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ORIGINAL ARTICLE

# Novel chaetosphaeriaceous hyphomycetes from aquatic habitats

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**Abstract** A survey of freshwater ascomycetes conducted in Thailand yielded a number of aquatic hyphomycetes. In this study, fresh collections of three chaetosphaeriaceous species from submerged wood in freshwater are characterized based on morphology and molecular phylogeny. *Dictyochaeta siamensis* sp. nov. and *Tainosphaeria siamensis* sp. nov. are introduced based on morphological and molecular data. A detailed description of *Menisporopsis theobromae* from the new collection is presented and the first molecular data for this genus are provided. Phylogenetic analysis of combined LSU and ITS sequence data was carried out to determine the phylogenetic placement of these species within the family Chaetosphaeriaceae. *Menisporopsis theobromae* showed a close phylogenetic relationship with *Rattania*, but has significantly different morphology; *D. siamensis* clustered together with *Codinaeopsis*, *Menispora*, and *Zignoëlla*; while *T. siamensis* clustered together with *T. crassiparies* but presented as a distinct clade. The phylogenetic relationship between the new taxa and their relatives are compared and discussed.

**Keywords** Asexual morph · New genus · Phylogeny · Sordariomycetes · Taxonomy

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## Introduction

Hyphomycetes are asexual fungi producing conidia and conidiogenous cells directly on the mycelium (Seifert et al. 2011). Currently, 1800 genera comprising 9000 species have been described worldwide (Kirk et al. 2008). Despite the large number of hyphomycetes documented, little is known connecting them to their sexual forms and, hence, their systematic placement. However, it is possible to link asexual morph taxa to their sexual morphs using molecular phylogenetic analysis.

Aquatic hyphomycetes are a dominant and diverse group of asexual fungi which are involved in litter degradation in freshwater ecosystems (Ho et al. 2001; Cai et al. 2003; Tsui and Hyde 2004; Krauss et al. 2011; Hyde et al. 2016). Taxa in this habitat are generally saprobic; however, some may also be pathogens or symbionts (Wong et al. 1998). The present study deals with three saprobic hyphomycetes in Chaetosphaeriaceae, a family which was established for *Chaetosphaeria* and its allies (Réblová et al. 1999). The type genus *Chaetosphaeria* was introduced by Tulasne and

Tulasne (1863) based on the type species, *C. innumera*. Species of *Chaetosphaeria* produce both perithecial ascomata and conidia, while some species apparently produce only conidia. Among those species for which both sexual and asexual morphs are known, the species differences are more readily seen in the asexual morphs than in the sexual morphs. Therefore, the diagnostic taxonomic value of individual morphological characters of the asexual morphs, particularly those of conidia and conidiogenous cells, has been discussed several times (Gams and Holubová-Jechová 1976; Kendrick 1980; Arambarri and Cabello 1989; Réblová 2000; Li et al. 2012). However, there are no review papers addressing the characters and phylogeny of asexual morphs. Réblová (2000) suggested that ontogenetic characters, such as conidiogenous cells and conidia, are useful to show the phylogenetic relationships and classify the asexual morph genus *Chaetosphaeria*. Lumbsch and Huhndorf (2010) listed ten genera (mostly sexual genera) in the family Chaetosphaeriaceae and Maharachchikumbura et al. (2015) listed 35 genera, which included most of the sexual and asexual genera, based on the literature.

In this study, we collected freshwater chaetosphaeriaceous hyphomycetes in Thailand and our studies yielded two novel species and one collection of *Menisporopsis theobromae* (type of the genus). The new species *Dictyochaeta siamensis* and *Tainosphaeria siamensis* are introduced, along with the first sequence data for *M. theobromae*. For those genera in Chaetosphaeriaceae that have been sequenced, phylogenetic analyses are presented to provide further evidence for the uniqueness of these taxa, and the new taxa are described and compared with related taxa.

## Materials and methods

### Isolation and morphology

Specimens of submerged decaying wood were collected from Chiang Rai and Prachuap Khiri Khan Provinces, Thailand during November to December 2014 and returned to the laboratory in plastic bags. The samples were incubated in plastic boxes lined with moistened tissue paper at room temperature for one week. The samples were processed and examined following the methods described by Taylor and Hyde (2003). Morphological observations were made using a Motic SMZ168 Series stereomicroscope and photographed by a Nikon E80i microscope camera system. Measurements were made with Tarosoft (R) Image Frame Work (Liu et al. 2010).

