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Research Article

Scytalidium parasiticum sp. nov., a New Species Parasitizing on *Ganoderma boninense* Isolated from Oil Palm in Peninsular Malaysia

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Abstract A mycoparasite, *Scytalidium parasiticum* sp. nov., isolated from the basidiomata of *Ganoderma boninense* causing basal stem rot of oil palm in Johor, Malaysia, is described and illustrated. It is distinct from other *Scytalidium* species in having smaller asci and ascospores (teleomorphic stage), longer arthroconidia (anamorphic stage), hyaline to yellowish chlamydospores, and producing a fluorescent pigment. The phylogenetic position of *S. parasiticum* was determined by sequence analyses of the internal transcribed spacers and the small-subunit ribosomal RNA gene regions. A key to identify *Scytalidium* species with teleomorphic stage is provided.

Keywords Biocontrol, Chlamydospore, *Elaeis guineensis*, Mycoparasitism

The adjectives "fungicolous" and "mycophilic" were used to describe consistent inter-fungal or fungus-fungus associations, but the biology and mechanisms of these interspecific relationships are still not clearly determined [1]. Fungicolous fungi are quite common in nature and can be found in various ecosystems, namely from terrestrial to aquatic environments [2]. Inter-fungal relationships or mycophilic associations between a fungicolous fungus and its fungal host can be classified into a few categories, namely neutralism, mutualism, antagonism (competition), and mycoparasitism [3, 4].

Fungus-fungus associations have been reported among different fungal groups, ranging from oomycetes to basidiomycetes, and even including the lichens. More than 50% of the reported fungicolous fungi were members of the Ascomycota (mostly in the orders Dothideales and Hypocreales); followed by members of the Basidiomycota [2, 5]. Most of the fungicolous species were ascomycetes and their anamorphs, and were found to colonize basidiomycetes, mostly on members of the order Aphyllophorales [2].

The basidiomycete genus *Ganoderma* Karst. is well known as one of the most widespread wood decaying fungi. To date there are more than 250 species of *Ganoderma* described worldwide [6], including those that are plant pathogenic [7], and cause white rot of wood. Certain species, however, are found to be beneficial, including those with certain medicinal values [8] and those producing lignin-degrading enzymes for industrial and biotechnology uses [9]. Some *Ganoderma* species were reported to harbour a number of ascomycetous and basidiomycetous fungicolous fungi. *Sporophagomyces chrysostomus* (Berk. & Broome) K. Põldmaa & Samuels [10], *Hypomyces pseudopolyporinus* Samuels & Rogerson [11], *Acremonium lindtneri* (Kirschst.) Samuels & Rogerson [12], *Hypocrea atrogelatinosa* Dingley [13], and *Cladobotryum semicirculare* G. R. W. Arnold, R. Kirschner & Chee J. Chen [14] from the order of Hypocreales, *Melanospora lagenaria* (Pers.) Fuckel from the order of Melanosporales [2], and *Xylogone ganodermophthora* Kang, Sigler, Y. W. Lee & S. H. Yun (anamorph *Scytalidium ganodermophthorum*

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Kang, Sigler, Y. W. Lee & S. H. Yun) from the order of Helotiales [15] have been observed to grow on or parasitize a few *Ganoderma* species, namely *G. lucidium* (Curtis) P. Karst., *G. applanatum* (Pers.) Pat., and *G. tsugae* Murrill [2, 16].

In Taiwan, the hyphomycete *C. semicirculare* was found to infect the commercially cultivated *G. tsugae* and hindered the growth as well as development of the host basidiocarps. In addition, the same hyphomycete has also been isolated from species of polypores and agarics in Cuba [14]. Recently reported in Korea, the yellow rot is a major lethal disease of commercially grown *G. lucidum*. This disease, which is caused by the ascomycetous fungal pathogen, *X. ganodermophthora*, has led to drastic losses of yield in *G. lucidum* fruit body production [15, 17]. The yellow rot pathogen *X. ganodermophthora* has the ability to produce massive amount of ascospores and arthroconidia. Hence, this fungal parasite is able to spread effectively to disease-free area by means of soil-borne and air-borne inocula to infect healthy *G. lucidum* [15, 18].

In one of our previous experiments conducted to determine the aggressiveness of twelve *Ganoderma boninense* Pat. isolates harvested from oil palms (*Elaeis guineensis*) with basal stem rot disease (BSR) found in various locations of the Malaysia peninsular (MP) [19], an undescribed ascomycete (isolate AAX0113) producing brown to dark brownish cleistothecia was detected to thrive aggressively on one of the *G. boninense* isolate collected from southern MP. Growth of this *Ganoderma* isolate was completely suppressed by this ascomycete under *in vitro* conditions. We have isolated this *Ganoderma*-philic, cleistothecial ascomycete in culture and extracted its DNA for molecular analysis. To determine its identity, sequences of the internal transcribed spacer regions (ITS) and the small subunit ribosomal RNA gene (SSU rRNA) of this fungus were generated and compared with the existing sequences from GenBank. Data based on phylogenetic analyses together with morphological features (including both sexual and asexual stages) elucidated that this mycoparasitic ascomycete was best accommodated in the genus *Scytalidium* Pesante. There are two reported species of *Scytalidium* [20] with teleomorphic stage of *Xylogone*, *X. ganodermophthora* [15], and *X. sphaerospora* Arx & T. Nilsson [21] (teleomorphs of *S. ganodermophthorum* and *S. sphaerosporum*, respectively). Our species (isolate AAX0113) however, differs from the two existing species in its ascospore, ascus, and arthroconidium morphology, and hence we describe it as new in this paper. A synopsis of and a key to these three *Scytalidium* species with teleomorphic stage are provided. We also describe the growth of this fungus on various commercial media in this paper. To the best of our knowledge, this is the first report of *Scytalidium* association with *Ganoderma boninense*.

