New species of Sporoschisma (Chaetosphaeriaceae) from aquatic habitats in Thailand

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Abstract

Collections of submerged wood in streams in Prachuap Khiri Khan Province, Thailand yielded three sporoschisma-like taxa. Morphological examination and phylogenetic analysis of LSU sequence data support the separation of two isolates as new species (S. palauense and S. longicatenatum) and the other as collection of S. hemipsila. The sexual morph (Melanochaeta) is again linked to the asexual genus Sporoschisma by molecular data. The new species are introduced with descriptions and illustrations and compared with the most similar species.

Key words: asexual fungi, new species, phylogeny, taxonomy

Introduction

We are carrying out a survey of freshwater fungi on submerged wood along a north / south gradient in the Asian /Australasian region (Hyde et al. 2016). In this study we collected several sporoschisma-like taxa in Prachuap Khiri Khan Province, Thailand.

The ascomycete genus Sporoschisma Berk. & Broome was introduced by Berkeley (1847) with S. mirabile Berk. & Broome as the type, and is assigned to the family Chaetosphaeriaceae, order Chaetosphaeriales (Maharachchikumbura et al. 2015, 2016). There are 23 epithets in Index Fungorum (2016). The genus is characterized by reduced, unbranched, brown conidiophores; phialidic conidiogenous cells with deep, cylindrical collarettes; phragmosporous, brown conidia in basipetal chains; vesiculate, capitulate setae with mucilaginous apices among the conidiophores and a stromata is sometimes present (Seifert et al. 2011). Most Sporoschisma species occur on submerged wood in freshwater (Goh et al. 1997, Ho et al. 2001, 2002, Zelski et al. 2014). Major revisions of the genus have been provided by Hughes (1949, 1966) and Goh et al. (1997). Hughes (1949, 1966) excluded five taxa which were species of blue-green alga, namely, S. juniperi Lind & Vleugel, S. mirabile var. lichenica Gonz. Frag., S. mori Sawada & Katsuki, S. stilboideum Bat. & J.L. Bezerra and S. tracyi Earle. Sporoschisma ampullula Sacc., S. connari Bat. & Peres, S. insigne Sacc., S. montellicum Sacc. and S. paradoxum De Seynes were excluded because of their hyaline phialoconidia or lack of capitate setae. Sporoschisma mirabile var. attenuatum Cavara was regarded as synonymous with the generic type (Hughes 1949). Recently, Sporoschisma australiense (Goh & K.D. Hyde) Réblová was transferred from Sporoschismopsis australiensis Goh & K.D. Hyde following the delimitation of the genus (Réblová 2014). The sexual genus Melanochaeta established by Müller et al. (1969) accommodates lignicolous species that bear superficial ascomata with capitate setae, arising from the entire perithecial surface (Mugambi & Huhndorf 2008). Melanochaeta and its species have been linked with Sporoschisma via cultural and molecular studies and were thus synonymized under Sporoschisma (Réblová et al. 2016).

During studies of freshwater fungi on submerged wood, three species of Sporoschisma were collected. Phylogenetic
analysis of LSU sequence data and morphological characters strongly support the placement of the four isolates within the genus *Sporoschisma*. Two collections match *Sporoschisma hemipsila* and the other two isolates represent new taxa. We therefore introduce *Sporoschisma palauense* and *S. longicatenatum* as new species, with an illustrated account, and support from molecular data.

**Materials and methods**

**Collection and examination of specimens**

Specimens of submerged, decaying wood were collected from streams flowing from waterfalls in Hua Hin, Prachuap Khiri Khan Province, Thailand, in December 2014. Specimens were brought to the laboratory in plastic bags and incubated in plastic boxes lined with moistened tissue paper at room temperature for one week. The samples were processed and examined following the method described in Taylor & Hyde (2003). Morphological observations were made using a Motic SMZ 168 Series dissecting microscope for fungal fruiting bodies. The fungi were examined using a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 600D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS6 software. Single spore isolations were made onto potato dextrose agar (PDA) and later transferred onto malt extract agar (MEA) following the method of Chomnunti et al. (2014). Specimens (dry wood material with fungal material) are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Axenic cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and the Guizhou Culture Collection (GZCC). Facesoffungi and Index Fungorum numbers are registered as outlined in Jayasiri et al. (2015) and Index Fungorum (2016).

