analysis of sequences of Eurotiomycetes including *Dactylospora* based on ITS and LSU sequence data. Maximum likelihood bootstrap values  $\geq 60\%$  and Bayesian Posterior Probabilities  $\geq 0.90$  are given above the nodes. Strain/culture numbers are given after the taxon names. The tree was rooted with *Teloschistes flavicans* (Sw.) Norman Tflav103.

1985, *D. suburceolata* Coppins & Fryday, 2012, *D. imperfecta* (Ellis) Hafellner, 1979 and *D. pertusariicola* (Willey ex Tuck.) Hafellner, 1979 differ from *D. chiangraiensis* sp. nov. by having ascospores with more than 1 septa (Ihlen *et al.* 2004). *Dactylospora rimulicola* (Müll. Arg.)

Hafellner, 1979, *D. ahtii* Zhurb. & Pino-Bodas, 2017, *D. saxatilis* (Schaer.) Hafellner, 1979, *D. homoclinella* (Nyl.) Hafellner, 1979, *D. australis* Triebel & Hertel, 1989 and *D. amygdalariae* Triebel, 1989 differ from *D. chiangraiensis* sp. nov. by having stipitate apothecia

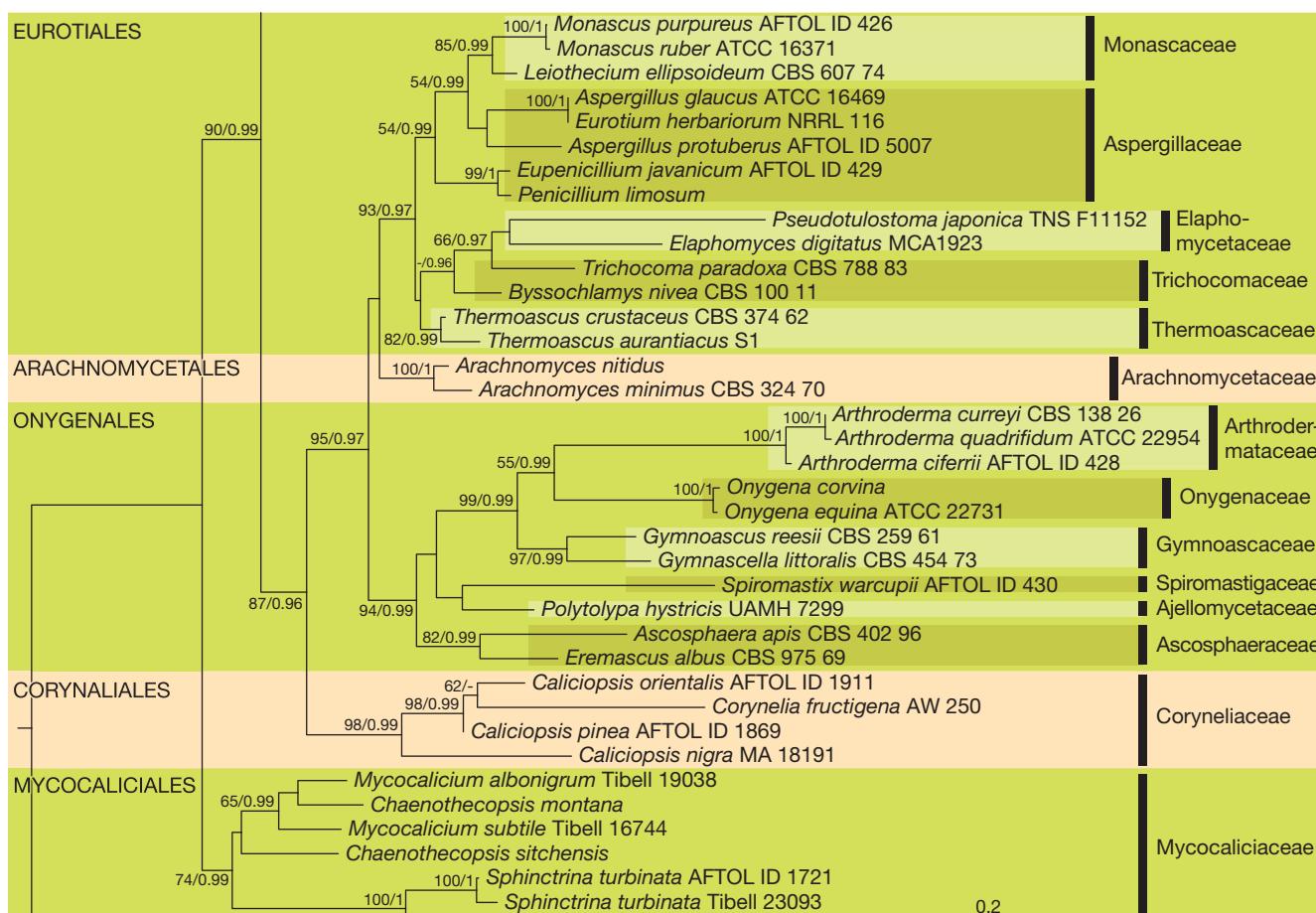


FIG. 3. — Continuation.

(Ihlen *et al.* 2004). *Dactylospora purpurascens* Triebel, 1989 and *D. athallina* (Müll. Arg.) Hafellner, 1979 differ by having an epiphymenium with K+ purple reaction (Ihlen *et al.* 2004). *Dactylospora lobariella* (Nyl.) Hafellner, 1979 and *D. protothallina* (Anzi) Hafellner, 1979 differ by having a lichenized lifestyle (Ihlen *et al.* 2004). *Dactylospora inopina* Döbbeler & W.R. Buck, 2017 differs by having polyporous ascospores (Döbbeler & Buck 2017). *Dactylospora aeruginosa* Holien & Ihlen, 2004 differs by having an epiphymenium with patches of violet-blue pigment (Ihlen *et al.* 2004). *Dactylospora microspore* Etayo, 1991 differs from *D. chiangraiensis* sp. nov. by having smaller ascospores (4–7 × 2–3 µm) (Ihlen *et al.* 2004; Joshi *et al.* 2010). *Dactylospora vrijmoediae* K.L. Pang, Sheng Y. Guo, Alias, Hafellner & E.B.G. Jones, 2014 and *D. canariensis* Kohlm. & Volkm.-Kohlm., 1998 differs from *D. chiangraiensis* sp. nov. by having ascospore appendages (Jones *et al.* 1999; Pang *et al.* 2014). *Dactylospora mangrovei* E.B.G. Jones, Alias, Abdel-Wahab & S.Y. Hsieh, 1999 and *D. haliotrepha* (Kohlm. & E. Kohlm.) Hafellner, 1979 differ from *D. chiangraiensis* sp. nov. by having ascospore wall ornamentation (Au *et al.* 1996; Jones *et al.* 1999).

*Dactylospora fusiformis* Ekanayaka,  
E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov.  
(Fig. 5)

Black pulvinate apothecia, ectal excipulum of textura angularis to globulosa, paraphyses with slightly swollen apices, unitunicate, short sessile, cylindric-clavate asci and smooth one-septate ascospores.

HOLOTYPE. — MFLU 16-0593.

ETYMOLOGY. — With reference to the ascospore shape.

INDEX FUNGORUM NUMBER. — IF555305.

FACESOFFUNGI NUMBER: FOF 04854.

TYPE LOCALITY. — Thailand.

