Asia-Pacific Journal of Science and Technology: Volume: 24. Issue: 03. Article ID.: APST-24-03-03. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Research and Technology Transfer Affairs Division,
Khon Kaen University, Thailand

A novel fungus, *Mycodomus formicartus* associated with black ant, *Dolichoderus thoracicus* (Smith) on bamboo

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Received 1 July 2018
Revised 7 June 2019
Accepted 10 June 2019

Abstract

A fungus on a bamboo plant in Dan Sai district, Loei province, Thailand was collected for identification. Its appearance consisted of a grey-to-black matter with pores on the surface attached on a bamboo branch, and a population of black ants, *Dolichoderus thoracicus* (Smith) was associated with this fungus. Inside its fruiting body, there was a cavity functioning as a nest. With very dense hyphal mass, perithecia with periphyses were produced below the surface of the fruiting body. Asci contained 8 partascospores. The phylogenetic trees using three DNA regions, 18s rDNA, 28s rDNA and internal transcribed spacer suggested that it was in Dothideomycetes, Capnodiaceae but did not fit in any reported genus. Therefore, a new genus *Mycodomus* and species, *Mycodomus formicartus* were proposed and described.

Keywords: Ant nest, Ant fungus, Black ant, Capnodiaceae, Perithecia

1. Introduction

Fungal samples were observed to have gray-to-black lumps with pores on the surface on bamboo branches and collected for identification. Each sample was surrounded by a group of black ants, *Dolichoderus thoracicus* (Smith). Thus, it was assumed that the fungus was where the ants lived or a nursery for the ants. The body of the samples consisted of various shapes and the mycelium was very dense, which caused the hardness of the fruiting structure. There were also some pores on the surface which served as the means of the ants to travel in and out of the fungal body. The bamboo plant was unharmed by the fungus because it only clung to the branch in different heights, from less than one meter above ground to several meters on top of the bamboo tips. Inside the fruiting body, there was a cavity, which is expected to be where the ants resided in during their early stage. Perithecia were found beneath the surface of the fruiting body containing asci with 8 partascspores arranging themselves spirally. These features were the main unique characteristics of this bambosicolous fungus.

Most of the fungal genera are in Sordariomycetes especially in Hypocreaceae, Xylariaceae, Lasiosphaeriaceae, and Clavicipitaceae [1]. There are also Dothideomycete fungi reported on bamboo e.g. *Bambusicola massarinia* Dai & Hyde, *B. bambusae* Dai & Hyde, *B. irregulispora* Dai & Hyde and *B. splendid* Dai & Hyde in northern Thailand which are Trematosphaeriaceae [2-3]. However, some publications have already reported this. Unspecified fungi from ant nests were found in various tropical regions [4]. The fungi were called Capnodiales spp. functioning as the nest of black ants, *Crematogaster*. In addition, another unidentified fungal isolate was derived from the nest wall of *Dendrolasius* and *Chthonolasius* ants and the fungus in Capnodiales displays mutualism with the ants [5]. However, in both reports, no perfect stage of the fungi was exhibited; only mycelial and conidia were available. A question arose whether the collected sample from Thailand was one of them. Therefore, molecular data of three ribosomal DNA regions, 18s rDNA, 28s rDNA and internal transcribe spacer were employed to perform phylogenetic inferences as well as identify its morphological features.

Due to its uniqueness relative to other fungi reported on bamboo and the insufficient number of reported ant-associating fungi, the fruiting bodies of the fungus were subjected to identification and characterization using morphology and molecular data, three rDNA sequences to construct phylogenetic trees.

2. Materials and methods

2.1 Collecting site

Fugal samples were found on bamboo branches with different heights. It was found on the branch on top of the tree and only a meter from the ground. The location of the collected samples was in Dan Sai district, Loei Province in April 2015. Black ants were always around the fungal samples. The bamboo branches with the fungal samples including ants were collected for identification.

2.2 Fungal isolation and culture

The fruiting bodies of the fungus was dissected in half and attached to the upper side of the Petri dishes containing Water Agar (WA) and left overnight at room temperature to discharge ascospores. The obtained ascospores on WA surface were transferred onto a new Potato Dextrose Agar (PDA) and incubated for 7 days at 25 °C before being transferred into Potato Dextrose Broth (PDB) and incubated for 7 days at 25 °C without agitation. In addition, the morphological features of the fruiting bodies were examined and the fungal isolates were aerially cultured to investigate the morphological characters of its teleomorphs for 14-21 days in moist chambers at 25 °C.

2.3 DNA extraction Polymerase chain reaction (PCR) and DNA sequencing

The fungal colonies on PDB were taken and washed in sterile distilled water for DNA isolation. The genomic DNA of was extracted using the standard method [6]. Of the collected colonies, 1 g were grounded in liquid nitrogen by using a sterile mortar and pestle in lysis buffer consisting of 200 mM Tris-HCl, pH 8.0, 250 mM NaCl, 25 mM EDTA, pH 8.0 and 2% sodium dodecyl sulfate for 700 µL with 2 µL ß-mercaptoethanol. Then, the mixture was transferred into tubes and incubated at 60 °C for 1 hr. After that the mixture of chloroform: isoamyl alcohol (24:1) for 700 µL were added and gently mixed before centrifuged at 12,000 rpm for 5 min at 4 °C. Only supernatant was transferred to new tubes. Isopropanol, 0.7 times of collected supernatant volume was added then the tubes were kept at -20 °C for 20 min. The centrifugation at 12,000 rpm for 5min was to obtain DNA pellets from the tubes then 70% ethanol for 500 µL was used to wash the pellets twice and left at room temperature to be air-dried. The dried DNA pellets were dissolved in 50-µL TE buffer made up of 10 mM Tris-HCl and 1 mM EDTA then followed by the addition of RNase A, 2 µL before being incubated for 20 min at 37 °C and 1 µl of Proteinase K was respectively added before 20 min incubation. Chloroform: isoamyl alcohol (24:1) was added, centrifuged 12,000 rpm for 4 min again to clean the DNA solution. The supernatant was transferred to new tubes and they were added with 3 µL of 3M sodium acetate and 150 µL of absolute ethanol and left at -20 °C for 30 min. The cleaned DNA pellets were obtained through centrifugation at 12,000 rpm for 10 min and the pellets were cleaned with 70% ethanol then dried at room temperature before re-suspended in TE buffer and kept at -20 °C for further experiments.