MATERIALS AND METHODS

Fungal isolate, growth and culture condition. The presence of cleistothecia (ascomata) of this mycoparasitic ascomycete (AAX0113) was spotted on mycelium of *Ganoderma boninense* grown on malt extract agar (MEA) and rubber wood block (RWB) inoculated with *G. boninense* in 2013. Cleistothecia were collected, separated and detached from *Ganoderma* mycelial mat using flame-sterilized needles under the dissecting microscope. The cleistothecia were then treated and incubated to obtain single-spore isolates according to the method proposed by Kang *et al.* [15]. Pure cultures from single-spore isolates were maintained on MEA supplemented with antibiotics as outlined in Vujanovic and Goh [22]. Other two existing *Xylogone* species, *X. sphaerospora* UAMH10840 and *X. ganodermophthora* UAMH10321 with anamorphic stage of *S. sphaerosporum* and *S. ganodermophthorum,* respectively, were purchased from University of Alberta Microbial Herbarium (UAMH) and the pure cultures were maintained on MEA amended with antibiotics. Mycelial plugs of all the three *Scytalidium* (anamorphic of *Xylogone*) strains excised from the pure cultures were kept in 10% glycerol and stored at −80°C.

Fungal growth was evaluated on various commercial media: potato dextrose agar (PDA), Czapek solution agar, MEA, corn meal agar (CMA), ME + A (12.75 g of malt extract amended with 15 g of agar in 1 L of sterilized distilled water), ME + A + P (12.75 g of malt extract, 15 g of agar and 0.78 g of Bacto peptone in 1 L of sterilized distilled water), oatmeal agar A (OAA) [23], rose bengal agar (Oxoid Ltd., Hampshire, UK), and water agar. Unless indicated otherwise, pH for all the media was adjusted to 5.6. Mycelial growth rates of isolate AAX0113 inoculated on MEA (pH 5.6) and incubated at 8°C, 20°C, 30°C, and 40°C were determined according to the approach proposed by Kok *et al.* [19]. For fungal growth measurement on media with different pH, MEA was used and the pH was adjusted to 5, 6, 7, and 8, using either 0.1 N HCl or NaOH. The media were prepared according to manufacturer's instructions. All the media used in this study were purchased from Difco (Becton Dickinson Diagnostics, Sparks, MD, USA), if not specified. Radius growth measurements (radii in four perpendicular directions were measured from the edge of mycelial plug to the periphery of fungal colony, and the average of these four readings was taken as the radius for the respective replicate) (in mm) were recorded daily for 7 days for assessing linear mycelial growth rate of the fungal isolate on different media [19]. There were five replicates per treatment in each individual experiment.

After two weeks of incubation at 24°C using the media cereal agar [15] and oatmeal agar [24], morphological studies of ascomata, ascospores, asci, mycelia, chlamydospore-like structures, and anamorphic features were conducted using the Optika B-1000BF microscope with a OPTIKAM Pro Cool 5 mount camera (OPTIKA SRL, Ponteranica, Italy). Fungal materials were mounted in water or 10% glycerol with lactophenol blue dye for microscopic observations. Diameters of ascomata were measured with 40× magnification objective lens. Measurements on ascospores, asci, arthroconidia, and mycelia were investigated and measured with 100×

magnification (oil immersion) objective lens. All the measurements were performed using OPTIKA View version 2 software (OPTIKA SRL). A small amount of the fungal samples mounted in water was also investigated under RaxVision IBS-100 inverted microscope with blue light filter and equipped with a RaxVision C mount camera (RaxVision, Chicago, IL, USA).

Statistical analysis. Differences in means for the linear mycelial growth (in mm/day) on nine different media, on MEA with five different temperatures, and under four different pH values, were analyzed separately using analysis of variance (ANOVA) and Tukey's test at $p = 0.05$ [25].

Cellulolytic and xylanolytic activities. Cellulolytic and xylanolytic activities of isolate AAX0113 on agar plates were investigated using the Gram's iodine [26] and the Congo red [27] methods. Radius measurements of the clearing zone (halo) (radii in four perpendicular directions were measured from the periphery of fungal colony to the edge of the halo zone, and the average of these four readings was taken as the radius for the respective replicate) (in mm) were measured and recorded on day 7 to determine the cellulolytic and xylanolytic activities of isolate AAX0113. There were 5 replicates for the respective treatment.

Scanning electron microscopy (SEM). Samples were prepared according to the methods outlined in Kirschner *et al.* [28], with a few minor modifications: cleistothecia were cracked by placing them in between two cover slips. All the samples were coated with platinum and viewed under the scanning electron microscope (FESEM, Model: JSM-6701F; Jeol, Tokyo, Japan).

Chlamydospore formation. Mycelial plugs of isolate AAX0113 were excised from 7-day-old cultures and placed onto three commercial media: namely PDA, CMA, and OAA; and two chlamydospore conversion media: minimal conversion medium (MCM) and MCM amended with mannitol (MCM + mannitol) [29]. All the plates were incubated at 24°C (room temperature) and 28°C in the dark for 2 wk. Two sets of Petri dishes with three commercial and two chlamydospores conversion media were prepared and incubated at two separate temperatures, namely room temperature (24°C) and 28°C, respectively.

DNA extraction, amplification and sequencing. *Scytalidium* isolate AAX0113 was cultured on PDA supplemented with antibiotics at 24°C for a week prior to DNA extraction. Genomic DNA was extracted with the FastDNA Spin Kit (MP Biomedicals, Solon, OH, USA) as per manual instruction. ITS and SSU rRNA fragments were amplified using primer sets ITS1/ITS4 and NS1/NS8, respectively [30]. Polymerase chain reaction (PCR) was performed according to protocols outlined in Kok *et al.* [19]. Purified PCR products were sent to Macrogen (Daejeon, Korea), for sequencing. ITS and SSU rRNA sequences obtained from the specimen were submitted to GenBank under the accession Nos. KF925450 and KF925449, respectively.