Isolations were made from single spores as described by Liu et al. (2010). Type material is deposited at the herbarium of Guizhou Academy of Agriculture Sciences (GZAAS), Guiyang, China and Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Fungi isolated in our study were deposited at Mae Fah Luang University Culture Collection (MFLUCC) and Guizhou Culture Collection, China (GZCC). Facesoffungi numbers and Index Fungorum numbers are provided as outlined by Jayasiri et al. (2015) and in Index Fungorum (2016).

### DNA extraction, PCR amplification, and sequencing

Fungal isolates were grown on PDA for 21 days at 28 °C in the dark. Genomic DNA was extracted from the fresh mycelium using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®), following the manufacturer's protocol (Hangzhou, P.R. China).

DNA amplification was performed by polymerase chain reaction (PCR). Two partial gene portions were used in this study: the internal transcribed spacers (ITS) and the large subunits of the nuclear ribosomal RNA genes (LSU). The primers used were ITS5 and ITS4 (White et al. 1990) for ITS, and LROR and LR5 (Vilgalys and Hester 1990) for LSU. The PCR thermal cycle program for ITS and LSU amplification was as follows: initially denaturing step of 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 50 s, elongation at 72 °C for 1 min, and a final extension at 72 °C for 10 min.

PCR products were purified using minicolumns, purification resin, and buffer according to the manufacturer's protocols (Amersham product code: 27-9602-01). Sequence analysis was carried out by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, P.R. China).

### Phylogenetic analysis

Sequences generated from different primers were analyzed with other sequences obtained from GenBank. The related sequences were determined by using a BLAST search to reveal the closest matches with taxa in Chaetosphaeriaceae and recent relevant publications (Lumbsch and Huhndorf 2010; Crous et al. 2012; Hashimoto et al. 2015a, b). Sequences were aligned using BioEdit 7.2.5 (Hall 1999) and ClustalX v.1.83 (Thompson et al. 1997). The alignments were checked visually and improved manually where necessary. Phylogenetic analyses were performed by using PAUP v.4.0b10 (Swofford 2002) for maximum parsimony (MP) and MrBayes v.3.0b4 (Huelsenbeck and Ronquist 2001) for Bayesian analyses.

A maximum likelihood (ML) analysis was performed at the CIPRES web portal (Miller et al. 2010) using RAxML v.7.2.8 as part of the "RAxML-HPC2 on TG" tool (Stamatakis 2006). A general time-reversible model (GTR) was applied with a discrete gamma distribution and four rate classes. Fifty thorough ML tree searches were done in RAxML v.7.2.7 under the same model. One thousand non-parametric bootstrap

iterations were run with the GTR model and a discrete gamma distribution. The resulting replicates were plotted onto the best scoring tree obtained previously.

MP analyses were performed using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight, and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis and Bull 1993). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v.3.0b4 (Huelsenbeck and Ronquist 2001). Six simultaneous Markov chains were run for 3,000,000 generations and trees were sampled every 1000th generation a tree was sampled. The MCMC heated chain was set with a "temperature" value of 0.15. All sampled topologies beneath the asymptote (20 %) were discarded as part of a burn-in procedure; the remaining trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree.

Phylogenetic trees were drawn using TreeView (Page 1996) and MEGA5 (Tamura et al. 2011). The sequences derived in this study are deposited in GenBank (Table 1).

## Results

### Phylogenetic analysis

Three isolates of hyphomycetes obtained from the incubated specimens of submerged wood were identified in the family Chaetosphaeriaceae. ITS and LSU sequence data and morphological characters were used to assign the species or genus and to describe novel taxa with a comparison with similar taxa.

The combined LSU and ITS dataset comprised 45 taxa, with *Lasiosphaeria ovina* (SMH 4605) as the outgroup taxon. The dataset comprises 1648 characters after alignment: 1112 characters were constant and 367 characters were parsimony informative, while 169 variable characters were parsimony-uninformative. RAxML, MP, and Bayesian analysis of the combined dataset resulted in phylogenetic reconstructions with largely similar topologies, and the Bayesian tree is shown in Fig. 1.

Representatives of the sequenced genera (with molecular data) of Chaetosphaeriaceae (Réblová and Winka 2000; Lumbsch and Huhndorf 2010; Crous et al. 2012; Hashimoto et al. 2015a, b; Liu et al. 2015; Maharachchikumbura et al. 2015) are included in our phylogenetic analysis (Fig. 1). Twenty-six genera are represented by at least one species in Chaetosphaeriaceae. Some were introduced or studied recently, such as *Neopseudolachnella* and *Pseudodinemasporium* (Hashimoto et al. 2015b), *Brunneodinemasporium*, *Dendrophoma*, and *Dinemasporium* (Crous et al. 2012), *Infundibulomyces* (Somrithipol et al. 2008), *Tainosphaeria* (Fernández and Huhndorf 2005), and *Pyrigemmula* (Magyar et al. 2011). Most of them are asexual taxa. Our three isolates were also included in the analysis of combined LSU and ITS sequence data. One isolate was identified as *Menisporopsis theobromae* and clustered with *Rattania* with high statistical support, but is quite different morphologically. *Dictyochaeta siamensis* formed a sister clade to the *Codinaeopsis*, *Menispora*, and *Zignoëlla* clade in Chaetosphaeriales. The third isolate clustered with *Tainosphaeria crassiparies* in a well-supported clade. It was, however, phylogenetically and morphologically distinct and is introduced as *T. siamensis* sp. nov. in this paper.

