

# Notes Worthy Aquatic Hyphomycetes from Thailand–I

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## Abstract

Taxonomy of asexual fungi is subjected to rapid changing with the use of DNA. Re-collecting and following DNA based taxonomy of old species is one of the important approaches in modern mycology. During a collection program of aquatic fungi, six known species were collected from different areas of Thailand. In here *Conlarium aquaticum*, *Dictyocheirospora nabanheensis*, *Distoseptispora rayongensis*, *Gliocladiopsis pseudotenuis*, *Spadicoides cordanoides* and *Sporoschisma longicatenatum* were illustrated, described, and their new ITS and LSU sequences were deposited to NCBI GenBank.

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**Keywords:** aquatic fungi, asexual, DNA sequences, hyphomycetes, taxonomy

**Abbreviations:** PDA: potato dextrose agar; ITS: internal transcribed spacer; LSU: large subunit ribosomal RNA

## Introduction

Hyphomycetes is one of the largest groups of asexual fungi which show different life modes including saprobes and pathogens [1]. They occur in different ecosystems of environments such as terrestrial, aquatic, etc. Further, some hyphomycetes can be found in extreme environments such as on rocks [2–4]. In morphology-based taxonomy, hyphomycetous taxa were differentiated from coelomycetous taxa as they lack closed conidiomata (*i.e.* pycnidia or acervuli) [1]. However, sometimes it is difficult to distinguish acervuli from sporodochia, thus essential to study vertical sections very carefully [5].

Aquatic hyphomycetes most commonly occur on dead leaves in streams and rivers and sporulate under water [6]. They are important in litter degradation in freshwater ecosystems [7, 8] and as parasites, *e.g.*, *Dwayaangam heterospora* [9]. Based on biological behaviour such as sporulation methods and mycelial growth, Thomas [10] and Goh and Hyde [11] classified freshwater hyphomycetes into four ecological groups *viz.* ingoldian, aero-aquatic, terrestrial-aquatic and submerged-aquatic species. So far, more than 300 species have been reported [12] and most recorded from temperate and fewer from tropical and subtropical regions [11–14].

In the present study, we illustrate six aquatic hyphomycetes collected from Thailand. Further, detailed taxonomic descriptions, classification and notes are also provided. We intend to publish interesting aquatic hyphomycetes as a series of papers from our ongoing research works in Thailand and China.

## Materials and Methods

### Collection and morphology

During a survey, to study the diversity of aquatic fungi, decaying woody specimens were collected from eastern Thailand in December 2018, following the procedures described in Kurniawati et al. [15]. Specimens were placed in ziploc plastic bags with sterile wet cotton and taken to the laboratory. Specimens were morphologically observed using a Nikon (Model SMZ-171, Japan) dissecting microscope to locate fruiting bodies. Photomicrographs were made with a Nikon (Model ECLIPSE Ni, Japan) compound microscope with a Cannon EOS 600D camera. Tarosoft (R) Image Frame Work program was used to measure the fungal structures and Adobe Photoshop CS6 Extended version 12.0 software (Adobe Inc., USA) was used to make the photo plates.

### Isolation

Isolations were made from single conidium on potato dextrose agar (PDA) following the methods of Chomnunti et al. [16], and then incubated overnight in an incubator (25°C). Single germinating conidia were transferred to new PDA plate. Cultures were examined and colony characteristics were recorded every seven days.

Dry specimens were deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Living cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection (KUMCC).

### DNA extraction, PCR amplification and sequencing

Total DNA was extracted from cultures grown on PDA for 30 days at 25°C using a Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China) according to the manufacturer's instructions. Two molecular markers were used in this study for molecular identification: the internal transcribed spacer (ITS) and the large subunit ribosomal RNA gene (LSU) and their respective primer pairs used were ITS5/ITS4 [17] and LROR/LR5 [18]. The amplifications were carried out in a 25 µL reaction volume containing 9.5 µL ddH<sub>2</sub>O, 12.5 µL 2 × PCR Master Mix, 1 µL of DNA template, 1 µL of each primer (10 µM). The PCR thermal cycles for amplification of the gene regions followed according to Su et al. [19]. The PCR products were examined on 1.0 % agarose gel stained with Gel Red ethidium bromide. Sequencing reactions were conducted by TSINGKE Biological Engineering Technology and Services Co., Kunming, P.R. China.