The desired genomic DNA of fungal specific regions informative for fungal classification and identification e.g. 18s rDNA using primers NS1-GTAGTCATATGCTTGTCTC and NS4-CTTCCGTCAATTCCTTTAAG (White *et al.* 1990), 28s rDNA amplified with primers NL1-GCATATCAATAAGCGGAGGAAAAG and NL4-GGTCCGTGTTTCAAGACGG [7] and 5.8s rDNA using primers, ITS1-TCCGTAGGTGAACCTGCGG, ITS4-TCCTCCGCTTATTGATATGC and ITS5- GGAAGTAAAAGTCGTAACAAGG [6]. The total volume of a PCR mixture was 50 µL containing 100 ng of total DNA, 0.6 mM dNTPs, 2.5 mM $MgSO_4$, 1 µM of each 20 pmol primer, 1x PCR buffer (Thermo Scientific), and 1.5 U of Taq polymerase (Thermo Scientific). The PCR conditions were used as follows. For NS1/NS4 primers, predenaturation was at 95 °C for 5 min and 30 cycles of then 95 °C for 1 min followed by annealing process at 54 °C at 1 min then extension at 72 °C for 1 min and final extension at 72 °C for 7 min (White *et al.* 1990). For NL1/NL4, the temperature used for predenaturation was at 94 °C for 5 min then followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s then 72 °C for 1 min and final extension at 72 °C for 7 min (O'Donnell 1993). To amplify the ITS regions, the predenaturation temperature was at 95 °C for 3 min followed by 35 cycles of 95 °C for 1 min, 55 °C (ITS1/ITS4), 57 °C (ITS4/ITS5) for 1 min then 72 °C for 2 min and 72 °C for 10 min [6]. Then, 1 µL of PCR products was loaded in 1% agarose gel through the electrophoresis in TBE buffer (1 M Tris, 0.9 M boric acid, and 0.01 M EDTA, pH 8.3) for 1 h then stained with ethidium bromide solution then visualized in gel documentation to determine whether the PCR yields were successful. The PCR products were in-gel purified and sequenced using a BigDye® Terminator v3.1 cycle sequencing kit by First BASE Laboratories, Seri Kembangan, Selangor, Malaysia.

2.4 Phylogenetic analysis

Sequence Scanner Software v2.0 was used to check unclear chromatogram signals of the sequences which were removed before the alignment. The 18s, 28s and 5.8s rDNA sequences were retrieved from GenBank (www.ncbi.nlm.nih.gov) as shown in Table 1. The accession numbers of DNA sequences of the fungus were as follows, 9 ITS sequences (KT454973, KT454974, KT454975, KT454976, KT454977, KT454978, KT454979, KT454980 and KT454981), 4 28s rDNA sequences (KT454982, KT454983, KT454984 and KT454985) and 4 18s rDNA sequences (KT454986, KT454987, KT454988 and KT454989). Available data from the database of fugal representatives in Arthoniomycetes, Dothideomycetes, Eurotiomycetes, Geoglossomycetes, Lecanoromycetes, Leotiomycetes, Lichinomycetes, and Sordariomycetes including different genera in Capnodiaceae were aligned with Clustal X and manually edited using MEGA 6 [8]. The analysis consisted of two data sets, the combined sequence of 18s and 28s rDNA and ITS. Outgroups for these datasets 1 and 2 were respectively *Orbilia auricolor* (A. Bloxam) Sacc. and *Scorias leucadendri* Crous. Then, both datasets of the aligned sequences were deposited in TreeBASE (link). Three methods for constructing phylogenetic trees were employed, Neighbour-Joining, Maximum parsimony and Baysian analysis. Firstly, a maximum parsimony method was performed to construct the tree of dataset 1 using PAUP v. 4.0b10 [9]. The analytic conditions for the analysis were set as follows: The heuristic search as the optimality criterion and stepwise addition with tree-bisection-reconnection (TBR) as the branch-swapping algorithm set to yield starting trees were selected. The Multrees option was in effect and gaps were treated as missing data. The number of bootstrap was done for 1000 replicates once the stepwise addition process reached 100 to test the confidences of the tree branches [10]. The final tree was illustrated using PAUP* version 4.0 b10 [9]. The confidence evaluation was 1000 replicates of bootstrap. The yielded tree was portrayed using PAUP* version 4.0 b10 [9]. Aside from these, Baysian analysis was also conducted using MrBayes version 3.2.1. to calculate the posterior probabilities of the tree through Markov chain-Monte Carlo (MCMC) method to both datasets [11]. The conditions of the analysis were as follows. Two million and 500,000 generations with 1,000 generations of sampled tree were performed in dataset 1 and 2 respectively. The general time-reversible model with invariant sites and gamma distribution (GTR + I + Γ) were used in searches and the 25% burn-in to approximate the posterior probabilities was applied (Huelsenbeck and Rannala, 2004). Tree nodes obtained from the phylogenetic analyses were considered as being well supportive by posterior probabilities and bootstrap values greater than or equal to 95% and 60 respectively [12].