## Taxonomy

***Dictyochaeta siamensis*** J. Yang, K.D. Hyde & J.K. Liu, sp. nov. **Fig.** 2

Index Fungorum number: IF552464; Facesoffungi number: FoF 02188

Holotype: MFLU 15-1149

**Etymology** Named after the country from where this fungus was collected, Thailand.

*Saprobic* on decaying plant twigs in freshwater. *Colonies* effuse, brown, with long hairy mycelium, with white glistening conidial mass. *Mycelium* partly immersed, partly superficial, consisting of branched, septate, smooth, thin-walled, brown hyphae. ***Asexual morph***: *Setae* erect, straight, dark brown at the base, fading towards the apex, acerose to subacerose, septate, unbranched, smooth, fertile, and tapering at distal ends, 165–365 × 3–6 μm. *Conidiophores* mononematous, macronematous, brown at the base, fading to pale brown towards the apex, 4–6 septate, unbranched, cylindrical, straight or slightly curved, erect, smooth, arising singly or in groups from the mycelial knots from the bases of setae, 60–100 × 2–5 μm. *Conidiogenous cells* mostly monophialidic or rarely polyphialidic, sometimes percurrently proliferating, integrated, terminal, and determinate, with conspicuous collarettes. *Conidia* 15.5–21 × 2.5–4 μm ($\bar{x}$ = 17.5 × 3 μm, n = 20), aggregated in slimy mass at the apex of the conidiophore, acrogenous, enteroblastic, hyaline, aseptate,

**Table 1** Isolates used in this study and their LSU and ITS GenBank accession numbers ([T]: ex-type/ex-epitype isolates)

| Taxon | Culture accession no. | GenBank accession no. | |
|---|---|---|---|
| | | LSU | ITS |
| *Brunneodinemasporium brasiliense* | CBS 112007[T] | JQ889288 | JQ889272 |
| *Chaetosphaeria innumera* | SMH 2748 | AY017375 | AY906956 |
| *Chaetosphaeria preussii* | CBS 262.76 | AF178561 | |
| *Chloridium lignicola* | CBS 143.54 | AF178544 | AF178544 |
| *Codinaeopsis gonytrichoides* | CBS 593.93 | AF178556 | AF178556 |
| *Dendrophoma cytisporoides* | CBS 223.95[T] | JQ889289 | JQ889273 |
| *Dictyochaeta siamensis* | MFLUCC 15-0614[T] | KX609952 | KX609955 |
| *Dictyochaeta simplex* | CBS 966.69 | AF178559 | AF178559 |
| *Dinemasporium decipiens* | CBS 592.73 | JQ889291 | JQ889275 |
| *Dinemasporium morbidum* | CBS 129.66[T] | JQ889296 | JQ889280 |
| *Dinemasporium morbidum* | CBS 995.97 | JQ889297 | JQ889281 |
| *Dinemasporium nelloi* | MFLUCC 13-0482[T] | KP711363 | KP711358 |
| *Dinemasporium polygonum* | CBS516.95[T] | JQ889292 | JQ889276 |
| *Dinemasporium pseudoindicum* | CBS 127402[T] | JQ889293 | JQ889277 |
| *Ellisembia brachypus* | HKUCC 10555 | DQ408563 | |
| *Exserticlava vasiformis* | TAMA 450 | AB753846 | |
| *Infundibulomyces cupulata* | BCC11929[T] | EF113979 | |
| *Infundibulomyces oblongisporus* | BCC13400[T] | EF113980 | |
| *Lasiosphaeria ovina* | SMH4605 | AY436413 | |
| *Lecythothecium duriligni* | CBS 101317 | AF261071 | |
| *Melanochaeta aotearoae* | SMH 3551 | AF466082 | |
| *Melanochaeta hemipsila* | SMH 2125 | AY346292 | |
| *Melanochaeta taitensis* | GKM156N | EU583220 | |
| *Melanochaeta taitensis* | GKM150N | EU583219 | |
| *Melanopsammella gonytrichii* | SMH 3785 | AF466085 | |
| *Melanopsammella vermicularioides* | FC 404 | AF466087 | |
| *Menispora tortuosa* | DAOM 231154 | AY544682 | KT225527 |
| *Menispora tortuosa* | CBS 214.56 | AF178558 | AF178558 |
| *Menisporopsis theobromae* | MFLUCC 15-0055 | KX609954 | KX609957 |
| *Neopseudolachnella magnispora* | MAFF 244359[T] | AB934042 | AB934066 |
| *Neopseudolachnella uniseptata* | MAFF 244360[T] | AB934043 | AB934067 |
| *Pseudodinemasporium fabiforme* | MAFF 244361[T] | AB934044 | AB934068 |
| *Pseudolachnea fraxini* | CBS 113701[T] | JQ889301 | JQ889287 |
| *Pseudolachnella asymmetrica* | MAFF 244366[T] | AB934049 | AB934073 |
| *Pseudolachnella botulispora* | MAFF 244367[T] | AB934050 | AB934074 |
| *Pyrigemmula aurantiaca* | CBS 126743[T] | HM241692 | HM241692 |
| *Pyrigemmula aurantiaca* | CBS 126744 | HM241693 | HM241693 |
| *Rattania setulifera* | GUFCC 15501 | HM171322 | GU191794 |
| *Striatosphaeria codinaeophora* | SMH 1524 | AF466088 | |
| *Tainosphaeria crassiparies* | SMH 1934T | AF466089 | |
| *Tainosphaeria siamensis* | MFLUCC15-0607[T] | KX609953 | KX609956 |
| *Thozetella nivea* | | EU825200 | EU825201 |
| *Umbrinosphaeria caesariata* | CBS 102664 | AF261069 | |
| *Zignoëlla pulviscula* | MUCL 15710 | AF466090 | |
| *Zignoëlla pulviscula* | SMH 3289 | AF466091 | |