We provide the similarity of ITS sequences of each taxon in BLASTn search as a percentage or compared ITS sequences between our new isolates and type strains. Classification of each taxon is also provided based on Wijayawardene et al. [20].

### Taxonomy and discussion

- *Conlarium aquaticum* W. Dong, H. Zhang & K.D. Hyde, Fungal Diversity 85: 92 (2017) (Figure 1)

Index Fungorum Registration Identifier 553759

*Classification:* Annulatasceae, Annulatascales, Diaporthomycetidae, Sordariomycetes

*Saprobic* on decaying submerged wood in freshwater. **Sexual morph:** undetermined. **Asexual morph:** hyphomycetes, *Colonies* sporodochial, broadly and irregularly punctiform, gregarious or scattered, raised, dark brown to black, velvety. *Mycelium* mostly immersed in natural substratum, consisting of branched, septate, thin-walled, smooth, pale brown to brown hyphae. *Conidiophores* absent or reduced to conidiogenous cells. *Conidiogenous cells* up to 18 µm long, monoblastic, holoblastic, integrated, determinate, cylindrical, hyaline to pale brown, smooth. *Conidia* acrogenous, solitary, dry, subglobose, ellipsoidal, oblong or irregular, brown, clathrate, muriform, 6–12-transversely

septate, 4–10-longitudinally septate, slightly constricted at septa, smooth, thin-walled,  $47\text{--}72 \times 25\text{--}54 \mu\text{m}$  ( $\bar{x} = 61.5 \times 36 \mu\text{m}$ ,  $n = 30$ ).

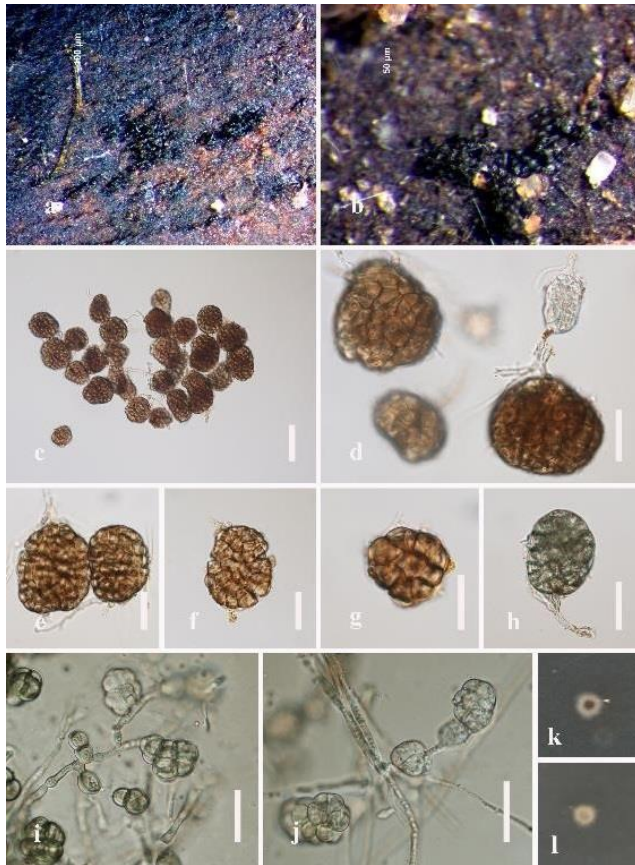
**Culture characteristics:** On PDA, colonies circular, reaching 10 mm in 30 days at  $25^\circ\text{C}$  in the condition of 12 h dark and 12 h light; gray to dark brown from above, dark brown from below, umbonate, rough, wrinkled, dry, edge undulate. *Conidiophores* macronematous, branched, sometimes reduced to a single conidiogenous cell, hyaline. *Conidiogenous cells* holoblastic, monoblastic, cylindrical, smooth-walled. *Conidia* acrogenous, solitary, dry, subglobose, ellipsoidal, oblong or irregular, muriform, smooth, thin-walled, hyaline when young, olivaceous at maturity,  $42\text{--}65 \times 25\text{--}56 \mu\text{m}$  ( $\bar{x} = 53 \times 34 \mu\text{m}$ ,  $n = 20$ ).

**Material examined:** Thailand, Mukdahan Province, small river of Nong Bo Na Kae,  $16^\circ 84' 96''\text{N}$ ,  $104^\circ 33' 12''\text{E}$ , on dead submerged decaying wood of unidentified plants, 13 December 2018, Hao Yang, t35 (MFLU 19–0545), living in culture = KUMCC 19–0209.