MATERIAL EXAMINED. — Thailand. Mae Fah Luang University, Chiang Rai Province, on dead stems, 20 XII 2015, A.H. Ekanayaka, (HD0047) (holo-, MFLU [MFLU 16-0593]; iso-, HKAS). Sequence data: ITS-MH718441, LSU-MH718434, Thailand, Mae Fah Luang University, Chiang Rai Province, on dead stems, 11 VII 2016, A.H. Ekanayaka, (HD0061), MFLU 18-0678. Sequence data: ITS-MH718442.

DESCRIPTION

Saprobic on dead stems.

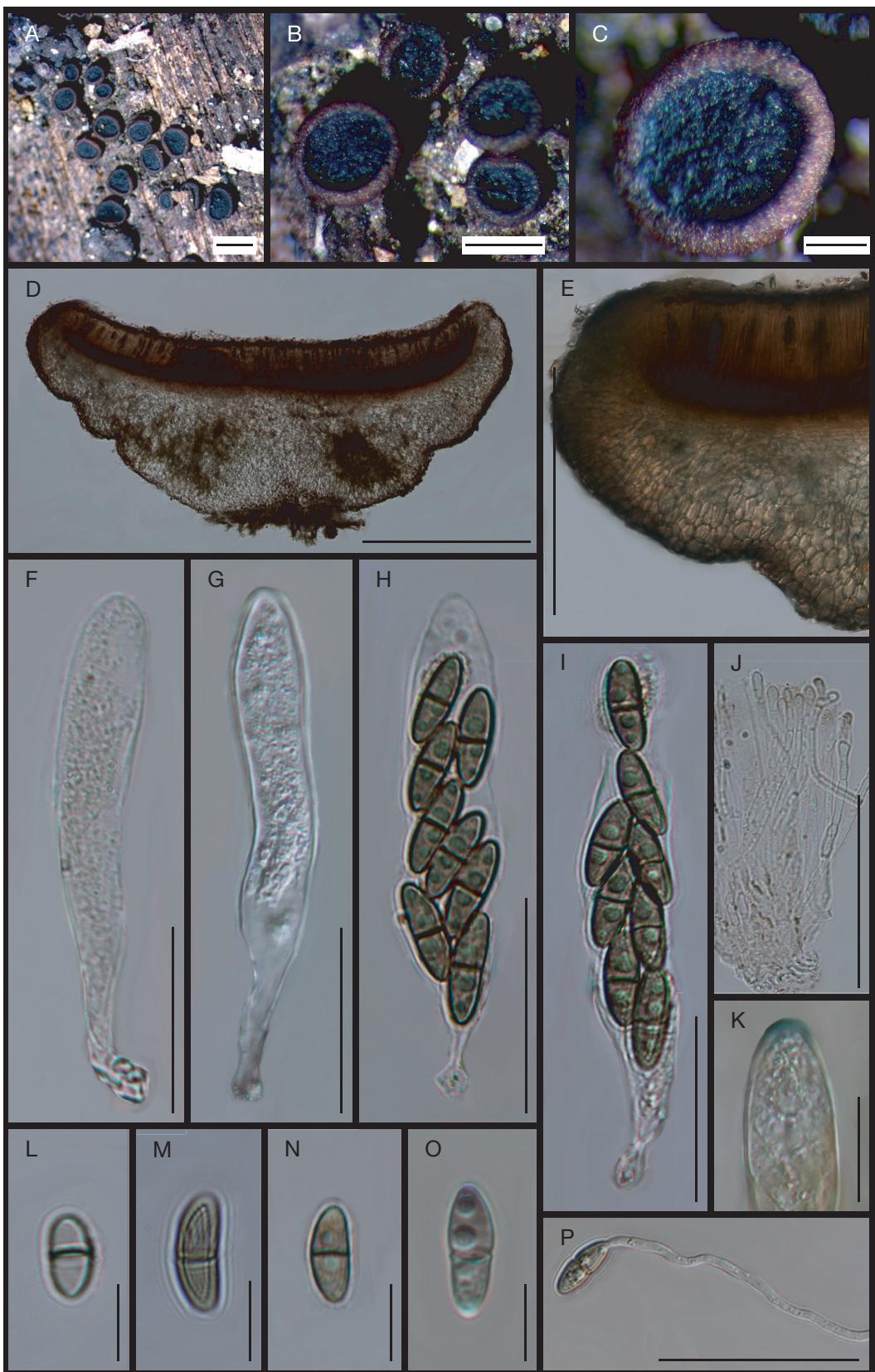


FIG. 4. — Morphology of *Dactylospora chiangraiensis* Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov. (Holotype MFLU 16-0570): **A**, **B**, ascocarps in wood; **C**, ascus in wood; **D**, cross section of a ascoma; **E**, peridium of ascoma; **F-I**, asci in water; **J**, paraphyses in water; **K**, ascus apex with gelatinous amyloid cap (in Melzer's reagent); **L-O**, ascospores in water; **P**, germinated ascospore. Scale bars: A, 1000 µm; B, 500 µm; C, D, 200 µm; E, 100 µm; F-I, 25 µm; J, P, 50 µm; K-O, 10 µm.

### Sexual morph

**Apothecia.** 400-1200 × 200-255 µm ( $\bar{x} = 968 \times 223$  µm, n = 10) superficial, arising singly or in small groups, sessile.

**Hamathecium.** Pulvinate, outer surface black.

**Margins.** Not clearly differentiate from the disc, concolorous to receptacle.

**Disc.** Flat to slightly convex, black, smooth or finely granulated surface.

**Ectal excipulum.** 25-40 µm ( $\bar{x} = 35.3$  µm, n = 10) wide at lower flanks, composed of large, thin-walled, dark brown cells of *textura angularis* to *globulosa*, outer cell layer is brown, inner cells are hyaline to brownish.

**Medullary excipulum.** 10-15 µm ( $\bar{x} = 13$  µm, n = 10) wide at lower flanks, composed of hyaline intertwined hyphae, embedded in a gelatinous matrix.

**Hymenium.** Hyaline to brownish, enclosed in a thick gelatinous matrix.

**Pseudo epitheciun.** Composed of hyaline to brown amorphous matter surrounding the paraphysis tips.

**Paraphyses.** 1.4-2 µm wide ( $\bar{x} = 2.7$  µm, n = 20) at the middle, 2.5-3.5 µm wide ( $\bar{x} = 3.2$  µm, n = 20) at the tips, numerous, filiform, septate, swollen and slightly branched at the apex and pigmented, extending beyond the ascii and apices glued together to develop pseudo epitheciun.

**Asci.** 70-80 × 12-18 µm ( $\bar{x} = 73.03 \times 14.5$  µm, n = 30) 8-spored, unitunicate, short sessile, cylindric-clavate, rounded at the apex, inoperculate with an amyloid gelatinous cap.

**Ascospore.** 17-21 × 4-7 µm ( $\bar{x} = 19.1 \times 5.7$  µm, n = 40), multiseriate, long ellipsoid to fusoid, immature spores are non-septate, hyaline and mature spores are one-septate, greenish brown, guttulate, smooth, thin walled.

### REMARKS

*Dactylospora fusiformis* is characterized by black pulvinate apothecia, paraphyses with slightly swollen apices and smooth one-septate ascospores. *Dactylospora fusiformis* is phylogenetically close to *D. chiangraiensis* sp. nov. and *D. stygia*. However, *D. chiangraiensis* sp. nov. and *D. stygia* differ from *D. fusiformis* by having apothecia with raised margins and shorter ascospores (Berkeley 1875, Baral & Marson 2005).