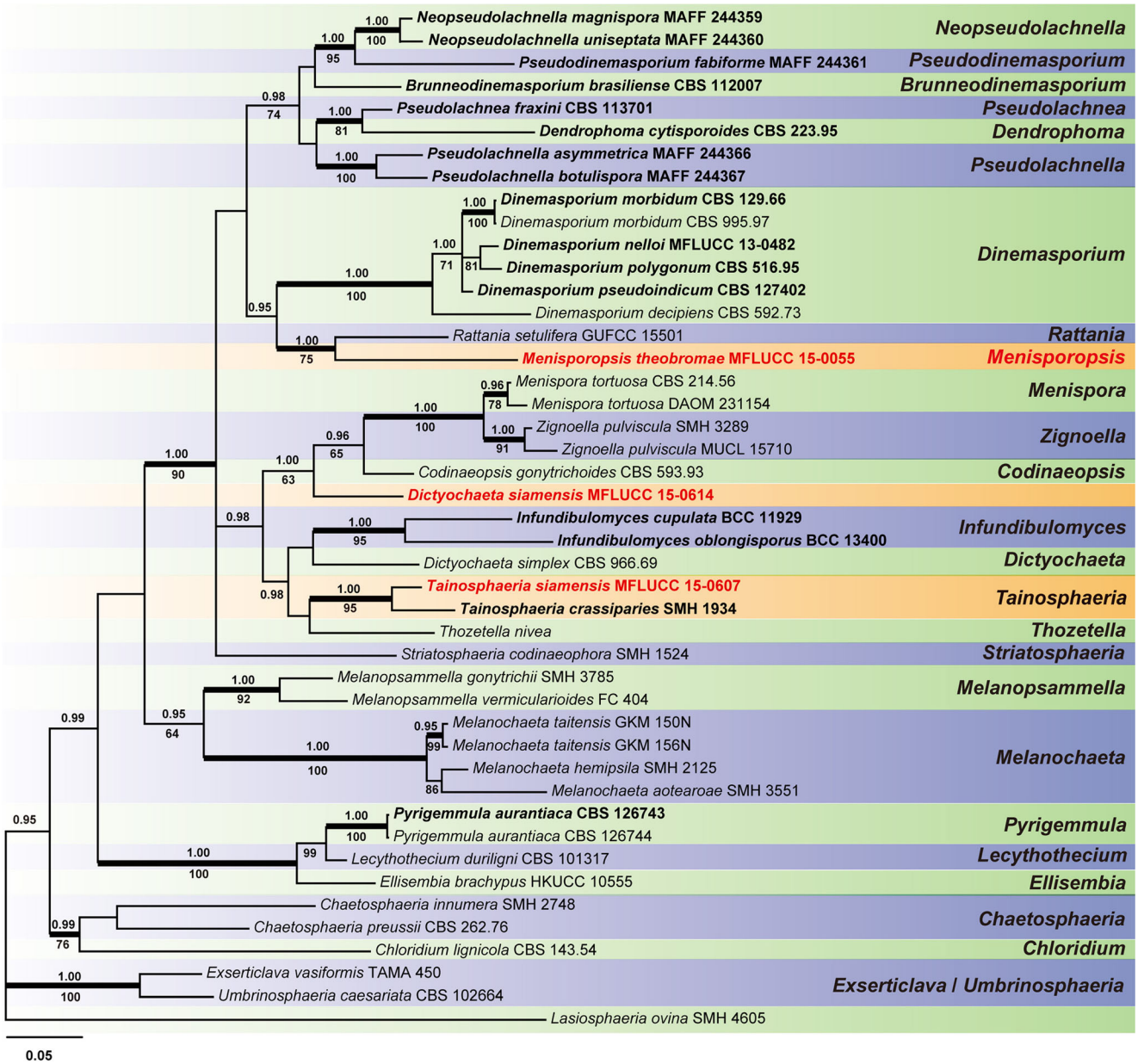


**Fig. 1** Consensus phylogram (50 %) majority rule resulting from a Bayesian analysis of a combined LSU and ITS sequence alignment of Chaetosphaeriaceae. Bayesian posterior probabilities (PP) above 0.95 and ML bootstrap proportion (BP) greater than 50 % are presented at the nodes as BP/PP. Branches with more than 75 % bootstrap (ML) are in **bold**. The original isolate numbers are noted after the species names, the new isolates are in *red*, and ex-type isolates are in **bold**. The scale bar shows 0.05 changes and the tree is rooted to *Lasiosphaeria ovina* (SMH 4605)

cylindrical or long fusiform, curved, lunar, with 7–12-μm-long hair-like appendages at both ends, smooth, rounded at apex. ***Sexual morph***: Not observed

**Material examined** THAILAND, Prachuap Khiri Khan Province, Hua Hin, Kaeng Krachan, on plant twigs in stream running from the national park, 25 December 2014, Jaap van Strien Site 4 15-2 (MFLU 15-1149, holotype); ex-type living culture MFLUCC 15-0614; GZCC 15-0060.

**Notes** The combined LSU and ITS phylogenetic analysis showed that *Dictyochaeta siamensis* clustered together with *Menispora*, *Zignoëlla*, and *Codinaeopsis*, and, although they are phylogenetically related, they are morphologically distinct


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◀ **Fig. 2** *Dictyochaeta siamensis* (MFLU 15-1149, holotype). **a** Substrate. **b** Colonies. **c** Conidiophore and conidiogenous cell. **d**, **e** Setae. **f**–**h** Conidiophores and conidiogenous cell. **i**–**n** Conidia. **o** Germinating conidium. **p**–**q** Culture. Scale bars: **b** = 200 μm; **c**, **d** = 50 μm; **e**, **f**, **h**, **o** = 30 μm; **g**, **l** = 20 μm; **j**–**n** = 15 μm