Sequence alignment and phylogenetic analyses. Sequences of ITS and SSU rRNA gene from the present study and sequences retrieved from GenBank were aligned using Clustal W [31], and edited in Bioedit [32]. Alignments are available on treebase.org at the following link: http://purl.org/phylo/treebase/phylows/study/TB2:S15654. Maximum likelihood (ML) analysis was performed with MEGA6 software [33]. The robustness of trees was validated using bootstrap analyses with 1,000 repetitions. Phylogenetic trees were prepared with sequences showing bootstrap values higher than 50%. Trees based on ITS and SSU rRNA sequences were rooted with sequences from *Sporendonema purpurascens* (Bonord.) E. W. Mason & S. Hughes strains (UAMH 1497 and KACC 41227).

RESULTS

***In vitro* growth of isolate AAX0113.** Three separate experiments were conducted to study the *in vitro* growth of isolate AAX0113: (1) on various commercial media; (2) on MEA incubated at different temperatures; and (3) on MEA adjusted to four different pH values. Linear mycelia growth rates for isolate AAX0113 were significantly faster ($p < 0.05$) on PDA, ME + A + P and OAA when compared with those on other media (Table 1). Production of cleistothecial ascomata was first spotted on ME + A on week 2 after inoculation. Ascomal production was slower

Table 1. Linear mycelial growth rate of *Scytalidium parasiticum* (isolate AAX0113) on various culture media

Medium	Radial growth rate (in mm/day) (± standard error)[a]
PDA	3.64 ± 0.09 a
MEA	2.49 ± 0.05 b
CSA	0.25 ± 0.04 de
RBA	0.65 ± 0.03 c
ME + A	2.36 ± 0.10 b
ME + A + P	3.85 ± 0.04 a
CMA	0.46 ± 0.07 cd
OAA	3.87 ± 0.12 a
WA	0 e

PDA, potato dextrose agar; MEA, malt extract agar; CSA, Czapek solution agar; RBA, rose bengal agar; ME + A, 12.75 g of malt extract amended with 15 g of agar in 1 L of sterilized distilled water; ME + A + P, 12.75 g of malt extract, 15 g of agar, and 0.78 g of Bacto peptone in 1 L of medium; CMA, corn meal agar; OAA, oatmeal agar A; WA, water agar.

[a]Means followed by the same letter are not significantly different at $p = 0.05$ after Tukey's test (SPSS 1990). There were 5 replicates per treatment.

Table 2. Effect of incubation temperature and pH on mycelial growth rate of *Scytalidium parasiticum* (isolate AAX0113) on malt extract agar

	Radial growth rate (mm/day)[a] (± standard error)
Temperature (°C)	
8	0 c
20	2.61 ± 0.20 b
24	2.49 ± 0.05 b
30	5.19 ± 0.10 a
40	0 c
pH	
5	36.75 ± 0.54 a
6	24.94 ± 0.73 b
7	12.00 ± 0.93 c
8	11.88 ± 0.68 c

[a]*Scytalidium parasiticum* mycelial growth rate on malt extract agar incubated at different temperature and medium with different pH was analysed separately. Means within each column followed by the same letter are not significantly different at $p = 0.05$ after Tukey's test (SPSS 1990). There were 5 replicates per treatment.

(3 to 4 wk after incubation) on PDA, ME + A + P and OAA, although the mycelial growth rates on these three media were faster than that on ME + A (data not shown). No mycelial growth was observed when isolate AAX0113 was inoculated on medium containing agar only (Table 1). The growth rate of isolate AAX0113 was significantly faster at 30°C, followed by 20°C and 24°C (Table 2). No growth was observed at 8°C and 40°C (Table 2). Among the four different pH values tested, isolate AAX0113 has the fastest growth rate at pH5 (Table 2).

Morphological analyses. Our species (isolate AAX0113) is typical of *Scytalidium* (*Xylogone*) (both the asexual and sexual stages), demonstrating the generic morphological characters such as having cleistothecial ascomata which are devoid of any appendages (Fig. 1A and 1B) and producing unicellular ascospores which are subglobose to globose, hyaline, and smooth-walled (Table 3, Fig. 1C and 1F). Ascomata of *Scytalidium* AAX0113 isolate (31~132 µm) were larger than those of *S. sphearosporum* (25~50 µm) but appeared to be comparable to those of *S. ganodermophthorum* (45~165 µm). Ascospores and asci (teleomorphic structures) in *Scytalidium* AAX0113, however, were found to be smaller when compared to those of *S. ganodermophthorum* and *S. sphaerosporum* (Table 3) (Fig. 1G~1I). When young cleistothecia were crushed, asci were seen in clusters. Each intact ascus was seen to contain 8 ascospores (Fig. 1D and 1E), but the ascal wall became evanescent at maturity. Both *Scytalidium* AAX0113 and *S. ganodermophthorum* had ascospores which were subglobose to globose, but the former were predominantly globose, with a length to width (L/l) ratio of 1.03 to 1.14. *Scytalidium ganodermophthorum* was having ascospores with L/l ratio of 1.08~1.10. In *S. sphaerosporum* however, ascospores were subglobose, with