Table 1 Accession numbers retrieved from the database for phylogenetic construction**.**

Taxa	GenBank Accession Number		
	18s	28s	ITS
Class Sordariomycetes			
Cainia graminis	AF431948	AF431949	
Chaetomium globosum	AB048285	AY346272	
Diatrype disciformis	DQ471012	DQ470964	
Sordaria fimicola	AY545724	AY545728	
Xylaria acuta	AY544719	AY544676	
Xylaria hypoxylon	AY544692	AY544648	
Class Leotiomycetes			
Botryotinia fuckeliana	AY544695	AY544651	
Bulgaria inquinans	DQ471008	DQ470960	
Crinula calciiformis	AY544729	AY544680	
Gelatinomyces siamensis	JX219377	JX219381	
Monilinia fructicola	AY544724	AY544683	
Neofabraea malicorticis	AY544706	AY544662	
Pezicula carpinea	DQ471016	DQ470967	
Potebniamyces pyri	DQ470997	DQ470949	

Taxa	GenBank Accession Number		
	18s	28s	ITS
Class Lecanoromycetes			
Diploschistes thunbergianus	AF274112	AF274095	
Lobaria scrobiculata	AY584679	AY584655	
Trapella placodioides	AF119500	AF274103	
Class Lichinomycetes			
Lempholemma polyanthes	AY548805	AF356691	
Peltula auriculata	DQ832332	DQ832330	
Peltula umbilicata	DQ782887	AF356689	
Class Eurotiomycetes			
Eremascus albus	M83258	AY004345	
Eurotium rubrum	U00970	AY004346	
Penicillium expansum	DQ912698	AF003359	
Exophiala dermatitidis	DQ823107	DQ823100	
Glyphium elatum	AF346419	AF346420	
Ramichloridium anceps	DQ823109	DQ823102	
Class Dothideomycetes			
Order Botryosphaeriales			
Botryosphaeria ribis	DQ678000	DQ678053	
Botryosphaeria stevensii	DQ678012	DQ67806 4	
Guignardia bidwellii	DQ678034	DQ678085	
Order Capnodiales			
Catenulostroma abetis	DQ678040	DQ678092	
Cercospora beticola	DQ678039	DQ678091	
Microxyphium citri	AY016340	AY004337	
Mycosphaerella punctiformis	DQ471017	DQ470968	
Scorias spongiosa	DQ678024	DQ678075	
Mycosphaerella punctiformis			EU343222
Mycosphaerella punctiformis			EU343223
Mycosphaerella punctiformis			EU343241
Scorias leucadendri			JQ044437
Antennariella placitaec			JN116688
Antennariella placitae			GQ303268
Capnodiales sp.			HQ634615

Taxa	GenBank Accession Number		
	18s	28s	ITS
Capnodiales sp.			HQ634609
Capnodiales sp.			HQ634627
Capnodiales sp.			HQ634618
Capnodiales sp.			HQ634625
Capnodiales sp.			HQ634624
Capnodium coffeae			DQ491515
Capnodium sp.			HQ914834
Capnodium sp.			KC180729
Capnodium sp.			HE584831
Capnodium sp.			HE584835
Heteroconium citharexylie			HM628776
Leptoxyphium glochidion			KF982307
Leptoxyphium kurandae			JF951150
Leptoxyphium madagascariense			HE584914
Polychaeton citri			GU214649
Teratosphaeria hortaea			FJ790281
Teratosphaeria hortaea			FJ790280
Order Dothideales			
Aureobasidium pullulans	DQ471004	DQ470956	
Delphinella strobiligena	AY016341	AY016358	
Discosphaerina fagi	AY016342	AY016359	
Dothidea ribesia	AY016343	AY016360	
Stylodothis puccinioides	AY016353	AY004342	
Order Myriangiales			
Cladosporium cladosporioides	DQ678004	DQ678057	
Davidiella tassiana	DQ678022	DQ678074	
Elsinoe centrolobi	DQ678041	DQ678094	
Myriangium duriaei	AY016347	DQ678059	
Order Pleosporales			
Arthopyrenia salicis	AY538333	AY538339	
Cucurbitaria elongata	DQ678009	DQ678061	
Neotestudina rosatii	DQ384069	DQ384107	
Pleospora herbarum	DQ247812	DQ247804	

Taxa	GenBank Accession Number		
	18s	28s	ITS
Setosphaeria monoceras	AY016352	AY016368	
Trematosphaeria heterospora	AY016354	AY016369	
Westerdykella cylindrical	AY016355	AY004343	
Order Insertae sedis			
Catinella olivecea			
Family Tubeufiaceae			
Helicomyces lilliputeus	AY856942	AY856899	
Helicomyces roseus	DQ678032	DQ678083	
Tubeufia cerea	AY856947	AY856903	
Class Arthoniomycetes			
Arthonia dispersa	AY571379	AY571381	
Dendrographa leucophaea	AY548803	AY548810	
Lecanactis abietina	AY548805	AY548812	
Outgroup			
Orbilia auricolor	DQ471001	DQ470953	

3. Results

3.1 Black ant

The ant samples were subjected to identification. They were found to be a population of *Dolichoderus thoracicus* (Smith) in subfamily Dolichoderinae generally found in South East Asia [13-15].

3.2 Taxonomy of the fungus

Mycodomus Jitjak & Sanoamuang, gen. Nov.
Biotec Collection no. TBRC1894
Diagnosis: *Ascostroma* gray to black, texture hard and tough, globose or rugby-ball shaped on bamboo branch, black ants living around ascostroma, cavity inside. *Ascomata* perithecia submerged beneath the surface of the ascostroma with black dots as ostiole tips containing periphyses and septate-ascospres.
Etymology: Referring to its role as a place for ants to reside, myco = fungus and domus = house.
Type: Mycodomus formicartus W. Jitjak and N.Sanoamuang 2019.
Description: *Stromata* present, gray to black, hard and tough in texture because of dense mycelium. *Ascomata* Perithecia arranging underneath the surface of stromata with periphysis at ostiole, *Perithecium* submerged underneath the surface containing periphyeses containing asci. *Asci* clavate or club-shaped, tapered at the base, bitunicate without operculum containing sepate ascospores. *Ascospores* fusiform and hyaline when young, 12-16 septates and brown when mature, ovoid with rough texture on external wall.
Colonies with slow-growing, dark-green colored, producing brown droplets on mycelium *Conidiophores* absent. *Conidia* thallospores due to mycelial fragmentation, ovoid, dark green to brown.