*Dactylospora fusiformis* differs from *D. rimulicola*, *D. ahtii*, *D. saxatilis*, *D. homoclinella*, *D. rhyparizae*, *D. australis* *D. amygdalariae* by having sessile apothecia (Ihlen et al. 2004). *Dactylospora fusiformis* differs from *D. protothallina* by having a saprobic lifestyle (Ihlen et al. 2004). *Dactylospora fusiformis* differs from *D. aeruginosa* in lacking epiphymenial pigments (Ihlen et al. 2004). *Dactylospora parellaria*, *D. borealis*, *D. rhy-*

*parizae*, *D. parasitica*, *D. attendenda*, *D. deminuta*, *D. urceolate*, *D. frigida*, *D. suburceolata*, *D. imperfecta* and *D. pertusariicola* differ from *D. fusiformis* by having ascospores with more than 1 septum (Ihlen et al. 2004). *Dactylospora fusiformis* differs from *D. inopina* by having 8-spored ascii (Döbbeler & Buck 2017). *Dactylospora purpurascens* and *D. athallina* differ by having an epiphymenium with K+ purple reaction (Ihlen et al. 2004). *Dactylospora vrijeimoeidae*, *D. mangrovei*, *D. lobariella* and *D. canariensis* differ from *D. fusiformis* by having ornamented ascospore walls (Jones et al. 1999, Pang et al. 2014). *Dactylospora microspora* differs from *D. fusiformis* by having smaller ascospores (4-7 × 2-3 µm) (Ihlen et al. 2004, Joshi et al. 2010).

### DISCUSSION

Dactylosporaceae is a widely distributed family in both terrestrial and mangrove ecosystems (Schoch et al. 2009; Pang et al. 2014). This paper introduces new *Dactylospora* species from Thailand and a new order for the family Dactylosporaceae (Dactylosporales Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, ord. nov.). We provided 3 phylogenetic analyses here. Phylogenetic analyses I and II (Figs 1; 2) represent the subdivision Pezizomycotina from 2 different gene combinations to provide more resolved phylogeny for the family Dactylosporaceae. Phylogenetic analysis I based on LSU, 5.8s and SSU gene regions while phylogenetic analysis II obtained from ITS, LSU, SSU, TEF, RPB1 and RPB2. The phylogenetic analysis I and II shows that the family Dactylosporaceae belongs to Eurotiomycetes as suggested by Schoch et al. (2009) and Pang et al. (2014). The phylogenetic analysis II shows the stable phylogenetic position of Dactylosporaceae within a new order Dactylosporales (Eurotiomycetes) introduced here. Moreover, it shows the phylogenetic relationships of the new species, *Dactylospora chiangraiensis* sp. nov. and *Dactylospora fusiformis* with other taxa of the family.

Moreover, Miadlikowska et al. (2014) showed the placement of family Dactylosporaceae within Lecanoromycetes and its close relationship with the genus *Strangospora*. However in our analysis I, we observed that the genus *Strangospora* is genetically related to Xylonomycetes.

Furthermore, Schoch et al. (2009) and Pang et al. (2014) found that the genus *Dactylospora* is polyphyletic. Similarly, in our phylogenetic analyses we found 2 clades that included *Dactylospora* species: *Dactylospora imperfecta* (Ellis) Hafellner and *Dactylospora lobariella* (Nyl.) Hafellner claded within Chaetothyriales, close to *Capronia*. However, there are no apothecial ascomycetes known within Chaetothyriales (Ekanayaka et al. 2017). Therefore more fresh collections and re-sequencing of samples are required to resolve the exact phylogenetic position for both *Dactylospora* species. Moreover both *Dactylospora* species may require the introduction of a new genus in the near future.

*Dactylospora* species are recorded from terrestrial, fresh water and marine habitats. Marine *Dactylospora* spp. include *D. vrijeimoeidae*, *D. haliotrepha*, *D. mangrovei* and *D. canariensis* while