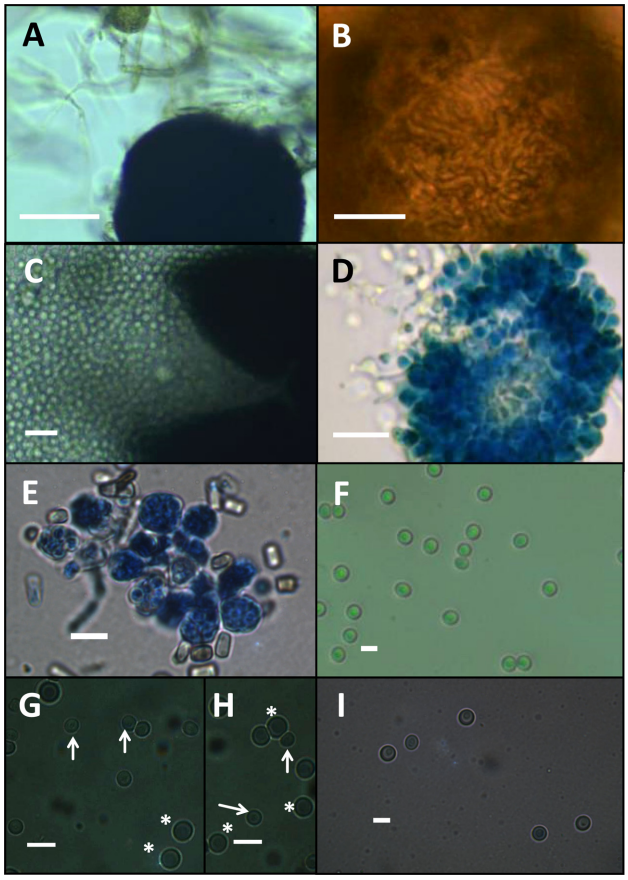

Fig. 1. *Scytalidium parasiticum* ex-type culture AAX0113 elucidating mature and immature ascomata (A); ornamentation on sexual fruiting body (B); ascospores from fruiting body (C); cluster of attached asci stained with lactophenol blue (D); asci stained with lactophenol blue (oil immersion objective) (E); and ascospores (F). G~H, Ascospores of *S. parasiticum* (arrows) (smaller in size) and *S. ganodermophthorum* UAMH10321 (asterisks); I, Ascospores of *S. sphaerosporum* UAMH10840 under oil immersion objective were included to illustrate the differences in sizes (scale bars: A = 30 µm, B~D = 10 µm, E, F = 3 µm, G~I = 5 µm).

an L/l ratio of 1.60 to 1.22 (Table 3) [15, 21]. A conidial (asexual structures) state was also observed in *Scytalidium* AAX0113, morphology of which conformed to other reported species in the hyphomycete genus *Scytalidium*, producing tremendous amount of arthroconidia in culture. These arthroconidia appeared to be morphologically comparable to those found in *Scytalidium ganodermophthorum*, *S. sphaerosporum,* and *S. cuboideum* (Fig. 2A~2C) [15]. However, arthroconidia in *Scytalidium* AAX0113 isolate (L/l ratio of 1.0 to 3.5) were generally longer than those produced by *S. ganodermophthorum* (L/l ratio of 1.0 to 1.3) and *S. sphaerosporum* (L/1 ratio of 0.9 to 1.8) (Table 3).

Cellulolytic and xylanolytic activities. Means of the radial clearing or halo zones by isolate AAX0113 for both the cellulolytic and xylanolytic tests were 7.69 and 1.50 mm

Table 3. Comparisons between *Scytalidium parasiticum* (AAX0113), *Scytalidium ganodermophthorum* and *S. sphaerosporum*, in term of fungal morphology, substratum, locality, fluorescence pigment, chlamydospores formation, and enzymatic activities

Species/Isolate		*S. parasiticum*	*S. ganodermophthorum*	*S. sphaerosporum*
Vegetative hyphae[a]	Diameter (μm)	1.3~6.5	1.2~6	1.5~5.5
Ascomata[b]	Size (μm)	31~132	45~165	25~50
Ascospores[b]	Size (μm)	2.5~3.5 × 2.2~3.4	3.3~4.3 × 3~4	4~5.5 × 2.5~4.5
	L/l ratio[e]	1.03~1.14	1.08~1.10	1.60~1.22
Ascus	Size (μm)	6~8.5 × 4~8	9~11 × 7~10.5	7.5~12 × 6.5~10
Arthroconidia[c]	L × l[f] (μm)	1.7~6.6 × 1.3~2.7	1.5~4 × 1.5~3	1.8~5.5 × 2~3
	L/l ratio[e]	1.0~3.5	1.0~1.3	0.9~1.8
Other characteristics	Substratum	Ganoderma boninense	Ganoderma lucidum	Wood chip
	Locality	Malaysia	Korea	Sweden
	Diffusible pigment	Dark yellow	Brownish yellow	Orange brown
	Chlamydospores[a,g]	+ (hyaline to yellowish solitary/catenulate)	-	+ (hyaline, solitary/catenulate)
Enzymatic activities[d] (clearing or halo zone) (± standard error, mm)	Cellulolytic	7.69 ± 0.25	NT[h]	NT
	Xylanolytic	1.50 ± 0.29	NT	NT

[a]On potato dextrose agar medium.
[b]Oatmeal agar.
[c]On cereal agar medium.
[d]Radius of clearing or halo zone was measured in the unit of mm. There were 5 replicates per experiment.
[e]L/l refers to the ratio of length/width of arthroconidia and ascospores.
[f]L × l refers to length × width of arthroconidia.
[g]Cornmeal agar medium.
[h]NT refers to not tested.

on day 7, respectively (Table 3).

Chlamydospores. Besides having smaller asci and ascospores, our *Scytalidium* AAX0113 isolate also differs from the other two *Scytalidium* species (reported with teleomorphic stage) in producing hyaline to yellowish, solitary or catenulate chlamydospores (Fig. 2D). Initiation of chlamydospores was spotted one week after inoculation on to PDA, CMA, and OAA incubated at both 24°C and 28°C (Table 4), and on MCM incubated at 28°C only. However, even after 2 wk of incubation, chlamydospores were not formed, neither on MCM incubated at 24°C nor on MCM + mannitol incubated at 24°C and 28°C (Table 4). MCM supplemented with mannitol has been proposed to induce chlamydospore formation in *Fusarium* species [29], but in this study, chlamydospore formation of isolate AAX0113 was not induced by cultivating on this amended medium.