**Sequences data:** ITS: MN513038, LSU: MN511740.

**Notes:** The genus *Conlarium* was established to accommodate the holomorphic species *C. dupliciascosporum* [21]. Zhang et al. [14] introduced the second species *C. aquaticum* from freshwater. A comparison of nucleotide sequences between our isolate and the type strain of *C. aquaticum* showed only 4 bp differences without gaps in ITS gene region (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), which means they are the same species according to Jeewon et al. [22]. In addition, they have similar morphology including conidial size. Thus, we confirm the identity of our isolate as *C. aquaticum*. The cultural characteristics of *C. aquaticum* are provided in this paper, which shows different conidial colour (brown on natural substrate vs. hyaline to olivaceous from culture).

**Known distribution:** Thailand



**Figure 1:** *Conlarium aquaticum* (MFLU 19–0545). (a), (b) Sporodochia on submerged wood. (c), (d) Conidia attach to conidiogenous cells. (e–h) Conidia. (i), (j) Conidia from PDA. (k), (l) Culture on PDA, k obverse and l reverse. Scale bars: c =  $50 \mu\text{m}$ , d–j =  $30 \mu\text{m}$ .

- *Dictyocheirospora nabanheensis* Tibpromma & K.D. Hyde, Fungal Diversity 93: 10 (2018) (Figure 2)

Index Fungorum Registration Identifier 554474

*Classification:* Dictyosporiaceae, Pleosporales, Pleosporomycetidae, Dothideomycetes

*Saprobic* on decaying wood in freshwater habitats. **Sexual morph:** undetermined. **Asexual morph:** hyphomycetous, *Colonies* sporodochial, scattered or in small groups, dark brown to black. *Mycelium* mostly immersed. *Conidiophores* micronematous, reduced to conidiogenous cell. *Conidiogenous cells* holoblastic, cylindrical, hyaline to pale brown, partly remaining attached to the conidia,  $9.5\text{--}14 \times 4\text{--}7 \mu\text{m}$ . *Conidia* solitary, oval to ellipsoid, cheiroid,  $43.5\text{--}55.5 \times 20\text{--}29.5 \mu\text{m}$  ( $\bar{x} = 50.0 \times 24.5 \mu\text{m}$ ,  $n = 40$ ), consisting of 40–48 cells, with a basal connecting cell, pale brown to yellow-brown, smooth-walled, individual cells discoid,  $28\text{--}37.5 \times 5\text{--}6 \mu\text{m}$ , arranged in 6 compact rows, with 6–10 cells per row; with 1–2 rounded to cylindrical appendages,  $5\text{--}10 \times 4\text{--}5 \mu\text{m}$ , arising from near middle of conidial rows, hyaline.

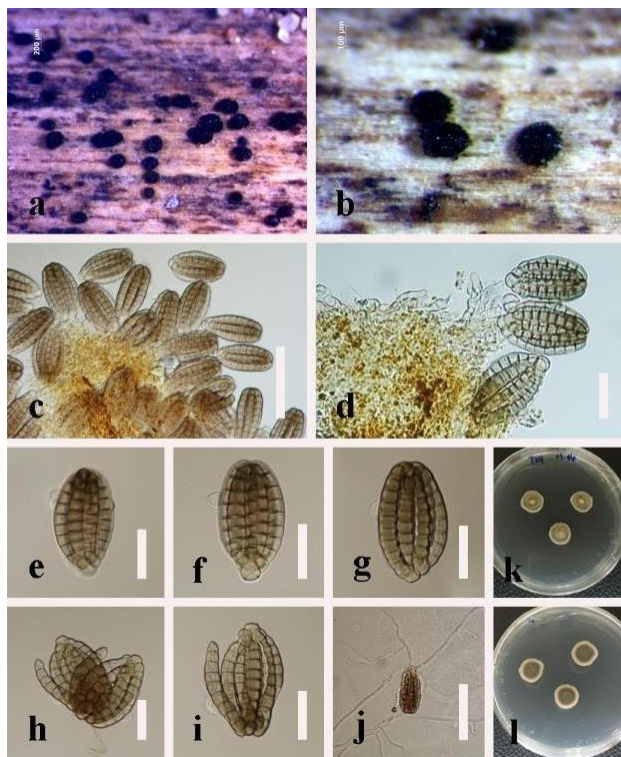
*Cultural characteristics:* *Conidia* germinated on PDA within 24 h. Germ tubes produced from the base cell. Colonies on PDA reaching 18–20 diameter in 18 days at 20–25°C in the condition of 12 h dark and 12 h light, with dense, yellow to brown mycelium on the surface; reverse brown to dark brown with white margin.