genera. *Codinaeopsis* has discrete conidiogenous cells produced in verticels along an erect seta; *Menispora* also has mainly discrete conidiogenous cells on conidiophores, the upper part of which is usually sterile. The new taxon *D. siamensis* has five or six conidiophores, each grouped around a single seta, and these morphological characters suggest that the taxon is a *Dictyochaeta* species. A key to *Dictyochaeta* species was provided by Whitton et al. (2000); however, *D. siamensis* cannot be assigned to any species. In addition, *D. siamensis* differs from the recently described species *D. aciculata* by its long fusiform, aseptate conidia, while the latter species has 3-septate, acicular conidia (Silva and Gusmão 2013). The molecular phylogeny of *Dictyochaeta* species is unresolved and there are few reliable sequences available in GenBank, although many species have been described in the genus. In our phylogenetic analysis, *D. simplex* (CBS 966.69) was included to represent *Dictyochaeta* and the result showed that the new taxon is not related; however, this is not an ex-type species. New collections and molecular data are required for *Dictyochaeta* species to determine their phylogenetic placement in Chaetosphaeriaceae, especially the type species. *Menispora* and *Zignoëlla* were reported as related genera (Fernández et al. 2006; Somrithipol et al. 2008); however, the relationship between these two genera requires further data for confirmation.