**DNA extraction, PCR amplification and sequencing**

Total genomic DNA was extracted from fresh fungal mycelia (500 mg) scraped from the margin of a colony on a MEA plate incubated at 25 °C for 14 days (Guo et al. 2000). The primer pairs LROR and LR5 as defined by Vilgalys & Hester (1990) were used to amplify a segment of the large subunit rDNA (LSU). The amplifications were performed in 25 μL of PCR mixtures containing 9.5 μL ddH₂O, 12.5 μL 2 × PCR Master Mix (TIANGEN Co., China), 1 μL of DNA template and 1 μL of each primer (10 μM). The amplification condition for LSU consisted of initial denaturation at 94 °C for 4 min; followed by 35 cycles of 45 s at 94 °C, 45 s at 56 °C and 1 min at 72 °C, and a final extension period of 10 min at 72 °C. The PCR product was observed on 1% agarose electrophoresis gel stained with ethidium bromide. Purification and sequencing of PCR product was carried out using the above mentioned PCR primer at Invitrogen Biotechnology Co., China.

**Phylogenetic analysis**

Sequences were optimized manually to allow maximum alignment and maximum sequence similarity. The sequences were aligned using the online multiple alignment program MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2013). The alignments were checked visually and improved manually where necessary.

Phylogenetic analysis of the sequence data consisted of maximum likelihood (ML) using RAxML-HPC v.8 (Stamatakis 2006, Stamatakis et al. 2008) on the XSEDE Teragrid of the CIPRES science Gateway (https://www.phylo.org ) (Miller et al. 2010) with rapid bootstrap analysis, followed by 1000 bootstrap replicates. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTRGAMMA substitution model. Maximum-parsimony 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 & Bull 1993). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains were run for 1000000 generations and trees were sampled every 100th generation (resulting in 10000 total trees). The first 2000 trees, representing the burn-in phase of the analyses, were discarded and the remaining 8000 trees used for calculating posterior probabilities (PP) in the majority rule consensus tree.
The resulting trees were printed with FigTree v. 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/), and the layout was created in Adobe Illustrator CS v. 6. Sequences generated in this study are deposited in GenBank (Table 1).

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Results

Phylogenetic analyses
The alignment comprised 22 strains, including four new isolates, with *Sordaria fimicola* (SMH 4106) as the outgroup taxon. The dataset comprised 830 characters including gaps. The genera included in our phylogenetic analysis were selected based on the BLAST searches of LSU sequence data and the best scoring RAxML tree is shown in Fig. 1. Based on the phylogenetic analysis of LSU sequence data, the placements of the four isolates are well supported within *Sporoschisma*. Two isolates formed distinct clades within *Sporoschisma* and these are described as new species, *S. palauense*, which represents a sister taxon to the type species *S. mirabile*, and *S. longicatenatum* as a sister taxon to *S. hemipsila*. The other isolates clustered with *S. hemipsila* and are morphologically similar.
FIGURE 1. Bayesian 50 % majority rule consensus tree for the analyzed Sporoschisma isolates based on a dataset of LSU sequence data. The Bayesian posterior probabilities greater than 0.90 and bootstrap support values for maximum likelihood greater than 50 are given at the nodes (PP/BP). The tree is rooted with Sordaria fimicola. The strains in this study are in black bold. The original isolate numbers are noted after the species names.

Taxonomy

*Sporoschisma hemipsila* (Berk. & Broome) Zelski, A.N. Mill. & Shearer, *IMA Fungus* 5(2): 433 (2014). Fig. 2


*Saprobic* on decaying plant substrates. **Asexual morph**: Colonies effuse, black, hairy. *Mycelium* immersed, composed of pale to dark brown hyphae. *Setae* scattered or in groups mixed with conidiophores, capitate, usually with hyaline mucilage at the swollen apex, smooth-walled, pale to medium brown, becoming paler towards the apex, straight or flexuous, 2–3-septate, 75–175 × 4.5–7 μm, 6–12 μm wide at the swollen apex. *Conidiophores*
FIGURE 2. Sporoschisma hemipsila (MFLU15-1150) a. Colony on wood. b–d. Conidia, setae and conidiophores. e, f. Portion of phialide producing conidia. g–j. Conidia. k. Germinating conidium. l, m. Cultures on MEA, l from above, m from below. Scale bars: a = 200 μm, b, c, f = 50 μm, c = 60 μm, d = 70 μm, g, h = 40 μm, i – k = 30 μm.
macronematous, mononematous, smooth, dark brown to black, paler at the apex, straight or slightly flexuous, solitary or in small groups of 2–3 with 1–4 setae, each composed of a bulbous base, a cylindrical stipe and a swollen venter with a long cylindrical neck, erect, 210–255 μm long, 9.5–16 μm wide below venter and 14–21 μm wide above, 17.5–26 μm wide at venter, with a vase-like apex. Conidiogenous cells monophialidic, integrated, terminal, determinate, brown, lageniform, with serrated, flared margin at free end. Conidia formed enteroblastically inside the tubular collarette of the conidiogenous cell and emerging in a chain, cylindrical to doliiform, 36.5–53 × 10–13 μm (X = 45.5 × 11.5 μm, n = 30), 1–5-euseptate, with conspicuously darkened septa, hyaline when young, olivaceous to brown at maturity, the two end cells paler and shorter than the central four cells. Sexual morph: not observed.