*Material examined:* Thailand, Mukdahan Province, small river of Sakon Nakhom, 16°53'30''N, 104°11'25''E, on dead submerged decaying wood of unidentified plants, 13 December 2018, Hao Yang, t24 (MFLU 19–0538), living culture = KUMCC 19–0208.

*Sequences data:* ITS: MN513035, LSU: MN511737.

*Notes:* *Dictyocheirospora nabanheensis* was introduced by Tibpromma et al. [23] and characterised by its cheiroid conidia consisting of 40–48 cells, 6–10 cells in each row and 1–2 rounded to cylindrical appendages. In a BLASTn search on NCBI GenBank, the closest match of our isolate in ITS region is 100% identical to the strain of *D. nabanheensis* (MK347756); thus, it is identified as *D. nabanheensis*.

*Known distribution:* Thailand



**Figure 2:** *Dictyocheirospora nabanheensis* (MFLU 19–0538). (a), (b) Colonies on substrate. (c) Squash of colonies. (d) Conidiogenous cells. (e–i) Conidia. (j) Germinating conidium on PDA. (k), (l) Culture on PDA, k obverse and l reverse. Scale bars: c, j = 50  $\mu\text{m}$ , d–i = 20  $\mu\text{m}$ .

- *Distoseptispora rayongensis* J. Yang & K.D. Hyde, Fungal Diversity (2019), in press. (Figure 3)

**Classification:** Distoseptisporaceae, Pleosporales, Pleosporomycetidae, Dothideomycetes

**Saprobic** on decaying wood submerged in freshwater. **Sexual morph:** undetermined. **Asexual morph:** hyphomycetous, **Colonies** on the substratum superficial, effuse, hairy or velvety, black. **Mycelium** mostly immersed, consisting of branched, septate, smooth, hyaline hyphae. **Conidiophores** macronematous, mononematous, solitary, unbranched, erect, cylindrical, straight or slightly flexuous, 2–4-septate, brown, smooth,  $62.5\text{--}73 \times 4\text{--}5.5 \mu\text{m}$  ( $\bar{x} = 68 \times 5 \mu\text{m}$ ,  $n = 10$ ). **Conidiogenous cells** monoblastic, integrated, determinate, terminal, cylindrical, brown. **Conidia** acrogenous, obclavate or obspathulate, rostrate, mostly 13–16-euseptate, rarely 16–24-septate, pale brown or pale olivaceous, becoming paler or hyaline towards the apex, truncate at the base, rounded at the apex, guttulate, smooth, thick-walled,  $85\text{--}163\text{--}(265) \times 10\text{--}14.5 \mu\text{m}$  ( $\bar{x} = 107 \times 12.5 \mu\text{m}$ ,  $n = 20$ ), with a darkened scar at the base, sometimes with percurrent proliferation and forming another conidium from the conidial apex.

**Culture characteristics:** *Conidia* germinated on PDA within 24 h. Germ tubes produced from the conidial top. Colonies on PDA reaching 16 mm diameter after 15 days at 25°C in the condition of 12 h dark and 12 h light, circular, aerial mycelium dense, brown; reverse dark brown with entire white margin.

**Material examined:** Thailand, Mukdahan Province, small river of Nong Bo Na Kae, 16°84'96"N, 104°33'12"E, on dead submerged decaying wood of unidentified plants, 13 December 2018, Hao Yang, t33 (MFLU 19–0543), living culture = KMUCC19–0334.

**Sequences data:** ITS: MN513037, LSU: MN511739.

**Notes:** *Distoseptispora rayongensis* was introduced by Hyde et al. [24] which was found in Rayong Province, Thailand. Our isolate was collected in Mukdahan Province of Thailand and its morphological characteristics, such as cylindrical, unbranched, septate conidiophores and distoseptate conidia, were identical to *D. rayongensis*. The nucleotide sequences of our isolate were 100% identical to the type strain of *D. rayongensis* in ITS gene region, therefore, we identified the strain as *D. rayongensis*.