***Tainosphaeria siamensis*** J. Yang, K.D. Hyde & J.K. Liu, sp. nov. **Fig.** 3

Index Fungorum number: IF552113; Facesoffungi number: FoF 02189

Holotype: MFLU 15-1142

**Etymology** Named after the country from where this fungus was collected, Thailand.

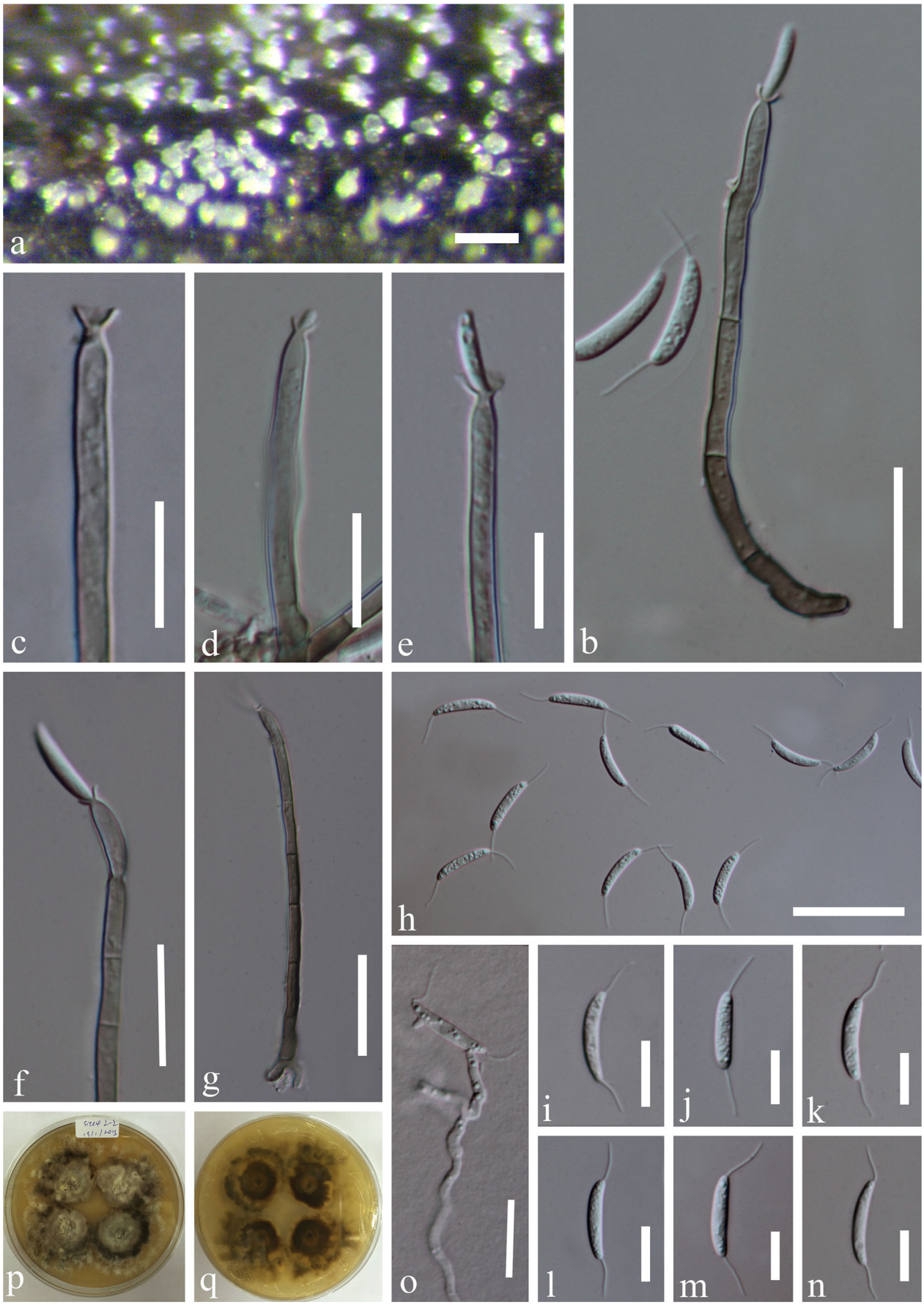
*Colonies* on submerged wood, effuse, aggregate, brown, as a hyaline, glistening conidial mass, hairy short. ***Asexual morph***: *Conidiophores* mononematous, macronematous, brown at the base fading to pale brown towards the apex, 40.5–86 μm long, 2.5–4 μm wide, 2–4 septate, unbranched, cylindrical, straight or slightly curved, erect, solitary, smooth. *Conidiogenous cells* monophialidic, integrated, terminal, determinate, with conspicuous collarettes. *Conidia* 13.5–19 × 2–3.5 μm ($\bar{x}$ = 16 × 2.5 μm, n = 20), aggregated in slimy mass at the apex of the conidiophore, acrogenous, enteroblastic, phialidic, hyaline, aseptate, cylindrical or long fusiform, with 3.6–10-μm-long hair-like appendages at both ends, smooth, apex rounded and slightly. ***Sexual morph***: Not observed.