Table 4. Effect of incubation temperature and type of media on chlamydospore formation in *Scytalidium parasiticum* (AAX0113)

Media	Temperature (°C)	Days after inoculation	
		7 days	14 days
PDA	24	+	+
	28	+	+
CMA	24	+	+
	28	+	+
OAA	24	+	+
	28	+	+
MCM	24	−	−
	28	+	+
MCM + Mannitol	24	−	−
	28	−	−

PDA, potato dextrose agar; +, chlamydospores formed; −, chalmydopores not formed; CMA, corn meal agar; OAA, oatmeal agar A; MCM, minimal conversion medium; MCM + Mannitol, MCM amended with mannitol.

Fluorescent pigment production. Mycelia together with both sexual and asexual spores of *Scytalidium* AAX0113 produced a greenish fluorescent pigment. Its mycelium, including both the sexual and asexual forms, fluoresced when observed under inverted microscope (RaxVision IBS-100) with blue light filter (Figs. 1F, 2A, and 2B). MEA plates fully grown with mycelia of *Scytalidium* AAX0113 were inspected under UV light (254 nm). Pure culture of *Scytalidium* grown on MEA appeared to be in yellowish to greenish fluorescence under UV (Fig. 2F) compared to plates under normal light (Fig. 2E). Methanol extract derived from *Scytalidium* culture inoculated on maize was extracted and the fungal extract was observed to be fluorescence on gel doc system equipped with blue L.E.D light (Fig. 2H) compared to extract under normal light (Fig. 2G).

Origins of the different *Scytalidium* species. Locality and substratum for *Scytalidium* AAX0113 isolated from

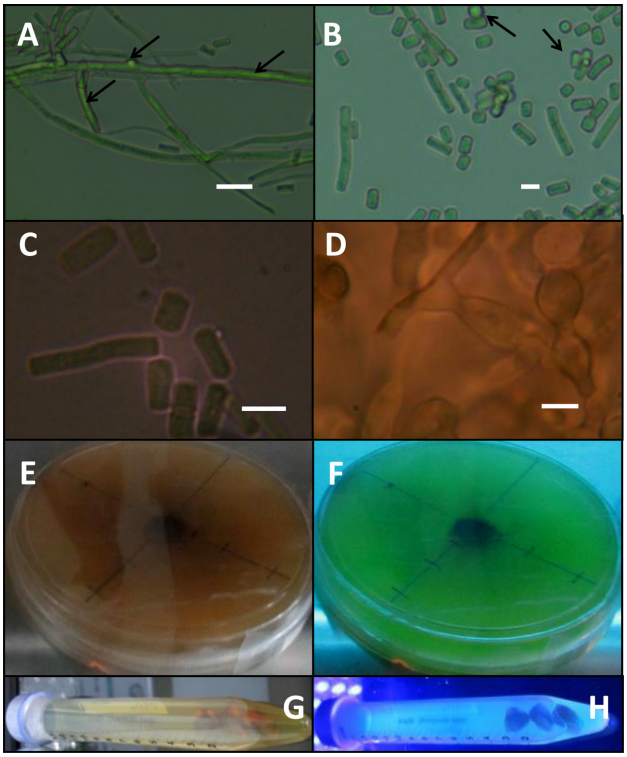

Fig. 2. *Scytalidium parasiticum* ex-type AAX0113 showing arthroconidia and mycelia with greenish fluorescence pigment (arrows) (A); arthroconidia with fluorescence pigment (arrows) (B) (A and B were examined using RaxVision IBS-100 inverted microscope with blue light filter); arthroconidia (oil immersion objective) (C); chlamydospores (oil immersion objective) (D); *S. parasiticum* inoculated on ME + A medium, without UV light (E) and under UV light (F); and methanol extract of *S. parasiticum* inoculated on maize or corn, without blue L.E.D light (G) and under blue L.E.D light (H) (scale bars: A = 10 µm, B~D = 3 µm). ME + A, 12.75 g of malt extract amended with 15 g of agar in 1 L of sterilized distilled water.

Malaysia on *G. boninense* were different compared to *S. ganodermophthorum* and *S. sphaerosporum,* which were isolated from Korea on *G. lucidum* and Sweden on wood chips, respectively (Table 3).

Phylogenetic analyses. Sequence alignments and ML analysis at 1,000× boostrap repetitions for the ITS sequences placed isolate AAX0113 within the same cluster containing strains of *Scytalidium* Pesante (hyphomycetes) with teleomorphic stage of *Xylogone* (ascomycetes) (Fig. 3). Isolate AAX0113 was also found to share the similar clade with all the strains of *Scytalidium ganodermophthorum* (*Xylogone ganodermophthora*) used in this analysis, with a 99% bootstrap support. Furthermore, isolate AAX0113 was also observed to group with all the Helotiales—*Scytalidium* species, namely *S. cuboideum* and *S. lignicola* (without *Xylogone* teleomorphic stage) and *Cryptosporiopsis* (Fig. 3). Based on the ML analysis using both ITS and SSU rRNA sequences, isolate AAX0113 significantly branched from the group containing different strains of *S. ganodermophthorum*, with 99% and 98% bootstrap supports in ITS and SSU rRNA trees, respectively (Figs. 3 and 4). In addition, both *S. ganodermophthorum* and isolate AAX0113 (associated with *Ganoderma* species) were separated from the clade of three other *Scytalidium* species, namely *S. sphaerosporum, S. lignicola,* and *S. cuboideum*, with a 97% bootstrap support (Fig. 4). All the sequences for both ITS and SSU rRNA regions (together with GenBank accession Nos.) used in the sequence analyses and alignments are available in TreeBASE (S15654).

Based on morphological differences and cultural characteristics, supported by phylogenetic analyses of the ITS and SSU rRNA, we describe the mycoparasite isolate AAX0113 as *Scytalidium parasiticum* sp nov., including both the teleomorphic and anamorphic features. The holotype was conserved at the herbarium of the Centre for Biodiversity Research, Faculty of Science, Universiti Tunku Abdul Rahman (Perak campus), Kampar, Malaysia (UTAR).

Taxonomy.

***Scytalidium parasiticum* Y-Kheng Goh, Goh, Y. K. Goh, K. J. Goh, sp. nov. (Figs. 1A~F, 2A~F, and 5A~H)**

MycoBank: MB810754.