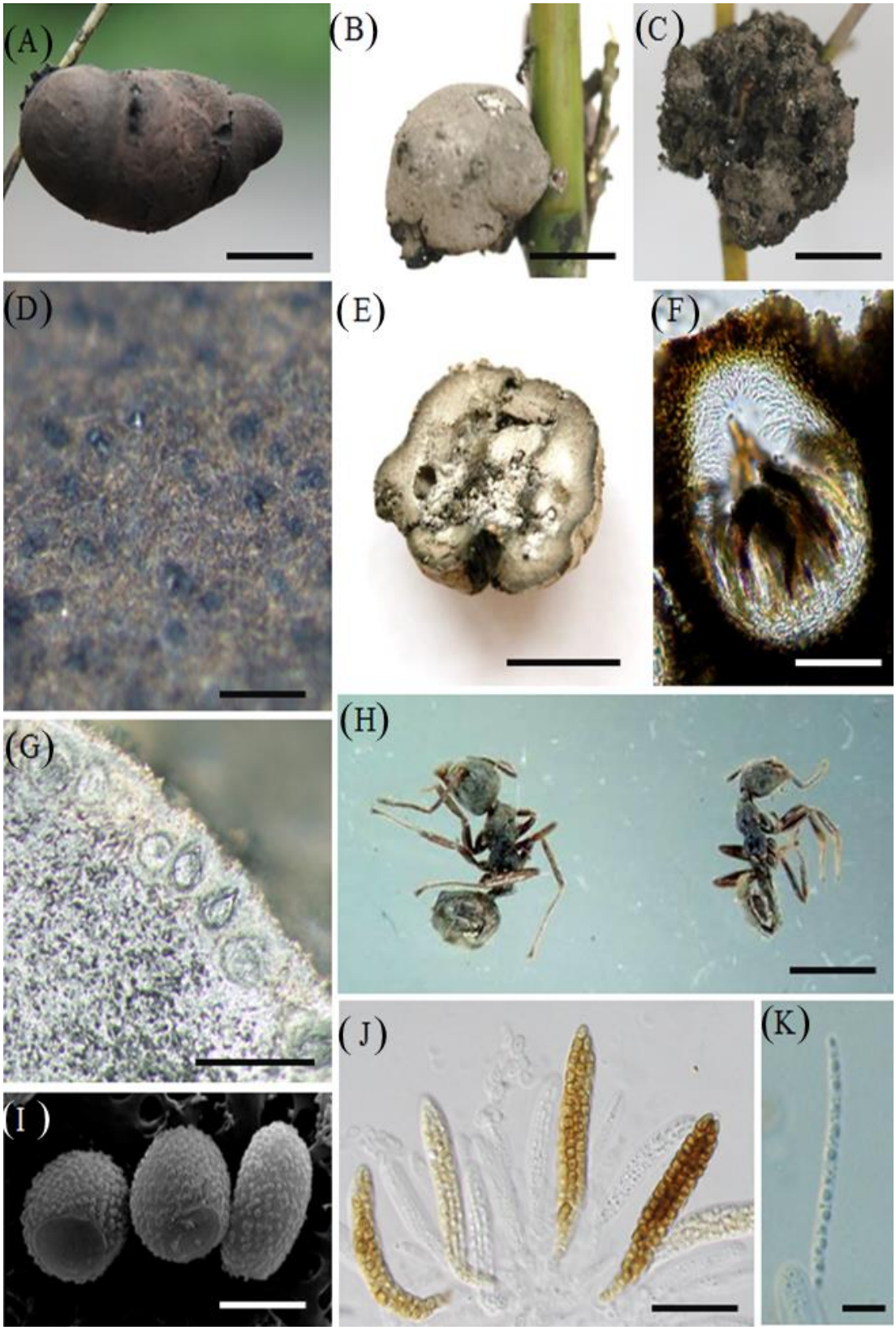

Figure 1 Fruiting bodies of *Mycodomus formicartus*. (A-B): Black and gray fugal bodies found on bamboo with superficial pores, (C): Degraded fruiting body revealing internal cavity, (D): Surface of fruiting body, (E): Fruiting body dissected in half, (F): A perithecium with periphyses, (G): A row of perithecia submerged underneath the fruiting- body surface, (H): Black ants, *Dolichoderus thoracicus* associated with fungal bodies, (I): Part-ascospores, (J): Asci containing ascospores, K: Young ascospore. Scale bars (A-C) = 1 cm, (D) = 1 mm, (E) = 1 cm, (F) = 20 µm, (G) = 1 mm, (H) = 1 mm, (I) = 1 µm, (J) = 10 µm, (K) = 1 µm.

Mycodomus formicartus Jitjak & Sanoamuang sp. Nov. Biotec Collection number**.** TBRC1894 **(**Figure 1-2**)**

Diagnosis: *Stromatal* hard and tough, globose, rugby- ball- shaped, various in size, having pertithecia submerged underneath the surface, cavity structure inside. *Asci* bitunicate, clavate, *Ascospores* fusiform, septate, rough wall. *Asexual morph* dark green to brown conidia produced by mycelial fragmentation, ovoid. *Colonies* slow-growing, dark-green.

Etymology: Due to its association with ants, formi=ant and cartus=container.

Type: Thailand**:** *Loei Province*: Dan-Sai district on bamboo branches, April 2015,

Jitjak & Sanoamuang (KKUMy – Holotype; KKUMy 1, 2, 3 -ex- holotype culture; Biotec Culture Collection code: TBRC1894)

Description: *Stromata* hard and tough, globule, rugby-ball-shaped, 3-4 × 1-2 cm in dimension, with miniature black dots and pores on surface, gray until black when mature, cavity structure inside connected with the pores on surface. *Ascomata* perithecia, dark and immerse beneath the stromatal surface, ostiole with periphyses, (40-) 46.75-75(-83) × (30-)45-70(-75) µm, L/W = 1.02: 1. *Asci* clavate, bitunicate without operculum, (10-) 14.45-31.1(-35) × (3-)3-5(-6) µm, L/W = 5.32: 1. *Peridium* thick and multi-layered, dark brown to black. *Ascospores* 12-16 fusiform, septate, globose to ovoid, hyaline when young and dark green to brown when mature, necklace-like, rough surface, (1.1-) 1.1-2.05(-2.5) × (0.9-)1-1.5(-2) µm, L/W = 1.48 :1.