**Material examined** THAILAND, Prachuap Khiri Khan Province, Hua Hin, Kaeng Krachan, on submerged wood, 25 December 2014, Jaap van Strien, Site 4 15-2 (MFLU 15-1142, holotype); ex-type living culture MFLUCC 15-0607; GZCC 15-0056.

**Notes** *Tainosphaeria* was introduced by Fernández and Huhndorf (2005) as a monotypic genus with *T. crassiparies* as the type species; both the asexual and sexual morphs were found from the substrate and described. *Tainosphaeria siamensis* was found on submerged wood with only the asexual morph; the morphology matches the genus *Tainosphaeria*. In a MegaBLAST search of GenBank, the closest hits for the LSU sequence data are *Tainosphaeria crassiparies* strain SMH 1934 [GenBank AF466089, identities 811/826 (98 %)] and *Pseudolachnea fraxini* strain CBS 113701 [GenBank JQ889301, identities 800/829 (97 %)]; for ITS, the closest hit is *Dictyochaeta simplex* strain ICMP 14613 [GenBank EF029193, identities 456/515 (89 %)]; the latter BLAST hits are probably because of the lack of ITS sequences for genera closely related to *Tainosphaeria*. The combined LSU and ITS sequence analysis showed that these two species of *Tainosphaeria* are phylogenetically distinct and form a well-supported (100 % ML, 1.00 PP) clade in the family Chaetosphaeriaceae. This also confirms *Tainosphaeria* as a well-supported genus of Chaetosphaeriaceae. Morphologically, *T. siamensis* differs from *T. crassiparies* by its larger conidia (13.5–19 × 2–3.5 μm versus 10.5–14.8 × 2–3 μm in *T. crassiparies*) (Fernández and Huhndorf 2005).

***Menisporopsis theobromae*** S. Hughes., *Mycol Pap* 48: 59 (1952) **Fig.** 4

*Colonies* on submerged wood, superficial, scattered, effuse, white to pale brown. Mycelium partly immersed, composed of brown hyphae. ***Asexual morph***: *Setae* central, 223 × 5.5 μm, solitary, erect, brown, septate, thick-walled, lower part encased tightly by compact conidiophores and obviously wider than each conidiophore. *Conidiophores* synnematous, macronematous, brown, smooth, thin to thick-walled, septate, unbranched, cylindrical, lower part narrow, upper part wider, erect, straight or slightly flexuous, up to 112.5 μm long. *Conidiogenous cells* terminal, monophialidic, integrated, pale brown, with collarettes. *Conidia* 14–19 × 2–3 μm ($\bar{x}$ = 17 × 2.5 μm, n = 20), hyaline, aseptate, thin-walled, smooth, acrogenous, aggregated in slimy masses at the apex of the synnemata, fusiform, gently curved or straight, with a single and unbranched, flexuous, hyaline, hair-like appendages at each end, 6–7.5 μm long. ***Sexual morph***: Not observed.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◀ **Fig. 3** *Tainosphaeria siamensis* (MFLU 15-1142, holotype). **a** Colonies. **b**–**g** Conidiophores and conidiogenous cells. **h**–**n** Conidia. **o** Germinating conidium. **p**–**q** Culture. Scale bars: **a** = 100 μm; **b**, **g**, **o** = 20 μm; **c**–**e**, **i**–**n** = 10 μm; **f** = 15 μm; **h** = 30 μm

**Material examined** THAILAND, Chiang Rai Province, stream flowing near Tham Luang Nang Non Cave, on submerged wood, 25 November 2014, Yang Jing (MFLU 15-1168, **reference specimen designated here**), living culture MFLUCC 15-0055; GUCC 16-0003.

**Notes** The production of a synnematous group of conidiophores around a conspicuous polar setulum places our new collection in *Menisporopsis*. *Menisporopsis* was introduced with *M. theobromae* as the type and was found on decaying leaves of *Theobroma cacao* in Ghana (Hughes 1952). Most *Menisporopsis* species were originally described as occurring on decaying leaves or wood with a pantropical distribution (Seifert et al. 2011), and *M. theobromae* is widespread. There are nine species accepted in the genus and keys were provided by Tsui et al. (1999) and Castañeda Ruiz et al. (2001). The newly collected fungus from submerged wood in Thailand is morphologically similar to *M. kobensis* and *M. theobromae*. However, its smaller conidia identifies this species as *M. theobromae*, while the conidia of *M. kobensis* are 16–32 × 3–5 μm and 14–19 × 2–3 μm in *M. theobromae*, which is in agreement with the original description by Hughes (1952). We, therefore, designate it as a reference species (*sensu* Ariyawansa et al. 2014).