**Known distribution:** Thailand



**Figure 3:** *Distoseptispora rayongensis* (MFLU 19–0543). (a), (b) Colonies on substrate. (c), (d) Conidiophores with conidia. (e) Conidiophore. (f) Conidiogenous cell. (g–l) Conidia. (m) Germinating conidium. (n), (o) Colony on PDA, n obverse and o reverse. Scale bars: c, l–m = 50  $\mu\text{m}$ , d = 30  $\mu\text{m}$ , e–f = 10  $\mu\text{m}$ , g–k = 20  $\mu\text{m}$ .



- *Gliocladiopsis pseudotenuis* L. Lombard & Crous, Persoonia 28: 31 (2012) (Figure 4)

Index Fungorum Registration Identifier 564403

*Classification:* Nectriaceae, Hypocreales, Hypocreomycetidae, Sordariomycetes

*Saprobic* on decaying wood in freshwater habitats. **Sexual morph:** undetermined. **Asexual morph:** hyphomycetous, *Colonies* on natural substrata effuse, scattered, white, upper part covered with distinct bright white mass of conidia. *Mycelium* partly immersed, partly superficial. *Conidiophores* synnematosus, micronematosus, penicillate, septate, smooth, hyaline,  $61\text{--}130 \times 3\text{--}6 \mu\text{m}$ , without stipe extensions and terminal vesicles. *Conidiogenous cells* polyblastic, terminal, phialides doliiform to cymbiform to cylindrical, hyaline,  $10\text{--}17 \times 2\text{--}3 \mu\text{m}$ . *Conidia* cylindrical,  $18\text{--}21 \times 3\text{--}4 \mu\text{m}$  ( $\bar{x} = 19 \times 3 \mu\text{m}$ ,  $n = 20$ ), hyaline, smooth with rounded ends, straight, with a single median septum.

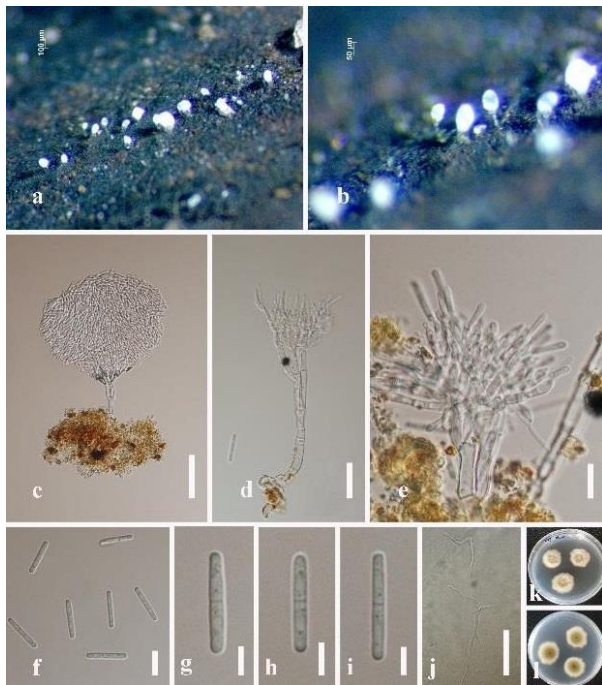
*Culture characteristics:* *Conidia* were germinated on PDA within 24 h. Colonies fast-growing, attaining 40 mm diameter in 13 days at  $20\text{--}25^\circ\text{C}$  in the condition of 12 h dark and 12 h light, floccose, rounded, white at first, brown at the center, becoming pale yellow when aged with dense mycelium on the surface, with undulate margin, colonies reverse yellow.

*Material examined:* Thailand, Mukdahan Province, small river of Nong Bo Na Kae,  $16^\circ 84' 96''\text{N}$ ,  $104^\circ 33' 12''\text{E}$ , on dead submerged decaying wood of unidentified plants, 13 December 2018, Hao Yang, t37 (MFLU 19-0547), living culture = MFLUCC 19-0337.

*Sequences data:* ITS: MN513039, LSU: MN511741.