Colonies with slowly growing, dark green, mycelia producing brown droplets, mycelial conjugation observed with necklace-liked hypha. *Conidiophores* absent. *Conidia*, thallospores, single-celled, ovoid, green to brown, (4)4-7(7) × (8)8.65-11.35(12) µm, L/W =1.78:1.

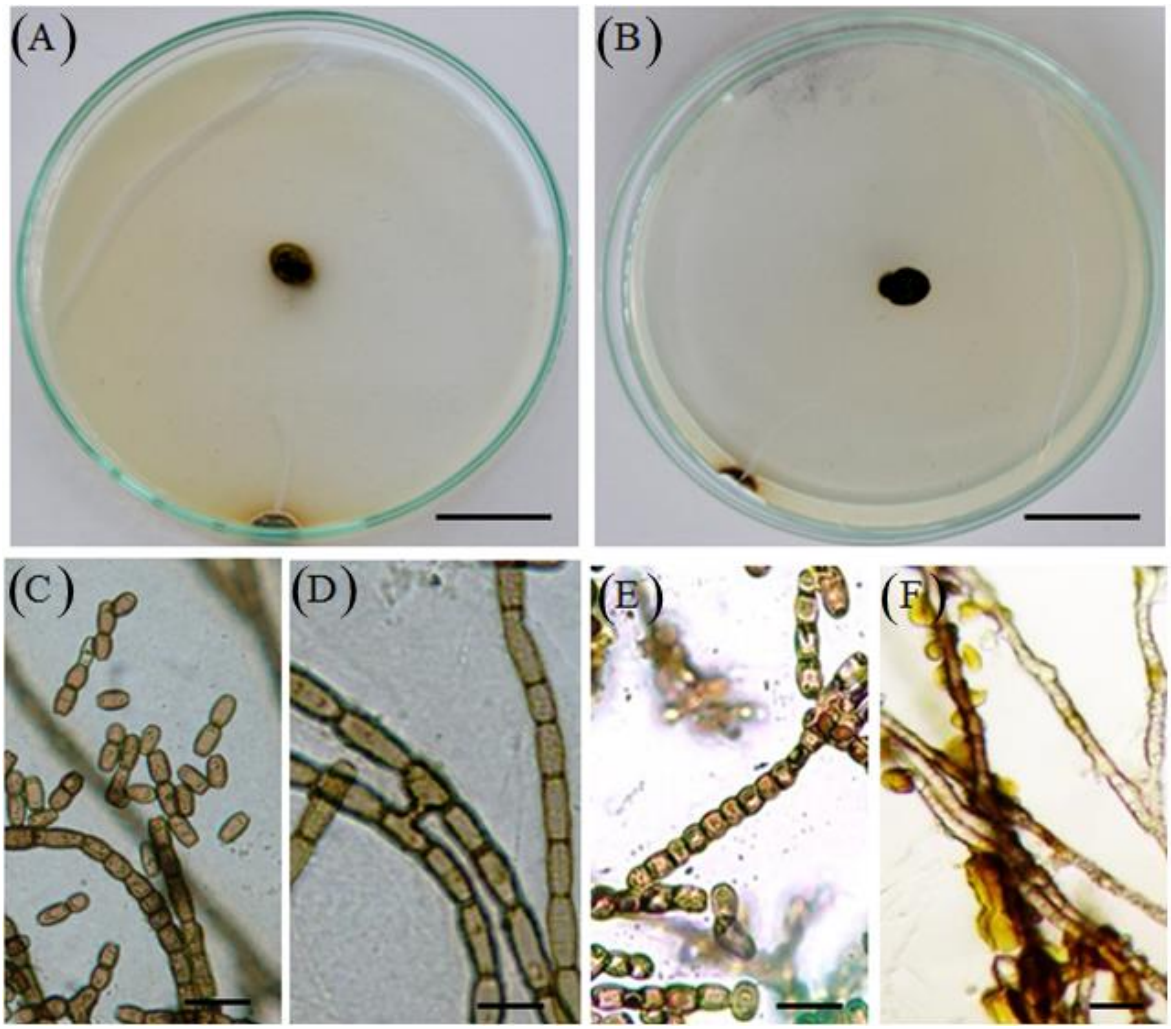

Figure 2 Teleomorphic characteristics of *Mycodomus formicartus*. (A - B): 7-day-old fungal colonies on PDA, (C): Conidia, (D): Mycelial fusion, (E): Necklace-liked hypha, (F): Brown droplets produced by the fungus, Scale bars (A-B) = 2 cm, (C-E) = 2 µm, (F) = 5 µm.

3.3 Phylogenetic trees

The analysis by means of maximum parsimony using the combination of 28s and 18s rDNA sequences with 2,345 total and 768 parsimony-informative characters (2,090 of minimum possible length, 7,537 of maximum possible length, consistency index (CI) = 0.516, retention index (RI) = 0.614, rescaled consistency index (RC) = 0.311, homoplasy index (HI) = 0.484) revealed that the fungal isolates, *M. formicartus* were clustered in the same branch with others in Capnodiales and Capnodiaceae, *Microxyphium citri* with potential bootstrap score at 100 (Figure. 3). Similarly, another phylogenetic inference, Bayesian analysis also indicated the same result. The fungal isolates were grouped in Capnodiales and related to *M. citri* with supportive posterior probability value, 100 (Figure 4).

As the results of analyses of both trees, the fungus could be clearly classified as Dothideomycetes, Capnodiales and Capnodiaceae. To further find which genus and species, ITS sequences of the fungus were used to perform the phylogeny with members in Capnodiaceae.