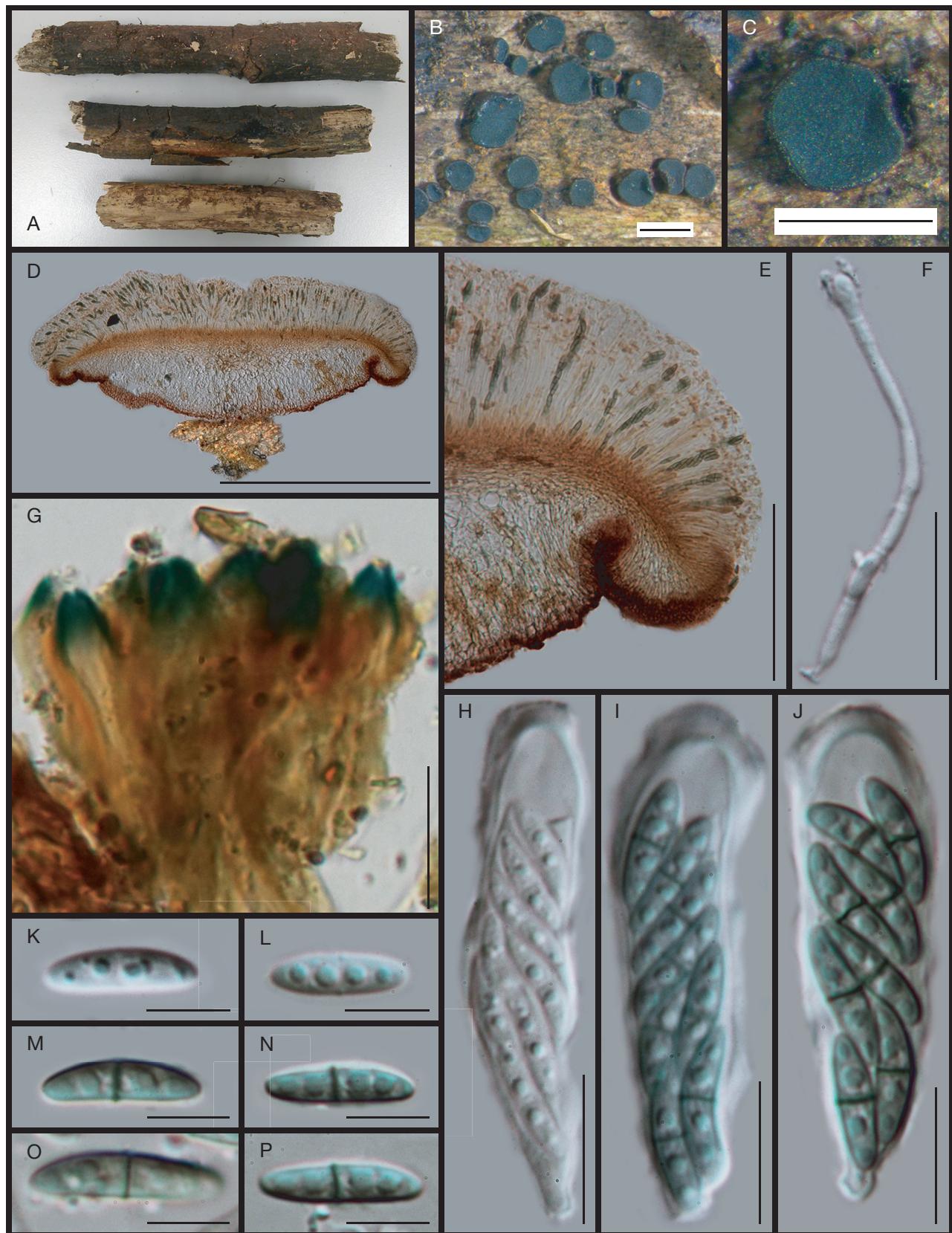


FIG. 5. — Morphology of *Dactylospora fusiformis* Ekanayaka, E.B.G. Jones, Q. Zhao & K.D. Hyde, sp. nov. (Holotype MFLU 16-0593): **A**, substrate; **B**, ascocarps on wood; **C**, ascocarp on wood; **D**, cross section of an ascoma; **E**, close up of a vertical section of the ascoma at margin; **F**, apically swollen paraphyses; **G**, ascospores with gelatinous amyloid cap (in Melzer's reagent); **H–J**, short sessile asci; **K–P**, ellipsoid to fusoid ascospores. Scale bars: B, C, 1000 µm; D, 400 µm; E, 200 µm; F, 30 µm; G, J, 20 µm; K–N, 10 µm.

*D. borealis* was recorded near fresh water streams and coastal lowlands. According to the literature all those non-terrestrial species are morphologically similar by having ascospore wall ornamentations and/or ascospore appendages. *Dactylospora vrijmoediae* and *D. canariensis* have appendaged ascospores, *D. haliotrepha*, *D. mangrovei* and *D. borealis* have striate, verrucose and granulated ascospore walls, while other terrestrial *Dactylospora* have smooth ascospore walls (Kohlmeyer & Volkmann-Kohlmeyer 1998; Jones et al. 1999; Ihlen et al. 2004; Fryday & Coppins 2012; Pang et al. 2014). Many aquatic ascomycetes have ascospore walls with ornamentations or appendages and these are thought to be an adaptation for their aquatic life (Hyde & Jones 1989; Jones 1994, Shearer & Raja 2010). Moreover, according to the phylogenetic analyses II, three marine *Dactylospora* species form a monophyletic lineage, sister to the *Sclerococcum*-*D. parasitica* clade. Hence, we suggest these aquatic *Dactylospora* are a phylogenetic distinct group from the terrestrial *Dactylospora* species. Furthermore, they may have evolved from terrestrial *Dactylospora* ancestors and adapted to an aquatic life style (Shearer 1993; Jones 2006).

Currently there are two discomycete families within Eurotiomycetes viz. Mycocaliciaceae and Dactyloporaceae. Taxa of these two families are similar in having sessile to stipitate apothecia, sparingly branched paraphyses, cylindric clavate asci and ellipsoid to fusiform ascospores. However, Dactyloporaceae taxa differ by having amyloid asci (Schoch et al. 2009; Jaklitsch et al. 2016; Ekanayaka et al. 2017).

*Dactylospora* is a sexual genus and there are no available records of its asexual morph. The asexual genus *Sclerococcum* claded with *Dactylospora* but its phylogenetic limitations are still unclear. Moreover, the genera *Sclerococcum* and *Dactylospora* share similar habitats and ecological affinities (Schoch et al. 2009; Pang et al. 2014; Diederich 2015). By considering the phylogenetic and ecological similarities, we suggest that *Sclerococcum* could be the asexual morph of *Dactylospora*. However, further taxon sampling, culture studies and molecular data are required to resolve the generic relationship of these two genera.

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## APPENDICES

APPENDIX 1. — GenBank accession numbers of strains used in phylogenetic analysis I and II of this study. New sequence data in this study are in bold.

| Species name                          | Strain number  | ITS      | LSU       | SSU       | TEF      | RPB2     | RPB1     |
|---------------------------------------|----------------|----------|-----------|-----------|----------|----------|----------|
| <i>Hyalorbilia inflatula</i>          | MFLU 15-0167   | MG599270 | MG599273  | —         | —        | —        | —        |
| <i>Orbilia stipitata</i>              | MFLU 15-0229   | MG599272 | MG599275  | —         | —        | —        | —        |
| <i>Orbilia auricolor</i>              | AFTOL-ID 906   | DQ491512 | DQ470953  | DQ471001  | DQ471072 | DQ470903 |          |
| <i>Orbilia vinosa</i>                 | AFTOL-ID 905   | DQ491511 | DQ470952  | DQ471000  | DQ471071 |          | DQ471145 |
| <i>Geoglossum nigritum</i>            | AFTOL_ID 56    | DQ491490 | AY544650  | AY544694  | DQ471044 | DQ470879 | DQ471115 |
| <i>Trichoglossum hirsutum</i>         | AFTOL_ID 64    | DQ491494 | AY544653  | AY544697  | DQ471049 | DQ470881 | DQ471119 |
| <i>Leucoglossum leucosporum</i>       | LE 291874      | KP272114 | KP272115  | —         | KX898413 | —        | —        |
| <i>Geoglossum difforme</i>            | ILLS 67349     | KC222124 | KC222137  | —         | —        | —        | KC222163 |
| <i>Ascobolus crenulatus</i>           | AFTOL ID 181   | DQ491504 | AY544678  | AY544721  | DQ471061 | DQ470893 | DQ471132 |
| <i>Ascodesmis sphaerospora</i>        | AFTOL ID 920   | —        | FJ176858  | FJ176804  | FJ238391 | FJ238346 | —        |
| <i>Caloscypha fulgens</i>             | AFTOL ID 152   | DQ491483 | DQ247799  | DQ247807  | DQ471054 | DQ247787 | —        |
| <i>Marcelleina persoonii</i>          | AFTOL ID 164   | —        | DQ470943  | DQ470991  | DQ471055 | DQ470887 | DQ471127 |
| <i>Pyronema domesticum</i>            | AFTOL ID 949   | DQ491517 | NG_027655 | NG_013185 | DQ471093 | DQ247795 | —        |
| <i>Cookeina tricholoma</i>            | HKAS87041      | KY094618 | MG871317  | MG859239  | MG980688 | —        | —        |
| <i>Trichaleurina javanica</i>         | HKAS 88981     | MG871291 | MG871326  | MG859241  | MG980693 | MG980716 | —        |
| <i>Morchella importuna</i>            | HKAS 55009     | MG871294 | MG871328  | MG859242  | MG980694 | MG980719 | —        |
| <i>Helvella pezizoides</i>            | HKAS 90006     | MG871299 | MG871333  | —         | MG980697 | MG980721 | —        |
| <i>Acervus flavidus</i>               | HKAS 90046     | MG871293 | KX765259  | —         | KX765258 | MG980718 | —        |
| <i>Chaenotheca furfuracea</i>         | Wedin 6366     | JX000101 | JX000087  | JX000068  | —        | —        | JX000137 |
| <i>Chaenotheca brachypoda</i>         | Prieto 3023    | —        | JX000086  | —         | —        | —        | JX000135 |
| <i>Chaenotheca trichialis</i>         | Prieto 3028    | JX000102 | JX000085  | JX000069  | —        | —        | JX000136 |
| <i>Lichenella iodopulchra</i>         | AFTOL_ID 896   | DQ842016 | DQ782916  | —         | DQ832327 | DQ832328 | DQ782857 |
| <i>Peltula hassei</i>                 | Buedel 14354a  | MF766365 | MF766406  | MF766283  | —        | —        | —        |
| <i>Peltula umbilicata</i>             | AFTOL_ID 891   | DQ832333 | DQ832334  | DQ782887  | DQ782919 | DQ832335 | DQ782855 |
| <i>Peltula auriculata</i>             | AFTOL_ID 892   | DQ832329 | DQ832330  | DQ832332  | —        | DQ832331 | DQ782856 |
| <i>Arthonia superpallens</i>          | KoLRI 038334   | —        | KX913671  | —         | —        | —        | —        |
| <i>Arthonia coreana</i>               | KoLRI 037694   | —        | KX913670  | —         | —        | KX913668 | —        |
| <i>Chrysotrichia candelaris</i>       | Frisch 11/Se45 | —        | KF707640  | —         | —        | KF707663 | —        |
| <i>Chrysotrichia flavovirens</i>      | Thor 26751     | —        | KJ851030  | —         | —        | —        | —        |
| <i>Lecanographa amylacea</i>          | Thor 26176     | —        | KF707639  | —         | —        | KF707659 | —        |
| <i>Didymella bryoniae</i>             | CBS 133.96     | GU237780 | GU301863  | —         | —        | GU371767 | —        |
| <i>Didymosphaeria rubi-ulmifoliae</i> | MFLUCC 14-0023 | —        | KJ436586  | KJ436588  | —        | —        | —        |
| <i>Dothidea hippophaes</i>            | CBS 188.58     | —        | DQ678048  | U42475    | DQ677887 | DQ677942 | —        |
| <i>Dothidea insculpta</i>             | CBS 189.58     | AF027764 | DQ247802  | DQ247810  | DQ471081 | AF107800 | —        |
| <i>Dothidotthia aspera</i>            | CPC 12933      | —        | EU673276  | EU673228  | —        | —        | —        |
| <i>Dothidotthia symphoricarpi</i>     | CBS 119687     | —        | EU673273  | EU673224  | —        | —        | —        |
| <i>Pleospora herbarum</i>             | CBS 191.86     | KC584239 | DQ247804  | DQ247812  | DQ471090 | DQ247794 | —        |
| <i>Camarosporium quaternatum</i>      | CBS 483.95     | KY929149 | GU301806  | GU296141  | GU349044 | —        | GU357761 |