*Notes:* *Gliocladiopsis pseudotenuis* was introduced to accommodate *G. tenuis* (Bugnic.) Crous & M.J. Wingf. [25]. Morphologically, our isolate shares the similar characters to that of *G. pseudotenuise* except the slightly larger conidia ( $\bar{x} = 19 \times 3 \mu\text{m}$  vs.  $17 \times 2 \mu\text{m}$ ). A comparison of nucleotide sequences between our isolate and the holotype of *G. pseudotenuis* showed a difference of only 3 bases in ITS gene regions, that means they are almost 100% identical, therefore, we validated our strain as *G. pseudotenuis*.

*Known distribution:* Hong Kong, Thailand



**Figure 4:** *Gliocladiopsis pseudotenuis* (MFLU 19-0547). (a), (b) Colonies on substrate. (c), (d) Conidiophores with conidia. (e) Conidiogenous cells. (f–i) Conidia. (j) Germinating conidium. (k), (l) Culture on PDA, k obverse and l reverse. Scale bars: c = 50  $\mu\text{m}$ , d, e = 20  $\mu\text{m}$ , f = 10  $\mu\text{m}$ , g–j = 5  $\mu\text{m}$ .

- *Spadicoides cordanoides* Goh & K.D. Hyde, Mycologia 88: 1023 (1997) (Figure 5)

Index Fungorum Registration Identifier 436825

*Classification:* Helminthosphaeriaceae, Sordariales, Sordariomycetidae, Sordariomycetes

*Saprobic* on submerged decaying wood. **Sexual morph:** undetermined. **Asexual morph:** hyphomycetous, *Colonies* on the substratum superficial, effuse, hairy, dark brown to black. *Mycelium* partly immersed, partly superficial, composed of pale brown, septate, smooth, branched hyphae. *Conidiophores* macronematous, mononematous, straight or slightly flexuous, solitary or in small groups, thick-wall, smooth, unbranched, multi-septate, pale brown to brown, paler towards the apex,  $224\text{--}565 \times 4\text{--}6 \mu\text{m}$  ( $\bar{x} = 424 \times 5 \mu\text{m}$ ,  $n = 20$ ), swollen or nodulose at the apex. *Conidiogenous cells* polytretic, integrated, terminal and intercalary, determinate. *Conidia* 1-septate, darkly banded at the septa, acropleurogenous, solitary, versicolorous, thick-walled, predominately 2-celled and obpyriform or ovoid,  $15\text{--}20 \times 9\text{--}13 \mu\text{m}$  ( $\bar{x} = 17.5 \times 11 \mu\text{m}$ ,  $n = 40$ ), base cell smooth, subhyaline to pale brown,  $4\text{--}5 \mu\text{m}$  long,  $5\text{--}6 \mu\text{m}$  wide, more or less obtrapezoidal or subcylindrical, with a truncate base, apical cell enlarged, subglobose,  $8\text{--}12 \mu\text{m}$  long,  $9\text{--}12.5 \mu\text{m}$  wide, dark brown or almost black, verrucous, with guttule when young.

*Material examined:* Thailand, Mukdahan Province, Nong Bo Na Kae,  $16^{\circ}84'96''\text{N}$ ,  $104^{\circ}33'12''\text{E}$ , on dead submerged decaying wood of unidentified plants, 13 December 2018, Hao Yang, t31 (IFRD 500–001).

*Notes:* *Spadicoides cordanoides* was introduced by Goh et al. [26]. They also provided a key to included 20 different species of *Spadicoides*. Subsequently, Ma et al. [27] updated the key of *Spadicoides* that included 40 species. Based on the distinguishing characteristics of the present strain in comparison to that of the 40 known species, we identify our isolate as *S. cordanoides*.

*Known distribution:* Australia, Thailand



**Figure 5:** *Spadicoides cordanoides* (IFRD 500–001). (a), (b) Colonies on substrate. (c–f) Conidiophores and Conidia. (g–i) Conidiogenous cells and conidia. (j–o) Conidia. Scale bars: c = 100 μm, d–f = 50 μm, g–h, k–o = 10 μm, i–j = 20 μm.