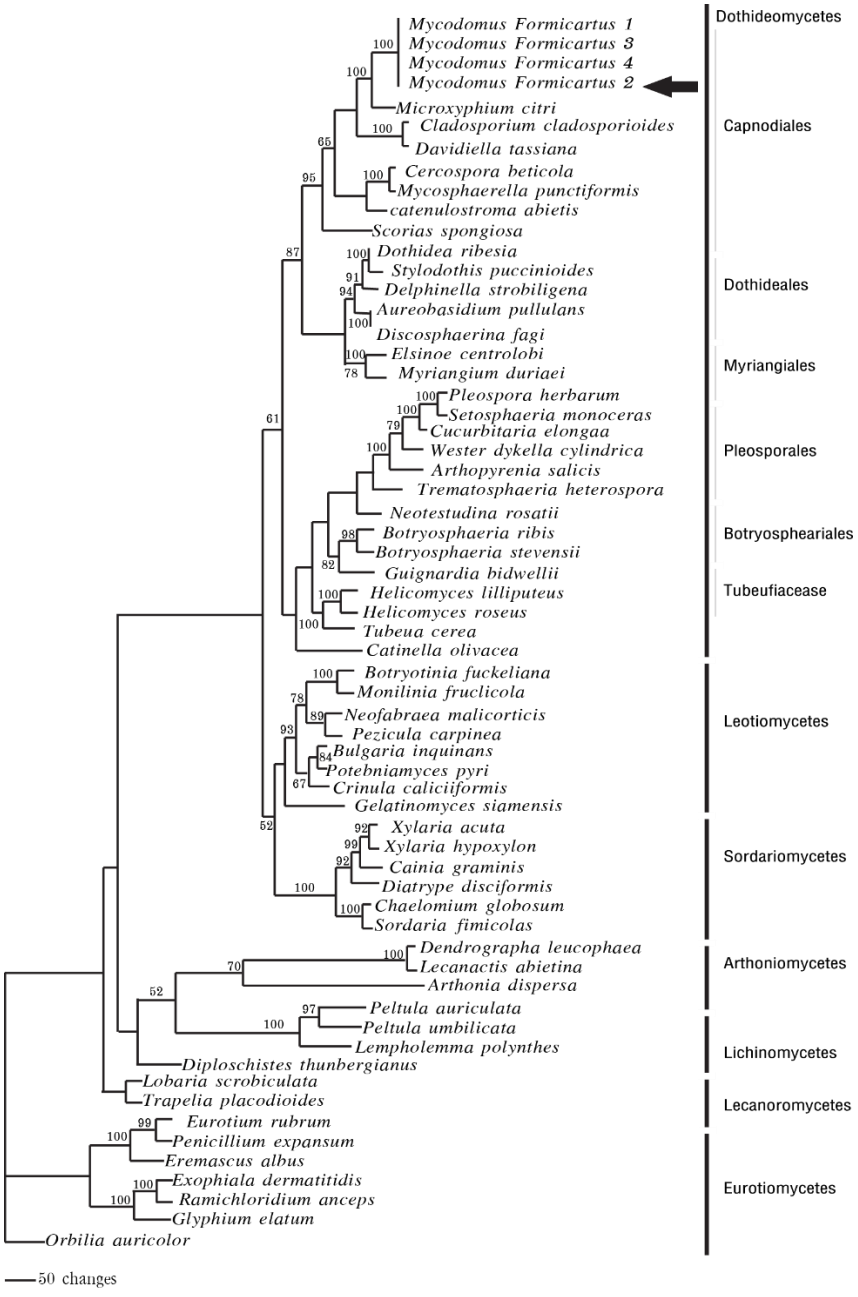

Figure 3 Phylogenetic tree of combined sequences of 18s and 28s rDNA obtained from Maximum Parsimony method. *Mycodomus formicartus* is grouped in Dothideomyctes, Capnodiales and Capnodiaceae with highly supportive bootstrap score, 100.