## APPENDIX 1. — Continuation.

| Species name                          | Strain number   | ITS       | LSU      | SSU       | TEF      | RPB2     | RPB1     |
|---------------------------------------|-----------------|-----------|----------|-----------|----------|----------|----------|
| <i>Capnodium coffeae</i>              | CBS 147.52      | AJ244239  | DQ247800 | DQ247808  | DQ471089 | DQ247788 | —        |
| <i>Dermea acerina</i>                 | CBS161.38       | AF141164  | DQ247801 | DQ247809  | —        | —        | —        |
| <i>Neobulgaria lilacina</i>           | M258            | EU940217  | EU940141 | EU940066  | —        | EU940352 | —        |
| <i>Medeolaria farlowii</i>            | —               | GQ406809  | GQ406807 | GQ406808  | —        | —        | —        |
| <i>Eleutheromyces pseudosubulatus</i> | CBS:458.88      | KJ710467  | EU754162 | EU754063  | —        | —        | —        |
| <i>Cudonia circinans</i>              | C316            | KC833156  | KC833182 | —         | KC833349 | KC833275 | —        |
| <i>Thelebolus ellipsoideus</i>        | CBS 113937      | AY957550  | FJ176895 | FJ176840  | —        | FJ238378 | FJ238445 |
| <i>Mollisia cinerea</i>               | AFTOL-ID 76     | DQ491498  | DQ470942 | DQ470990  | DQ471051 | DQ470883 | DQ471122 |
| <i>Loramyces macrosporus</i>          | AFTOL-ID 913    | JN033383  | DQ470957 | DQ471005  | DQ471076 | DQ470907 | DQ471149 |
| <i>Herpomyces periplanetae</i>        | 602c            | KT800041  | KT800010 | KT800025  | —        | —        | —        |
| <i>Herpomyces chaetophilus</i>        | 602b            | KT800039  | KT800009 | KT800023  | —        | —        | —        |
| <i>Laboulbenia pedicellata</i>        | H84-1           | —         | KY350537 | KY523244  | —        | —        | —        |
| <i>Pyxidiophora arvernensis</i>       | AFTOL-ID 2197   | —         | FJ176894 | FJ176839  | FJ238412 | FJ238377 | —        |
| <i>Xylona heveae</i>                  | TC269           | JQ838225  | JQ838239 | JQ838236  | —        | JQ838246 | JQ838243 |
| <i>Xylona heveae</i>                  | TC161           | JQ838232  | JQ838238 | JQ838237  | —        | JQ838244 | JQ838242 |
| <i>Symbiotaphrina buchneri</i>        | CBS:6902        | —         | KY109806 | —         | —        | —        | —        |
| <i>Symbiotaphrina microtheca</i>      | DVLH            | —         | KJ004453 | —         | —        | —        | —        |
| <i>Cordyceps militaris</i>            | OSC 93623       | JN049825  | AY184966 | AY184977  | DQ522332 | —        | DQ522377 |
| <i>Diaporthe eres</i>                 | AR 3519         | —         | AF362565 | —         | —        | —        | —        |
| <i>Diatrype disciformis</i>           | AFTOL-ID 927    | —         | DQ470964 | DQ471012  | DQ471085 | —        | DQ471158 |
| <i>Ophiocordyceps aurantiaca</i>      | OSC 128578      | JN049833  | DQ518770 | DQ522556  | DQ522345 | DQ522445 | DQ522391 |
| <i>Ophiocordyceps gracilisOSC</i>     | OSC 151906      | —         | KJ878890 | KJ878923  | KJ878969 | —        | —        |
| <i>Ophiodiaporthe cyathae</i>         | YMJ 1364        | JX570889  | JX570891 | JX570890  | KC465406 | JX570893 | —        |
| <i>Ophiocordyceps variabilis</i>      | OSC 111003      | —         | EF468839 | EF468985  | EF468779 | EF468933 | —        |
| <i>Monilinia laxa</i>                 | AFTOL-ID 169    | —         | AY544670 | —         | DQ471057 | DQ470889 | —        |
| <i>Melanconis stilbostoma</i>         | AR3501          | —         | AF408374 | NG_013198 | EU221886 | EU219299 | —        |
| <i>Plagiostoma euphorbiae</i>         | CBS 340.78      | EU199198  | AF408382 | DQ862055  | GU354016 | EU219292 | —        |
| <i>Cyphellophora laciniata</i>        | CBS 190.61      | EU035416  | FJ358239 | FJ358307  | —        | —        | FJ358370 |
| <i>Cyphellophora pauciseptata</i>     | CBS 284.85      | JQ766466  | JQ766515 | —         | —        | —        | JQ766415 |
| <i>Chaetothyrium agathis</i>          | MFLUCC 12 C0113 | KP744437  | KP744480 | —         | —        | —        | —        |
| <i>Ceramothyrium thailandicum</i>     | MFLU 13-0632    | KP324928  | KP324930 | —         | —        | —        | —        |
| <i>Trichomerium deniquatum</i>        | MFLUCC10-0884   | JX313654  | JX313660 | —         | —        | —        | —        |
| <i>Trichomerium foliicola</i>         | MFLUCC 10-0078  | NR_144963 | JX313661 | —         | —        | —        | —        |
| <i>Neophaeococomyces aloes</i>        | CPC 21873       | KF777182  | KF777234 | —         | —        | —        | —        |
| <i>Neophaeococomyces catenatus</i>    | CBS 650.76      | AF050277  | AF050277 | —         | —        | —        | —        |
| <i>Minimelanolocus obscurus</i>       | MFLUCC:15-0416  | KR215606  | KR215611 | —         | —        | —        | —        |
| <i>Minimelanolocus aquaticus</i>      | MFLUCC:15-0414  | KR215607  | KR215612 | —         | —        | —        | —        |
| <i>Exophiala dermatitidis</i>         | AFTOL-ID 668    | DQ826738  | DQ823100 | DQ823107  | DQ840566 | DQ840562 | DQ840555 |