Material examined:—THAILAND. Prachuap Khiri Khan Province: Hua Hin, stream outside Kaeng Krachan National Park, on submerged wood, 25 December 2014, Jaap van Strien, Site 4-16-6 (MFLU15-1150), living culture, MFLUCC15-0615, GZCC15-0069; Site 5-8-1 (MFLU16-1324), living culture, MFLUCC16-0177.

Sporoschisma palauense J. Yang, J.K. Liu & K.D. Hyde, sp. nov. Fig. 3

Index Fungorum Number: IF552181, Facesoffungi Number: FoF02242.

Etymology:—Referring to the Pala-U waterfall, near where the holotype was collected.

Holotype:—MFLU15-1151.

Saprobic on decaying plant substrates. Asexual morph: Colonies effuse, black, hairy, with long chains of conidia. Mycelium immersed, composed of pale to dark brown hyphae. Setae scattered or in groups among conidiophores, capitately, usually with hyaline mucilage at the swollen apex, smooth-walled, pale to medium brown, becoming paler towards the apex, rarely with a knob at the upper part, straight or curved, 2–4-septate, 115–215 × 3.5–6 μm, 4.5–8.5 μm wide at the swollen apex. Conidiophores macronematous, mononematous, smooth, dark brown to black, straight or slightly flexuous, scattered to gregarious, arising from dark bulbous base, composed of a cylindrical stipe and a swollen venter, with a long cylindrical erect neck, 170–310 μm long, stipes 6.5–10 μm wide below venter and 12–18 μm wide above, 13.5–27 μm wide at the venter. Conidiogenous cells monophialidic, integrated, terminal, determinate, brown, lageniform, consisting of a swollen venter and a tubular collarette. Conidia catenate, formed endogenously in basipetal succession, cylindrical to doliiform, hyaline and aseptate when young, becoming pale to dark brown, 1–3-euseptate, 26–58 × 10–14 μm (X = 45 × 12 μm, n = 40), darkened at the septa and both ends. Sexual morph: Undetermined.

Culture characters:—Conidia germinating on PDA within 24 h and germ tubes produced from both ends. Colonies on MEA reaching 7–10 mm diam. at 14 days, with dense, greyish green, sparse mycelium on surface initially, becoming dark green, white-grey at the undulant edge; in reverse with a dark green middle and yellowish margin. After one month of incubation, the colony on MEA produced the asexual morph.

Habitat and distribution:—On submerged wood in freshwater, Thailand.

Material examined:—THAILAND. Prachuap Khiri Khan Province: Hua Hin, stream outside Kaeng Krachan National Park, on submerged wood, 25 December 2014, Jaap van Strien, Site 4-17-1 (MFLU15-1151 holotype), ex-type living culture, MFLUCC15-0616; ibid. (HKAS95042 isotype).

Notes:—Sporoschisma palauense is morphologically similar to S. phaeocentron and S. parcicuneatum. However, S. palauense has longer conidiophores and setae and the conidia are larger than those of S. parcicuneatum. The conidia of S. palauense are brown, and mostly 3-septate, while in S. phaeocentron the central cells are dark brown with the end cells paler, and in S. parcicuneatum the conidia are mostly one septate. The fungus is distinguished from the type species S. mirabile by the shape of the conidia at the ends. Phylogenetic analysis showed that S. palauense represents a sister taxon to S. mirabile and is a distinct species in the genus.

Sporoschisma longicatenatum J. Yang, J.K. Liu & K.D. Hyde, sp. nov. Fig. 4

Index Fungorum Number: IF552182, Facesoffungi Number: FoF 02243.

Etymology:—Referring to the long chains of conidia.