The phylogenetic analysis placed the *Menisporopsis theobromae* isolate close to *Rattania*, which is a monotypic

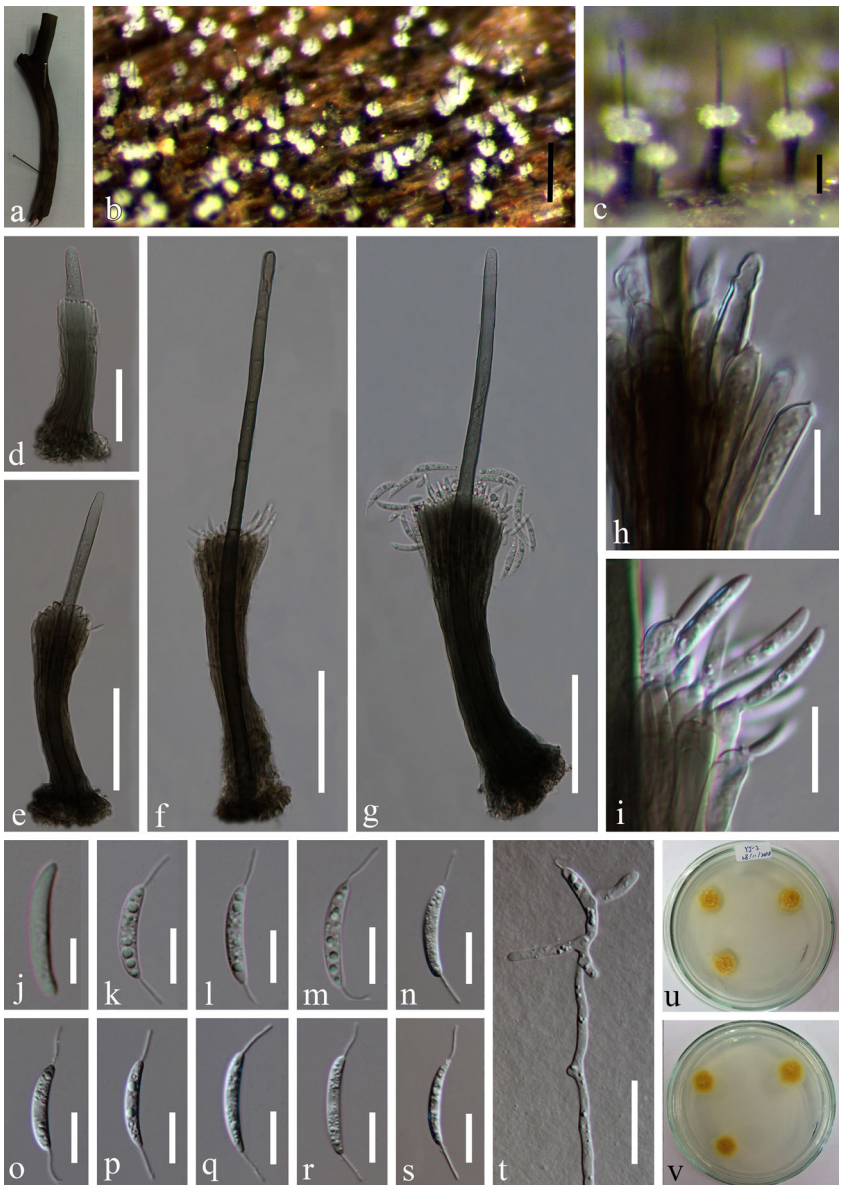


**Fig. 4** *Menisporopsis theobromae* (MFLU 15-1168). **a** Substrate. **b** Colonies. **c** Fruiting bodies. **d**–**f** Conidiophore. **g** Fruiting body. **h**, **i** Conidiogenous cells. **j**–**s** Conidia. **t** Germinating conidium. **u**, **v** Culture. Scale bars: **b** = 200 μm; **c** = 50 μm; **d** = 30 μm; **e**–**g** = 50 μm; **h**–**i**, **k**–**s** = 10 μm; **j** = 5 μm; **t** = 20 μm

genus. However, it differs from *Rattania* by its long synnematous conidiophores and the single seta associated with each synnema, while *Rattania* has very short conidiophores arranged in a sporodochium with multiple setae. *Menisporopsis theobromae* also shares similar conidia characters with *Dinemasporium* and *Pseudolachnella*, but the latter genera are coelomycetous. The isolate of *M. theobromae* formed a distinct clade in Fig. 1, and based on both the morphology and molecular phylogeny, this genus is distinct.

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