## APPENDIX 1. — Continuation.

| Species name                                | Strain number | ITS      | LSU      | SSU      | TEF      | RPB2     | RPB1     |
|---|---------------|----------|----------|----------|----------|----------|----------|
| <i>Capronia pilosella</i>                   | AFTOL-ID 657  | DQ826737 | DQ823099 | DQ823106 | DQ840565 | DQ840561 | DQ840554 |
| <i>Leptomeliola ptilidii</i>                | M186          | EU940200 |          | EU940051 | —        | EU940336 | —        |
| <i>Epibryon plagiochilae</i>                | M187          | EU940201 | EU940124 | —        | —        | EU940337 | —        |
| <i>Epibryon turfosorum</i>                  | M292          | EU940221 | EU940145 | —        | —        | EU940355 | —        |
| <i>Polyblastia viridescens</i>              | AFTOL-ID 2240 | —        | EF643771 | EF689855 | —        | —        | EF689774 |
| <i>Verrucaria rupestris</i>                 | SS043         | EU553501 | EU598724 | —        | —        | —        | EU723786 |
| <i>Pleostigma jungermannicola</i>           | M174          | EU940195 | EU940119 | EU940046 | —        | EU940331 | —        |
| <i>Celothelium cinchonarum</i>              | F 17105       | —        | DQ329020 | —        | —        | —        | —        |
| <i>Dolabria nepheliae</i>                   | CBS 122120    | —        | GU332517 | —        | GU332523 | —        | GU332521 |
| <i>Xenocylindrosporium kirstenboschense</i> | CBS 125545    | GU229890 | GU229891 | —        | —        | —        | —        |
| <i>Pyrenula chlorospila</i>                 | CG1520b       | JQ927452 | JQ927471 | —        | —        | —        | —        |
| <i>Pyrenula nitida</i>                      | F 5929        | JQ927458 | DQ329023 | —        | —        | —        | —        |
| <i>Rhynchostoma proteae</i>                 | —             | —        | AY230151 | —        | —        | —        | —        |
| <i>Granulopyrenis seawardii</i>             | CBS 109025    | —        | EF411062 | —        | —        | —        | —        |
| <i>Eurotium herbariorum</i>                 | NRRL 116      | EF652052 | U29553   | AB002069 | —        | —        | —        |
| <i>Aspergillus glaucus</i>                  | ATCC 16469    | —        | AY176751 | —        | —        | —        | —        |
| <i>Monascus ruber</i>                       | ATCC 16371    | AY498572 | AF364996 | —        | —        | —        | —        |
| <i>Leiothecium ellipsoideum</i>             | CBS 607.74    | —        | FJ358285 | FJ358350 | —        | —        | FJ358412 |
| <i>Byssochlamys nivea</i>                   | CBS 100.11    | FJ389934 | AY176750 | —        | —        | JF417414 | JN121551 |
| <i>Trichocoma paradoxa</i>                  | CBS 788.83    | —        | FJ358290 | FJ358354 | —        | —        | —        |
| <i>Pseudotulostoma japonica</i>             | TNS-F11152    | —        | AB161194 | —        | —        | —        | —        |
| <i>Elaphomyces digitatus</i>                | MCA1923       | —        | JN713148 | —        | —        | —        | —        |
| <i>Thermoascus crustaceus</i>               | CBS 374.62    | —        | FJ358289 | —        | —        | —        | —        |
| <i>Thermoascus aurantiacus</i>              | S1            | —        | KJ535692 | —        | —        | —        | —        |
| <i>Arachnomyces minimus</i>                 | CBS 324.70    | —        | FJ358274 | AJ315167 | —        | —        | —        |
| <i>Arachnomyces nitidus</i>                 | IFO 32048     | —        | AB075351 | —        | —        | —        | —        |
| <i>Gymnoascus reesii</i>                    | CBS 259.61    | —        | FJ358284 | FJ358349 | —        | —        | FJ358411 |
| <i>Gymnascella littoralis</i>               | CBS 454.73    | —        | FJ358272 | FJ358340 | —        | —        | FJ358404 |
| <i>Arthroderma curreyi</i>                  | CBS 138.26    | KT155805 | AY176726 | AJ315165 | —        | —        | —        |
| <i>Arthroderma quadrifidum</i>              | ATCC 22954    | —        | AY176728 | —        | —        | —        | —        |
| <i>Onygena equina</i>                       | ATCC 22731    | —        | AY176717 | —        | —        | —        | —        |
| <i>Onygena corvina</i>                      | JCM 9546      | —        | AB075355 | —        | —        | —        | —        |
| <i>Ascospaera apis</i>                      | CBS 402.96    | —        | FJ358275 | FJ358343 | —        | —        | FJ358406 |
| <i>Eremascus albus</i>                      | CBS 975.69    | —        | FJ358283 | FJ358348 | —        | —        | FJ358410 |
| <i>Polytolypa hystricis</i>                 | UAMH 7299     | AY527405 | AY176718 | —        | —        | —        | —        |
| <i>Caliciopsis nigra</i>                    | MA 18191      | —        | KP144011 | —        | —        | —        | —        |
| <i>Corynelia fructigena</i>                 | AW 250        | KP881704 | KP881716 | KP881720 | —        | —        | —        |
| <i>Sphinctrina leucopoda</i>                | Kalb 33829    | AY795875 | AY796006 | —        | —        | —        | —        |
| <i>Sphinctrina turbinata</i>                | Tibell 23093  | AY795877 | DQ009001 | —        | —        | —        | —        |