- *Sporoschisma longicatenatum* Jiao Yang, Jian K. Liu & K.D. Hyde. Phytotaxa 289: 152 (2016) (Figure 6)

Index Fungorum Registration Identifier 552182

*Classification:* Chaetosphaeriaceae, Chaetosphaeriales, Sordariomycetidae, Sordariomycetes

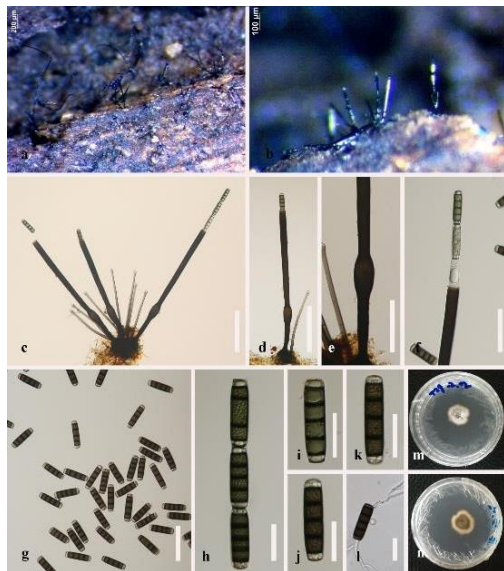
*Saprobic* on submerged decaying wood. **Sexual morph:** undetermined. **Asexual morph:** hyphomycetous, *Colonies* on the substratum superficial, effuse, hairy, gregarious, black, with long chains of conidia. *Mycelium* immersed, composed of dark brown hyphae. *Setae* scattered or in groups mixed with conidiophores, capitate, usually with hyaline, mucilaginous substances at the swollen apex, smooth-walled, pale brown, becoming paler towards the apex, straight or flexuous, 2–3-septate,  $113\text{--}168 \times 4.5\text{--}6 \mu\text{m}$ ,  $6\text{--}8.5 \mu\text{m}$  wide at the swollen apex. *Conidiophores* macronematous, mononematous, smooth, dark brown to black, straight or slightly flexuous, solitary or in small groups of 2–3, each composed of a bulbous base, a cylindrical stipe and a swollen venter with a long cylindrical neck, erect,  $259\text{--}316 \mu\text{m}$  long,  $7.5\text{--}12 \mu\text{m}$  wide below venter and  $13\text{--}15.5 \mu\text{m}$  wide above,  $16\text{--}21 \mu\text{m}$  wide at venter. *Conidiogenous cells* monophialidic, percurrent, integrated, terminal, determinate, brown, lageniform, frayed at the apex, with serrate, flared margin at free end. *Conidia* formed in chains, cylindrical to doliiform,  $31\text{--}47 \times 9\text{--}12.5 \mu\text{m}$  ( $\bar{x} = 41 \times 10 \mu\text{m}$ ,  $n = 30$ ), 5-euseptate, hyaline when young, olivaceous to brown at maturity, with conspicuously darkened septa, occasionally constricted at septa, centre cells brown, end cells paler coloured and shorter than the central four cells.

*Culture characteristics:* *Conidia* germinating on PDA within 24 h and germ tubes produced from both ends. Colonies on PDA attaining 15 mm diameter in 15 days at  $20\text{--}25^\circ\text{C}$  in the condition of 12 h dark and 12 h light, with fluffy, dense, white mycelium on the surface, colonies reverse dark brown at the centre, with white undulate margin.

*Material examined:* Thailand, Mukdahan Province, Nong Bo Na Kae,  $16^\circ 84' 96''\text{N}$ ,  $104^\circ 33' 12''\text{E}$ , small river, on dead submerged decaying wood of unidentified plants, 13 December 2018, Hao Yang, T29 (MFLU 19–0540), living culture = MFLUCC 19–0331.

*Sequences data:* ITS: MN513036, LSU: MN511738.

*Notes:* *Sporoschisma longicatenatum* was introduced by Yang et al. [28]. Even though, our isolate shares similar morphological characters with *S. hemipsilum* (Berk. & Broome) Zelski, A.N. Mill. and Shearer and *S. longicatenatum*. The closest match of ITS sequence of our strain is 100% identical to *S. longicatenatum* strain MFLUCC 16–0180 (KX505871) in the BLASTn search on NCBI GenBank, thus, we identified our strain as *S. longicatenatum*.



**Figure 6:** *Sporoschisma longicatenatum* (MFLU 19-0540). (a), (b) Colonies on submerged wood. (c), (d) Conidiophores and setae. (e) Portion of phialide producing conidium. (f) Conidiophore with conidia. (g–k) Conidia. (l) Germinating conidium on PDA. (m), (n) Culture on PDA, m from front, n from reverse. Scale bars: c, d =  $100 \mu\text{m}$ , e–g =  $50 \mu\text{m}$ , h–l =  $20 \mu\text{m}$ .



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