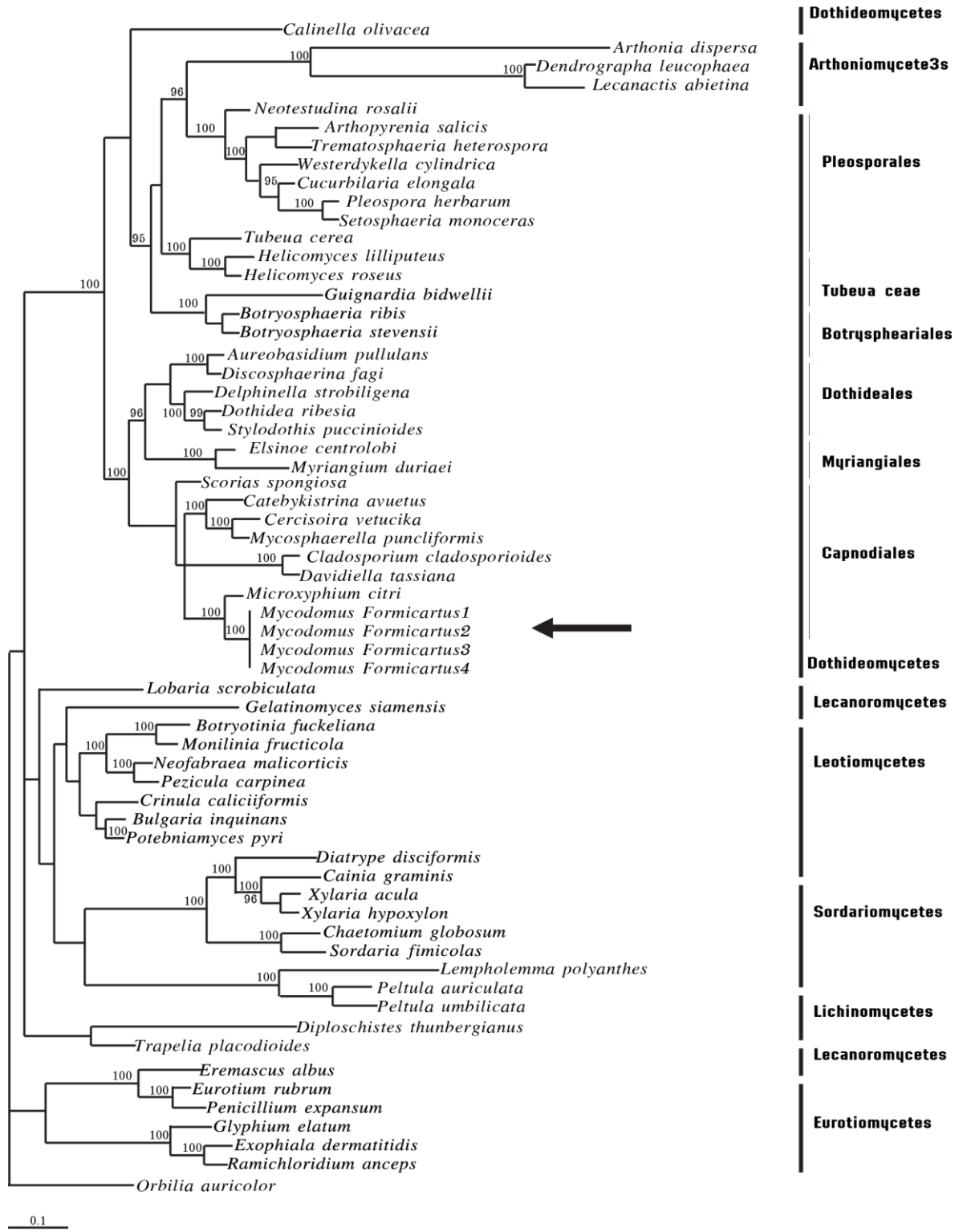

Figure 4 Phylogenetic tree of combined sequences of 18s and 28s rDNA obtained from Baysian analysis. *Mycodomus formicartus* is grouped in Dothideomyctes, Capnodiales and Capnodiaceae with high posterior probability, 100.

In Capnodiales, the previous phylogenetic analysis yielded *M. formicatus* with fungi in Capnodiaceae. Therefore, ITS sequences were selected to perform another phylogenetic analysis with candidates from this family, including *Capnodium*, *Leptoxyphiu*, *Mycosphaerella*, *Teratosphaeria* and unidentified Capnodiales spp. reported [4]. The result suggested that the *M. formicartus* isolates were clustered at the same branch as the fungus of this study. Both phylogenetic trees derived from Maximum Parsimony and Bayesian analysis suggested the

similar position of the fungal isolates. The trees indicated that *M. formicartus* isolates were situated with Capnodiales isolates with supportive statistical scores, 80 of bootstrap and 100 of posterior probability values (Figure 5 and 6). It suggested that the fungus of this study could likely be one of these Capnodiales species or another species related to them.

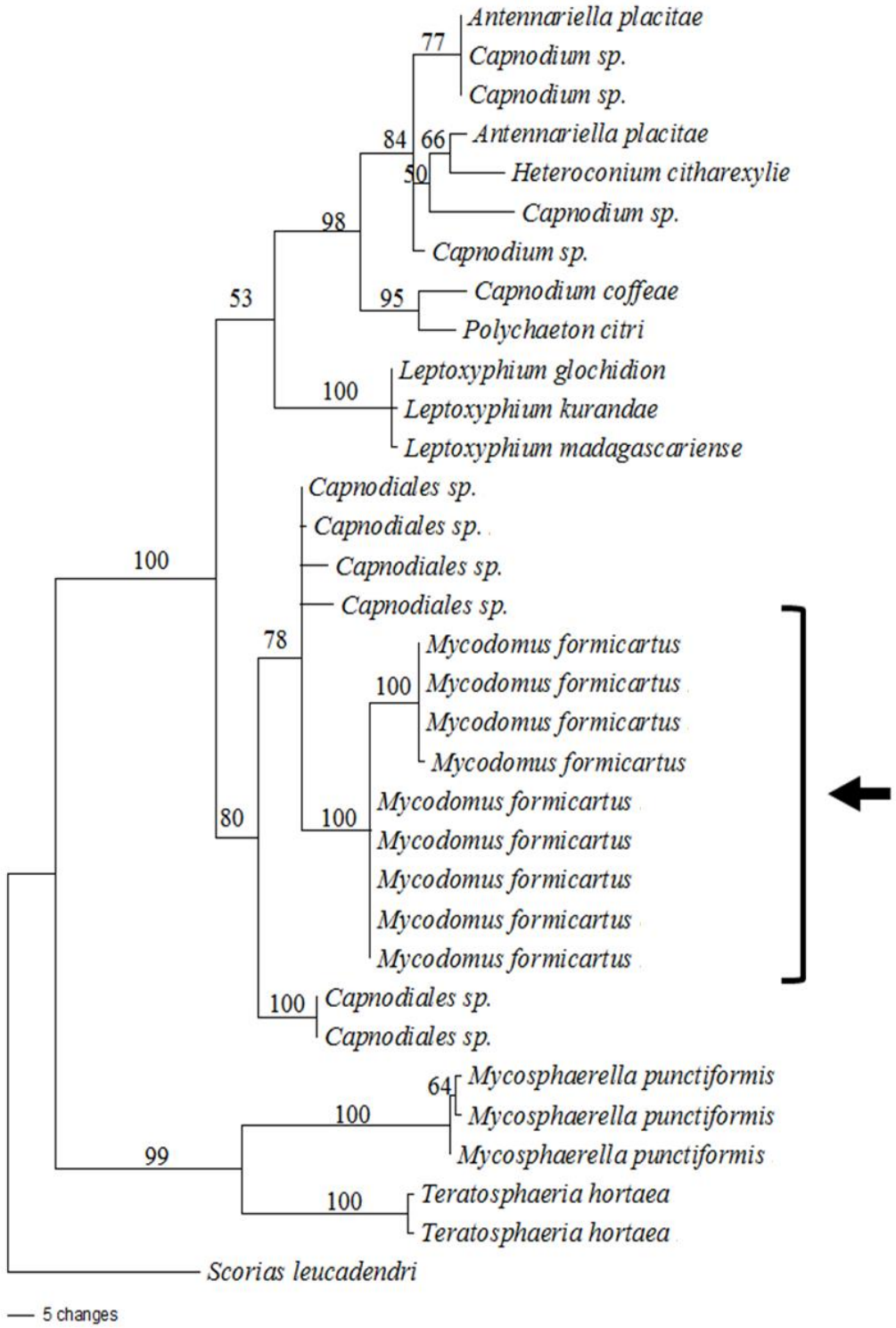

Figure 5 Phylogenetic tree of ITS sequences obtained from Maximum Parsimony method. *Mycodomus formicartus* is grouped in the same branch as an unnamed fungus, *Capnodiales* spp. with highly supportive bootstrap score, 80.