Description: Colonies on PDA at 24°C fast growing, floccose, initially light yellow, becoming yellowish brown to brownish orange (after about 7 to 10 days) with development of arthroconidia, becoming brown to dark brown (approximately after 3 to 4 wk) with development of abundant ascomata, producing a strong yellow diffusible pigment. Cellulolytic and xylanolytic. Ascomata cleistothecial, arising from coiled ascogenous hyphae, initially subhyaline to pale yellowish brown, becoming dark brown at maturity with wall of textura epidermoidea, lacking appendages, globose, with a 2-layered wall, 31~132 µm diameters. Asci dispersed in centrum, subglobose to globose, thin-walled, quickly evanescent, eight-spored, 6~8.5 × 4~8 µm. Ascospores hyaline, with a greenish fluorescence pigment, smooth with reflective walls, globose to subglobose, 2.5~3.5 × 2.2~3.4 µm. Anamorph hyphomycetous. Fertile hyphae borne laterally on simple conidiophores, fragmenting into arthroconidia. Arthroconidia hyaline to light yellow, smooth, cuboidal to oblong or cylindrical, 1.7~6.6 × 1.3~2.7 µm, occasionally wider than long, lacking separating cells or disjunctors.

Etymology: *Parasiticum*, referring to the hyperparasitic nature of this fungus.

Host range: Associated with *Ganoderma boninense* of BSR on oil palm.

Holotype: Malaysia, Selangor, Kota Damansara, on *G. boninense* cultured on RWB, preserved as dried material, UTAR(M)-0005 (AAX0113); the host *G. boninense* was originally collected from Kulaijaya, Johor, Malaysia, 10 April 2013, N. F. Marzuki and Yit Kheng Goh.

Paratype: Malaysia, Selangor, Kota Damansara, teleomorphic and anamorphic stages, preserved in polyvinyl alcohol

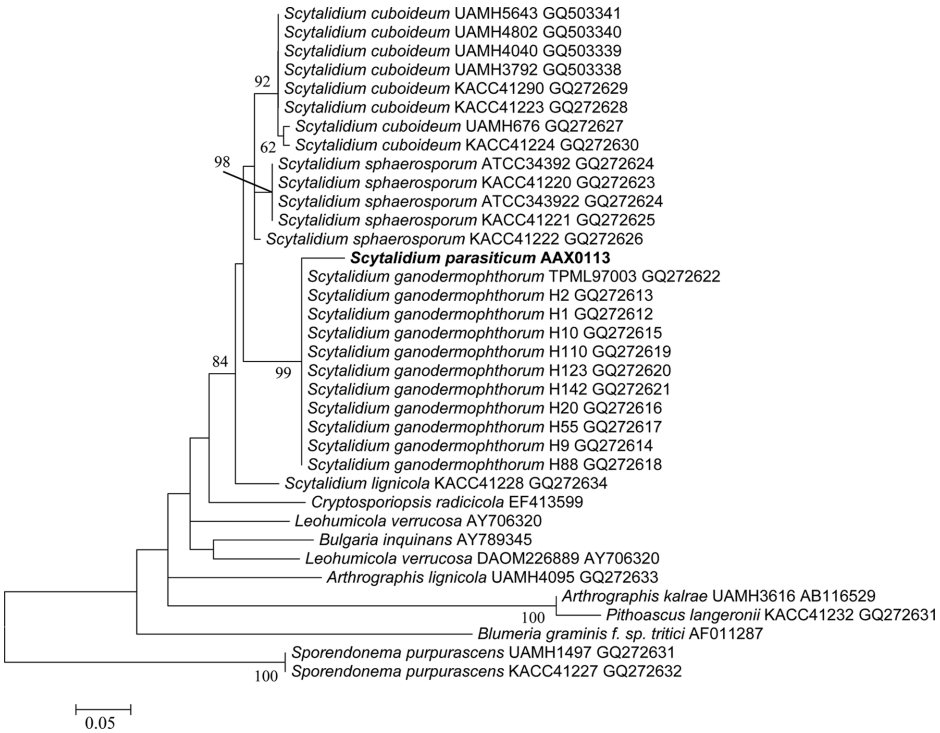

Fig. 3. Phylogenetic tree generated through maximum likelihood approach based on internal transcribed spacer sequences showing the position of *Scytalidium parasiticum* (in bold). Bootstrap values of 50% or greater from 1,000 bootstrap replications are indicated for the corresponding branches. Branch lengths are drawn to scale with the scale bar indicating 0.05 substitutions per nucleotide position.