## APPENDIX 1. — Continuation.

| Species name                           | Strain number    | ITS             | LSU             | SSU      | TEF      | RPB2     | RPB1     |
|--|------------------|-----------------|-----------------|----------|----------|----------|----------|
| <i>Mycocalicium subtile</i>            | Tibell 16744     | —               | AY796004        | —        | —        | —        | —        |
| <i>Mycocalicium albonigrum</i>         | Tibell 19038     | —               | AY796001        | —        | —        | —        | —        |
| <i>Acarospora schleicheri</i>          | Reeb VR 5-VII-98 | —               | AY640945        | AY640986 | —        | —        | —        |
| <i>Agyrium rufum</i>                   | Wedin 7931       | JX000097        | EF581826        | —        | —        | —        | EF581822 |
| <i>Coccotrema cucurbitula</i>          | AFTOL-ID 957     | —               | AF274092        | —        | —        | —        | —        |
| <i>Icmadophila ericetorum</i>          | AFTOL-ID 4846    | —               | KJ766573        | KJ766729 | —        | —        | —        |
| <i>Lobothallia radiosa</i>             | AFTOL-ID 1860    | —               | KJ766596        | KJ766746 | —        | KJ766935 | KJ766870 |
| <i>Ochrolechia tartarea</i>            | FR:DNA7          | JN943620        | JN941358        | —        | —        | —        | JN992649 |
| <i>Pertusaria amara isolate</i>        | AFTOL-ID 4874    | —               | KJ766623        | KJ766764 | —        | —        | —        |
| <i>Arctomia delicatula isolate</i>     | P166             | —               | KR017191        | KR017255 | KR017563 | —        | KR017488 |
| <i>Solorina saccata isolate</i>        | AFTOL-ID 1964    | HQ650625        | KJ766661        | KJ766797 | —        | —        | —        |
| <i>Peltigera dolichorrhiza isolate</i> | P348             | KX897182        | KM005745        | —        | KM005875 | —        | KM005937 |
| <i>Teloschistes flavidans isolate</i>  | Tflav103         | KT291472        | KT291565        | —        | —        | KT291662 | KT291604 |
| <i>Seirophora lacunosa voucher</i>     | SK B07           | KT220204        | KT220213        | —        | —        | —        | —        |
| <i>Anzia colpodes</i>                  | Lumbsch 4.VI.04  | —               | DQ923651        | —        | —        | —        | —        |
| <i>Canoparmelia caroliniana</i>        | AFTOL-ID 6       | —               | AY584634        | AY584658 | —        | AY584683 | —        |
| <i>Calicium salicium</i>               | CBS 100898       | —               | KF157982        | KF157970 | —        | KF157998 | —        |
| <i>Calicium viride</i>                 | Soechting 7475   | —               | AF356670        | —        | —        | —        | —        |
| <i>Taphrina deformans</i>              | AFTOL-ID 1234    | —               | DQ470973        | DQ471024 | DQ471097 | DQ470927 | DQ471170 |
| <i>Taphrina antarctica</i>             | CCFEE 5198       | NR_132870       | JX124717        | —        | —        | —        | —        |
| <i>Dactylospora haliotrepha</i>        | AFTOL-ID758      | —               | FJ176855        | FJ176802 | —        | FJ238344 | —        |
| <i>Dactylospora haliotrepha</i>        | AFTOL-ID798      | —               | FJ713617        | —        | —        | FJ713614 | —        |
| <i>Dactylospora imperfecta</i>         | AFTOL-ID 5006    | —               | FJ176896        | FJ176841 | —        | —        | —        |
| <i>Dactylospora lobariella</i>         | AFTOL-ID 2137    | —               | FJ176891        | FJ176837 | —        | —        | —        |
| <i>Dactylospora mangrovei</i>          | AFTOL-ID 2108    | —               | FJ176890        | FJ176836 | FJ238411 | FJ238375 | KJ766849 |
| <i>Dactylospora vrijmoediae</i>        | NTOU4002         | NR_138396       | KC692153        | KC692152 | —        | KC692154 | —        |
| <i>Dactylospora ahtii</i>              | RP23             | KY661630        | KY661659        | —        | —        | —        | —        |
| <i>Dactylospora parasitica</i>         | RP424            | —               | KY661667        | —        | —        | —        | —        |
| <i>Dactylospora parasitica</i>         | RP422            | KY661646        | KY661666        | —        | —        | —        | —        |
| <i>Dactylospora glaucomariooides</i>   | RP275            | KY661632        | KY661660        | —        | —        | —        | —        |
| <i>Dactylospora deminuta</i>           | RP235            | KY661629        | —               | —        | —        | —        | —        |
| <i>Dactylospora fusiformis</i>         | MFLU 16-0593     | <b>MH718441</b> | <b>MH718434</b> | —        | —        | —        | —        |
| <i>Dactylospora chiangraiensis</i>     | MFLU 16-0570     | <b>MH718440</b> | <b>MH718433</b> | —        | —        | —        | —        |

APPENDIX 2. — GenBank accession numbers of strains used in phylogenetic analysis II of this study. New sequence data in this study are in bold.

| Species name                                | Strain number   | ITS       | LSU      |
|---|-----------------|-----------|----------|
| <i>Cyphellophora laciniata</i>              | CBS 190.61      | EU035416  | FJ358239 |
| <i>Cyphellophora pauciseptata</i>           | CBS 284.85      | JQ766466  | JQ766515 |
| <i>Chaetothyrium agathis</i>                | MFLUCC 12 C0113 | KP744437  | KP744480 |
| <i>Ceramothyrium thailandicum</i>           | MFLU 13-0632    | KP324928  | KP324930 |
| <i>Trichomerium deniquulatum</i>            | MFLUCC10-0884   | JX313654  | JX313660 |
| <i>Trichomerium foliicola</i>               | MFLUCC 10-0078  | NR_144963 | JX313661 |
| <i>Neophaeococomyces aloes</i>              | CPC 21873       | KF777182  | KF777234 |
| <i>Neophaeococomyces catenatus</i>          | CBS 650.76      | AF050277  | AF050277 |
| <i>Minimelanolocus obscurus</i>             | MFLUCC:15-0416  | KR215606  | KR215611 |
| <i>Minimelanolocus aquaticus</i>            | MFLUCC:15-0414  | KR215607  | KR215612 |
| <i>Exophiala dermatitidis</i>               | AFTOL-ID 668    | DQ826738  | DQ823100 |
| <i>Capronia pilosella</i>                   | AFTOL-ID 657    | DQ826737  | DQ823099 |
| <i>Leptomeliola ptilidii</i>                | M186            | EU940200  | —        |
| <i>Epibryon plagiochilae</i>                | M187            | EU940201  | EU940124 |
| <i>Epibryon turfosorum</i>                  | M292            | EU940221  | EU940145 |
| <i>Polyblastia viridescens</i>              | AFTOL-ID 2240   | —         | EF643771 |
| <i>Verrucaria rupestris</i>                 | SS043           | EU553501  | EU598724 |
| <i>Pleostigma jungermannicola</i>           | M174            | EU940195  | EU940119 |
| <i>Celothelium cinchonarum</i>              | F 17105         | —         | DQ329020 |
| <i>Dolabria nepheliae</i>                   | CBS 122120      | —         | GU332517 |
| <i>Xenocylindrosporium kirstenboschense</i> | CBS 125545      | GU229890  | GU229891 |
| <i>Rhynchostoma proteae</i>                 | —               | —         | AY230151 |
| <i>Granulopyrenis seawardii</i>             | CBS 109025      | —         | EF411062 |
| <i>Eurotium herbariorum</i>                 | NRRL 116        | EF652052  | U29553   |
| <i>Aspergillus glaucus</i>                  | ATCC 16469      | —         | AY176751 |
| <i>Monascus ruber</i>                       | ATCC 16371      | AY498572  | AF364996 |
| <i>Leiothecium ellipsoideum</i>             | CBS 607.74      | —         | FJ358285 |
| <i>Byssochlamys nivea</i>                   | CBS 100.11      | FJ389934  | AY176750 |
| <i>Trichocoma paradoxa</i>                  | CBS 788.83      | —         | FJ358290 |
| <i>Pseudotulostoma japonica</i>             | TNS-F11152      | —         | AB161194 |
| <i>Elaphomyces digitatus</i>                | MCA1923         | —         | JN713148 |
| <i>Thermoascus crustaceus</i>               | CBS 374.62      | —         | FJ358289 |
| <i>Thermoascus aurantiacus</i>              | S1              | —         | KJ535692 |
| <i>Arachnomyces minimus</i>                 | CBS 324.70      | —         | FJ358274 |
| <i>Arachnomyces nitidus</i>                 | IFO 32048       | —         | AB075351 |
| <i>Gymnoascus reesii</i>                    | CBS 259.61      | —         | FJ358284 |
| <i>Gymnascella littoralis</i>               | CBS 454.73      | —         | FJ358272 |
| <i>Arthroderma curreyi</i>                  | CBS 138.26      | KT155805  | AY176726 |
| <i>Arthroderma quadrifidum</i>              | ATCC 22954      | —         | AY176728 |
| <i>Onygena equina</i>                       | ATCC 22731      | —         | AY176717 |