Holotype:—MFLU16-1325.
FIGURE 3. *Sporoschisma palauense* (MFLU15-1151, holotype) a. Colony on wood with long conidia chains. b. Conidia, seta and conidiophore. c, d. Conidiophores. e, i. Conidia. f. Chain of young conidia. g, h. Chains of mature conidia. j. Germinating conidium. k, l. Cultures on MEA, k from above, l from below. Scale bars: a = 200 μm, b, f = 100 μm, c–e, g, h = 50 μm, i, j = 30 μm.
Saprobic on decaying plant substrates. **Asexual morph:** Colonies effuse, black, hairy, with long chains of conidia. Mycelium immersed, composed of pale to dark brown hyphae. Setae scattered or in groups mixed with conidiophores, capitate, usually surrounded by hyaline mucilage at the swollen apex, smooth, pale to medium brown, paler towards the subhyaline apex, straight or flexuose, 2–3-septate, 95–185 × 4.5–6.5 μm, 6–8.5 μm wide at the swollen apex. **Conidiophores** macronematous, mononematous, smooth, dark brown to black, paler at the torn apex, straight or slightly flexuose, solitary or in groups of 2–3 with 0–4 setae, arising from dark brown to black bulbous base, composed of a cylindrical stipe and a swollen venter with a long cylindrical neck, erect, sometimes proliferating percurrently, 245–335 μm long, 7.5–12 μm wide below venter and 11–16.5 μm wide above, 15–22 μm wide at venter. **Conidiogenous cells** monophialidic, percurrent, integrated, terminal, determinate, brown, lageniform, frayed at the apex. **Conidia** cylindrical to doliiform, 35–45.5 × 9–11 μm (X = 40 × 10 μm, n = 35), 1–5-euseptate, hyaline when young, olivaceous to brown at maturity, with hyaline to pale brown end cells, which are much shorter than the four inner cells, conspicuously darkened at the septa, rounded at both ends. **Sexual morph:** Undetermined. **Culture characters:**—Conidia germinating on PDA within 24 h and germ tubes produced from both ends. Colonies on MEA reaching 5–10 mm diam. at 7 days, with fluffy, dense, white mycelium in the center, becoming sparse and hyaline at the smooth edge; in reverse with a yellowish middle, paler towards the smooth margin. **Habitat and distribution:**—On submerged wood in freshwater, Thailand. **Material examined:**—THAILAND, Prachuap Khiri Khan Province: Hua Hin, stream outside Kaeng Krachan National Park, on submerged wood, 25 December 2014, Jaap van Strien, Site 5-14-3 (MFLU16-1325 holotype; HKAS95044 isotype); ex-type living culture, MFLUCC16-0180, GZCC15-0072. **Notes:**—Phylogenetic analysis showed that *Sporoschisma longicatenatum* is close to *S. hemipsila*. Morphological comparison demonstrated that they share similar morphological characters. The conidia ends of *S. longicatenatum* are rounded, while they are subtruncate in *S. hemipsila*. In addition, percurrent proliferations of conidiophores were observed in *S. longicatenatum*. Discussion

*Sporoschisma* is a common genus reported on submerged wood. Eight species are now known from freshwater habitats, with *S. hemipsila*, *S. nigroseptatum* and *S. uniseptatum* being the most commonly found taxa (Tsui et al. 2000, Hu et al. 2010, 2014, Goh et al. 1997). *Sporoschisma* is morphologically similar to *Sporoschismopsis*. The shared characters are large conidiophores, or stalked phialides, and brown, septate conidia produced in long, basipetal, false chains. However, *Sporoschismopsis* is generally distinguished from *Sporoschisma* by the absence of capitate setae and the anatomy of the conidia. The shape of the collarette (swollen venter or apical funnel-shaped collarette) has also been considered an important character for the generic distinction (Ellis 1971, 1976). Goh et al. (1997) considered the repeating percurrent proliferation of conidiophores in *Sporoschismopsis* to have greater taxonomic significance. However, Réblová (2014) suggested that the percurrent regeneration of phialides has little taxonomic significance once a fungus has produced a certain number of conidia from a fixed point. Moreover, the presence of a swollen venter is not a fixed morphological character of *Sporoschisma* being affected by age, nutrient reserves, growth medium and other factors. Thus, some taxa may possess a mixture of morphological characters. Based on phylogenetic studies, however, *Sporoschismopsis* is placed in the Reticulascaceae (Glomerellales, Hypocreomycetidae) (Réblová et al. 2011, Réblová 2014), while *Sporoschisma* is placed in the Chaetosphaeriales (Sordariomycetidae).

The placement of the four collections in this study within *Sporoschisma* is supported by both molecular and morphological characters. Asexual morphs associated with the sexual species have been described for *S. hemipsila*, *S. mirabile* and *S. uniseptatum*. However, only the asexual morphs of the two new taxa were observed in this study.

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