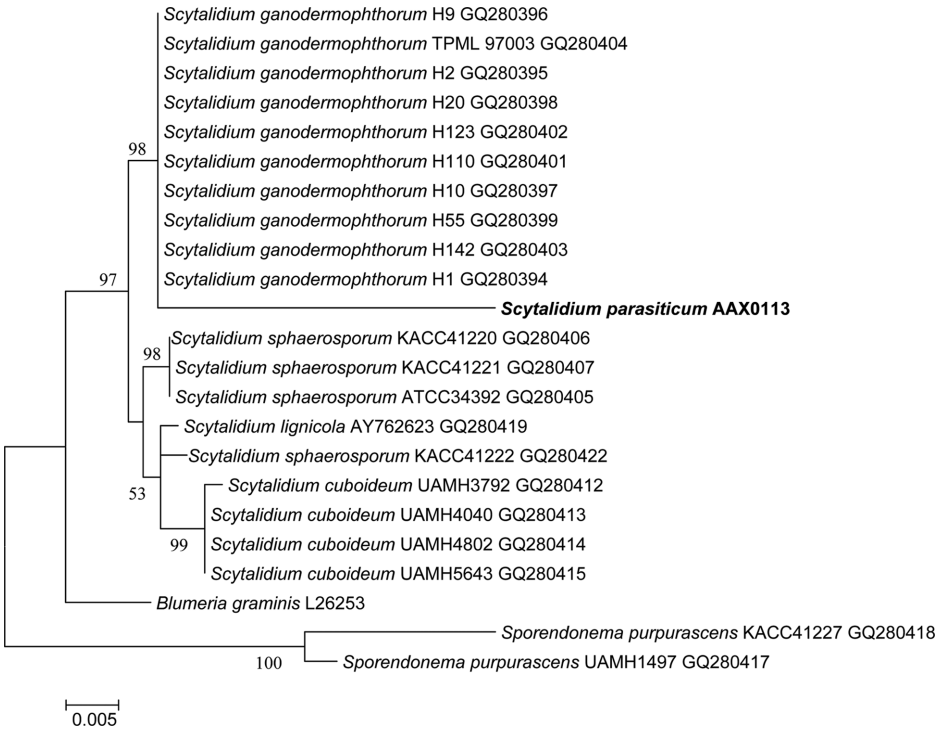

Fig. 4. Phylogenetic tree generated through maximum likelihood approach based on small subunit ribosomal RNA sequences showing the position of *Scytalidium parasiticum* (in bold) among the *Scytalidium* species. Bootstrap values of 50% or greater from 1,000 bootstrap replications are indicated for the corresponding branches. Branch lengths are drawn to scale with the scale bar indicating 0.005 substitutions per nucleotide position.

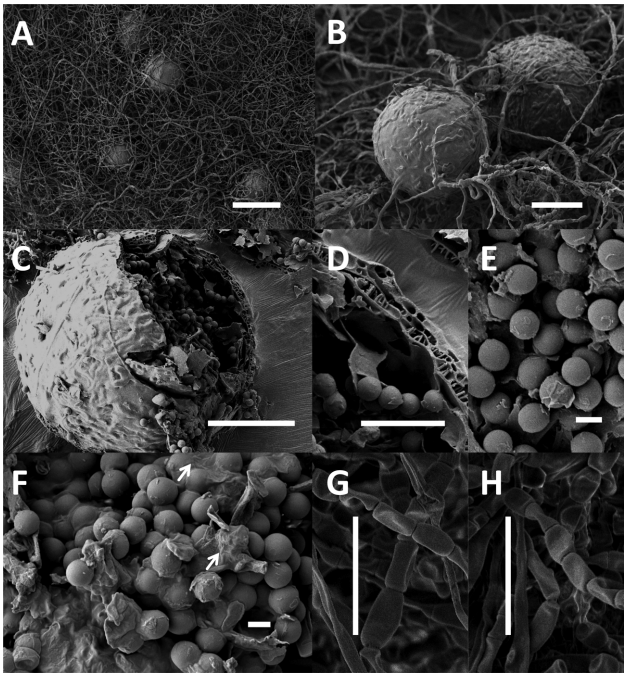

Fig. 5. Scanning electron photographs for *Scytalidium parasiticum* of ascomata (A, B); partially expose ascocarp (C); 2-layered ascocarp walls (D); smooth ascospores (E); membrane of asci on ascospores (arrows) (F); arthroconidia (G, H) (scale bars: A = 100 µm, B = 20 µm, C = 30 µm, D, G, H = 10 µm, E, F = 2 µm).

glycerol medium on glass slides, UTAR(M)-0005A.

Key to species of *Scytalidium* with teleomorphic stage

1. Ascospores subglobose (L/l = 1.22 to 1.60) ..... *S. sphaerosporum*
1. Ascospores subglobose to globose (L/l = 1.03 to 1.14) 2
2. Ascospore size, above 3 × 3 µm and with no chlamydospores ..... *S. ganodermophthorum*
2. Ascospore size, below 3 × 3 µm and with chlamydospores ..... *S. parasiticum*

DISCUSSION

In the most recent publications on adopting the recommendations for using the 'one name for one fungus' in naming the generic names, a list of fungal genera in Leotiomycetes has been recommended to be protected for fungal nomenclature determination [34]. In the paper by Johnston *et al.* [34], the genus name of *Scytalidium* Pesante (1957) (anamorphic stage) was recommended to be protected over the genus of *Xylogone* Arx & T. Nilsson (1969). Therefore, the genus name of *Scytalidium* was used to describe the new mycoparasite isolate AAX0113 in this study. Furthermore, *Scytalidium ganodermophthorum* Kang, Sigler, Lee & Yun and *S. sphaerosporum* Sigler & Kang instead of *Xylogone ganodermophthora* Kang, Sigler, Lee & Yun and *X. sphaerospora* Arx & T. Nilson were incorporated into this study for determining the *S. parasiticum* AAX0113.

Scytalidium parasiticum was first discovered to grow on pure *Ganoderma boninense* culture (cultured on MEA) and RWB inoculated with *G. boninense* in 2013. Both the *G. boninense* pure cultures and *G. boninense*-inoculated RWBs were incubated at room temperature (24°C) in the dark condition. This mycoparasite, however, has later also been found to infect and reproduce sexually on most of the other *Ganoderma* isolates or species available in our laboratory (unpublished data). The hosts now include *G. australe* (Fr.) Pat., *G. boninense*, and *G. lucidum*. However, the host-parasite interactions between *S. parasiticum* and various *Ganoderma* species are yet to be unraveled. We are currently working on investigating the unique relationship between *S. parasiticum* and different *Ganoderma* species, including the pathogenicity and the pathogenesis of *Scytalidium* on *Ganoderma.*