## APPENDIX 2. — Continuation.

| Species name                         | Strain number  | ITS             | LSU             |
|--------------------------------------|----------------|-----------------|-----------------|
| <i>Onygena corvina</i>               | JCM 9546       | —               | AB075355        |
| <i>Ascospaera apis</i>               | CBS 402.96     | —               | FJ358275        |
| <i>Eremascus albus</i>               | CBS 975.69     | —               | FJ358283        |
| <i>Polytolypa hystricis</i>          | UAMH 7299      | AY527405        | AY176718        |
| <i>Caliciopsis nigra</i>             | MA 18191       | —               | KP144011        |
| <i>Corynelia fructigena</i>          | AW 250         | KP881704        | KP881716        |
| <i>Sphinctrina leucopoda</i>         | Kalb 33829     | AY795875        | AY796006        |
| <i>Sphinctrina turbinata</i>         | Tibell 23093   | AY795877        | DQ009001        |
| <i>Mycocalicium subtile large</i>    | Tibell 16744   | —               | AY796004        |
| <i>Mycocalicium albonigrum</i>       | Tibell 19038   | —               | AY796001        |
| <i>Dactylospora haliotrepha</i>      | AFTOL-ID758    | —               | FJ176855        |
| <i>Dactylospora haliotrepha</i>      | AFTOL-ID798    | —               | FJ713617        |
| <i>Dactylospora imperfecta</i>       | AFTOL-ID 5006  | —               | FJ176896        |
| <i>Dactylospora lobariella</i>       | AFTOL-ID 2137  | —               | FJ176891        |
| <i>Dactylospora mangrovei</i>        | AFTOL-ID 2108  | —               | FJ176890        |
| <i>Dactylospora vrijmoediae</i>      | NTOU4002       | NR_138396       | KC692153        |
| <i>Dactylospora ahtii</i>            | RP23           | KY661630        | KY661659        |
| <i>Dactylospora parasitica</i>       | RP424          | —               | KY661667        |
| <i>Dactylospora parasitica</i>       | RP422          | KY661646        | KY661666        |
| <i>Dactylospora glaucomariooides</i> | RP275          | KY661632        | KY661660        |
| <i>Dactylospora deminuta</i>         | RP235          | KY661629        | —               |
| <i>Dactylospora stygia</i>           | BHI-F312a      | MF161218        | —               |
| <i>Dactylospora fusiformis</i>       | MFLU 18-0678   | <b>MH718442</b> | —               |
| <i>Dactylospora fusiformis</i>       | MFLU 16-0593   | <b>MH718441</b> | <b>MH718434</b> |
| <i>Dactylospora chiangraiensis</i>   | MFLU 16-0570   | <b>MH718440</b> | <b>MH718433</b> |
| <i>Arthroderma ciferrii</i>          | AFTOL-ID 428   | —               | EF413625        |
| <i>Aspergillus protuberans</i>       | AFTOL-ID 5007  | —               | FJ176897        |
| <i>Caliciopsis orientalis</i>        | AFTOL-ID 1911  | KP881690        | DQ470987        |
| <i>Caliciopsis pinea</i>             | AFTOL-ID 1869  | —               | DQ678097        |
| <i>Capronia mansonii</i>             | CBS 101.67     | AF050247        | AY004338        |
| <i>Capronia munkii</i>               | AFTOL-ID 656   | —               | EF413604        |
| <i>Capronia peltigerae</i>           | UAMH 11090     | HQ709322        | HQ613813        |
| <i>Ceramothyrium carniolicum</i>     | AFTOL-ID 1063  | —               | EF413628        |
| <i>Chaenothecopsis montana</i>       | Tuovila 07-086 | JX119105        | JX119114        |
| <i>Chaenothecopsis sitchensis</i>    | Tuovila 06-033 | JX119102        | JX119111        |
| <i>Dermatocarpon miniatum</i>        | AFTOL-ID 91    | DQ782837        | AY584644        |
| <i>Endocarpon pallidulum</i>         | AFTOL-ID 661   | DQ826735        | DQ823097        |
| <i>Epibryon bryophilum</i>           | M2             | —               | EU940090        |
| <i>Epibryon diaphanum</i>            | M122           | EU940178        | EU940101        |
| <i>Epibryon hepaticola</i>           | M224           | EU940212        | EU940136        |

## APPENDIX 2. — Continuation.

| <b>Species name</b>                   | <b>Strain number</b> | <b>ITS</b> | <b>LSU</b> |
|---------------------------------------|----------------------|------------|------------|
| <i>Epibryon intercapillare</i>        | M125                 | —          | EU940102   |
| <i>Epibryon interlamellare</i>        | M32                  | EU940174   | EU940097   |
| <i>Eupenicillium javanicum</i>        | AFTOL-ID 429         | KT232212   | EF413621   |
| <i>Eupenicillium limosum</i>          | CBS 339.97           | NR_111496  | EF411064   |
| <i>Exophiala pisciphila</i>           | AFTOL-ID 669         | DQ826739   | DQ823101   |
| <i>Exophiala salmonis</i>             | AFTOL-ID 671         | —          | EF413609   |
| <i>Monascus purpureus</i>             | AFTOL-ID 426         | DQ782847   | DQ782908   |
| <i>Phialophora verrucosa</i>          | AFTOL-ID 670         | —          | EF413615   |
| <i>Pyrenula aspista</i>               | AFTOL-ID 2012        | —          | EF411063   |
| <i>Pyrenula cruenta</i>               | CBS 132372           | KC592268   | AF279407   |
| <i>Pyrenula pseudobufonia</i>         | VR 14-VI-02/5        | —          | AY640962   |
| <i>Pyrgillus javanicus</i>            | AFTOL-ID 342         | DQ826741   | DQ823103   |
| <i>Sarcinomyces petricola</i>         | CBS 600.93           | AJ244274   | FJ176893   |
| <i>Sclerococcum sphaerale</i>         | Diederich 17283      | —          | JX081673   |
| <i>Sclerococcum sphaerale</i>         | Diederich 17292      | —          | JX081672   |
| <i>Sclerococcum sphaerale</i>         | Ertz 17425           | —          | JX081674   |
| <i>Sphinctrina turbinata</i>          | Tibell 22478         | AY795876   | AY796007   |
| <i>Spiromastix warcupii</i>           | AFTOL-ID 430         | DQ782848   | DQ782909   |
| <i>Staurothele frustulenta</i>        | AFTOL-ID 697         | DQ826736   | DQ823098   |
| <i>Teloschistes flavicans isolate</i> | Tflav103             | KT291472   | KT291565   |