The present study has shown that the *S. parasiticum* recorded on *G. boninense* grouped together in a similar cluster as other *Scytalidium* species within the order of Helotiales and the class of Leotiomycetes. *Scytalidium* and *Cryptosporiopsis* from Helotiales were observed to branch out from other selected fungal orders or classes, namely Leotiales (*Bulgaria* and *Leohumicola* species), Erysiphales (*Blumeria* species), Dothideomycetes (*Arthrographis* species), and Sordariomycetes (*Pithoascus* species) (Fig. 3). Fungicolous *S. ganodermophthorum* and *S. parasiticum* were found to form a separate clade within the *Scytalidium* group and both of these fungicolous fungi were separated from other lignicolous or wood-associated fungi, in particular, *S. sphaerosporum, S. cuboideum,* and *S. lignicola* (Figs. 3 and 4). Results are also in accordance to those of Kang *et al.* [15], i.e., confirm that *S. ganodermophthorum* strains form a separate clade from *S. sphaerosporum* strains and other *Scytalidium* species. In addition, Kang *et al.* [15] also reported that all the four *Scytalidium* species, in particular, *S. ganodermophthorum, S. sphaerosporum, S. cuboideum* were positioned within the clade that allied with the Leotiomycetous fungi, and separated from other fungal classes (Sordariomycetes, Dothideomycetes, and Eurotiomycetes). Based on the phylogenetic analyses generated with both ITS and SSU rRNA sequences, *S. parasiticum* branched from the other *S. ganodermophthorum* strains and supported by the bootstrap values of 99% and 98%, respectively (Figs. 3 and 4). In view of the *S. parasiticum* tight relationships to *Scytalidium*, we have established our fungus as a new member of this genus.

The results obtained from morphological and phenotypical analyses in the present study have provided us with additional evidence on differentiating *S. parasiticum* isolate from other *Scytalidium* species, in term of the sizes for arthroconidia (asexual stage), ascospores and asci (sexual stage); as well as substratum, locality, diffusible pigmentation, and chlamydospore formation (Table 3). Majority of the ascospores and asci were smaller in size compared to the

two existing *Scytalidium* species reported with teleomorphic stage. Morphology of *S. parasiticum* anamorphic stage is similar to existing *Scytalidium* species, especially both *S. ganodermophthorum* and *S. sphaerosporum* [15]. However, arthroconidia (anamorphic) for *S. parasiticum* were longer (1.7~6.6 µm) and with higher L/l ratio compared to anamorphic stages of both *S. ganodermophthorum* and *S. sphaerosporum*, 1.5~4 and 1.8~5.5 µm, respectively (Table 3). Abundance of *S. parasiticum* arthroconidia with the length of < 2, 2 to 4, and > 4 µm, was 3%, 55%, and 43%, respectively (unpublished data). Approximately 86% of the arthroconidia were found to have L/l ratio of 1 to 2 and 14% arthroconidia were showing L/l ratio of > 2 (unpublished data). L/l ratios of arthroconidia generated by *A. cuboidea*, *S. lignicola*, and *S. purpurascens* were 1.2 to 1.4, 2.7 to 3.6, and 1.0 to 1.7, respectively. The L/l ratios were produced based on the measurements obtained by Kang *et al.* [15].

In the present study, arthroconidia, ascospores and mycelia were found to illustrate greenish fluorescence pigmentation under microscope with blue light filter. Nevertheless, the metabolites released into solid medium and maize were observed to show greenish to bluish fluorescence pigmentation under UV or blue L.E.D light as well. There was no fluorescence pigmentation reported in the original non-transformed *S. ganodermophthorum* [18]. However, the property and function of the yellowish to greenish fluorescence pigment found in *S. parasiticum* are unknown. Yellow pigment released by *S. ganodermophthorum* was proposed to be crucial in host hyperparasitism as the diffusible yellowish pigment was noted to suppress growth of *Ganoderma lucidum* under *in vitro* conditions [35]. The pigment or metabolites released by *S. parasiticum* could be potential for controlling *Ganoderma* disease, namely BSR caused by *G. boninense*. In addition, the metabolites, pigments or chemical compounds with yellowish to greenish fluorescence could be toxigenic compounds or molecules, namely mycotoxin, as reported on *Aspergillus* and other toxin producers [36, 37]. In the same papers, Hara *et al.* [36] and Sekar *et al.* [37] described the use of fluorescence to assess mycotoxins production in *Aspergillus* and associated fungal species. Sekar *et al.* [37] highlighted that under long wave (365 nm) UV-light aflatoxin B producer generated blue fluorescence while aflatoxin G producer green fluorescence.

Scytalidium parasiticum was observed to demonstrate cellulolytic and xylanolytic activities when inoculated on media supplemented with cellulose or xylan (Table 3). Both cellulolytic and xylanolytic enzymes could be useful for *S. parasiticum* to thrive, colonize, and compete with *G. boninense* in oil palm ecosystem. Hence, *S. parasiticum* will be able to suppress and control *G. boninense*. Wide variety of cell wall degrading enzymes, namely cellulase, xylanase, and pectinase, released by many parasitic or saprophytic microorganisms were found to be useful for crop protection. Some of these hydrolysing enzymes were reported to act as important proteinaceous elicitors for triggering defence responses in plants [38, 39], which were found to play crucial roles in biological control [40] or facilitated extensive saprophytic growth for the purposes of colonization and competition [41] by the mycoparasitic fungi.

Under *in vitro* conditions on slide culture, when the *G. boninense* isolate was challenged with *S. parasiticum,* mycelia of the *Ganoderma* host were found to show the symptoms of shrinkage of cytoplasm and cell membrane from the cell wall (unpublished data). Most of the reported destructive or necrotrophic mycoparasites were observed to kill or degrade their fungal host cells with extracellular enzymes at the immediate contact zones or nearby regions prior to the colonization and nutrients acquisition from the dead cells [42, 43]. On the other hand, biotrophic mycoparasites obtain nutrients from their respective living host cells through specialized structures, namely haustoria, hypae, or hook-/clamp-like organs [1, 4, 42, 43]. Based on the preliminary observations using slide culture, *S. parasiticum* could be a potential necrotrophic mycoparasite. In conclusion, *S. parasiticum*, with its mycoparasitic growth habit and production of fluorescent pigments and/or metabolites, could serve as a potential biocontrol agent for suppressing the growth of *G. boninense*, the causal agent of BSR in oil palm.

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