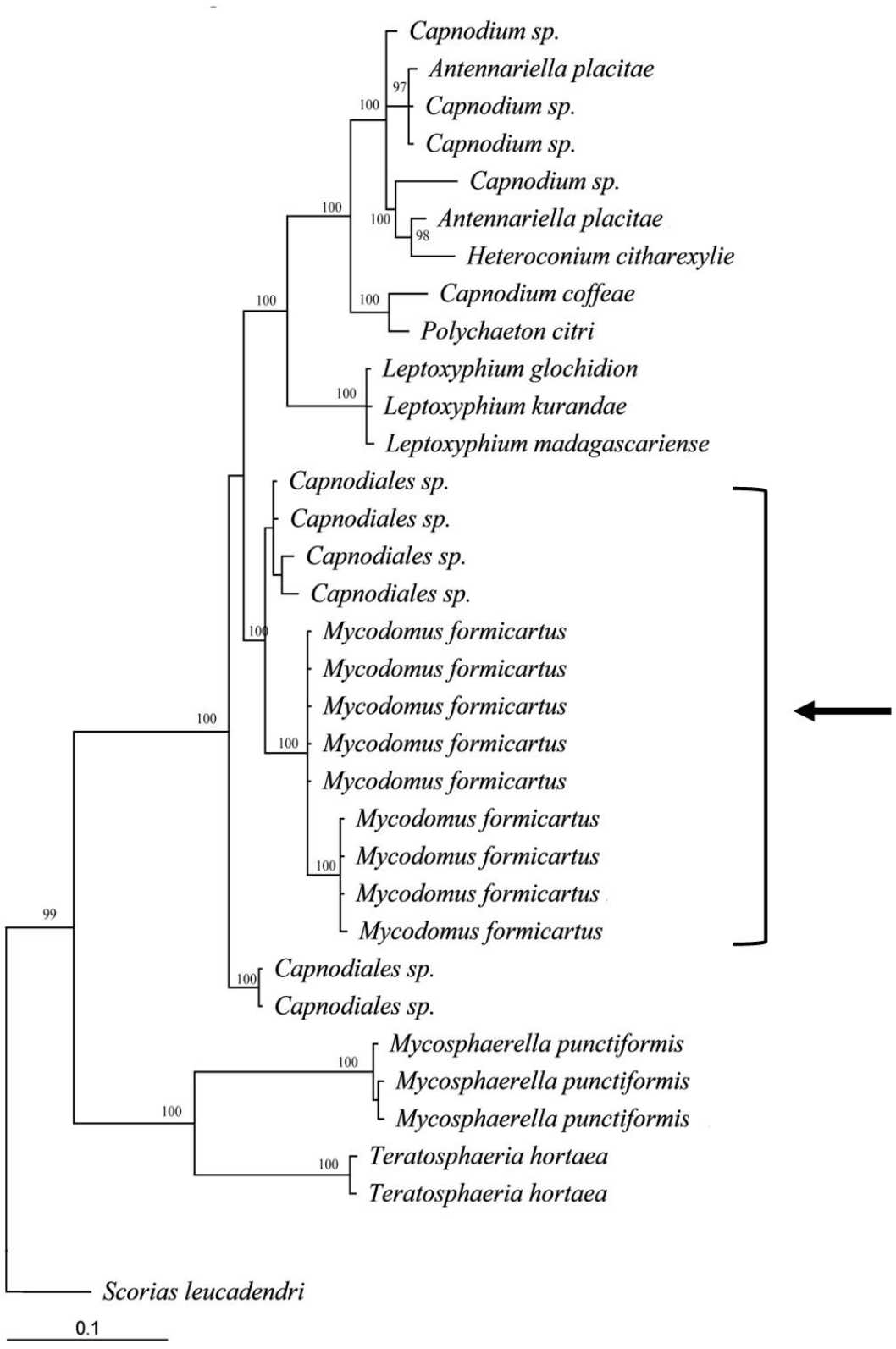

Figure 6 Phylogenetic tree of combined sequences of 18s and 28s rDNA obtained from Baysian analysis. *Mycodomus formicartus* is grouped in *Dothideomyctes, Capnodiales* and *Capnodiaceae* with high posterior probability, 100.

4. Discussion

The fungus had been believed to be one of bambusicolous fungi because it was always found on bamboo branches as black balls or lumps in different heights ranging from just above the ground to several meters on top of the canopy. According to literatures, a great number of bamboo-associating fungi are reported. More than 1,000 species of fungi on bamboo are documented and more than half of them are ascomycetes with different ecological roles to bamboo [1]. For example, *Bambusicola* species which are members in Trematosphaeriaceae, Dothideomycetes have reported on bamboo from the northeastern region of Thailand including *Shiraia bambusicola* (Dothideomycetes, Pleosporomycetidae) with pink- orange ascostromata [2-3,16]. Fungi in Sordariomycetes are also associated with the bamboo plant, *Acrospermum chilense*, *Engleromyces goetzei* and *Daldinia bambusicola* with distinctive fruiting bodies [17–19]. Other hypocrealean fungi such as *Ascopolyporus philodendrus Moelleriella gaertneriana*, *Mycomalus bambusinus* are also related to bamboo and have interactions with some insect species [20-22].

Another outstanding feature that could narrow the spectrum of fungal diversity was the relation to black ants, *D. thoracicus*, of the fungus. The ants were always close to the fungus. In the dissected fruiting body, there was a cavity structure and debris inside. This suggested that the fungus could have a certain association with the ants functioning as the habitat (Figure 1A-B). Even in the mature fungal sample, which was naturally degraded and exposed the carton-like structure, the ants were still observed around the sample (Figure 1C & E). *D. thoracicus* is an indigenous species widely populated throughout South East Asian region including Thailand, and they are capable of forming nests in different substrates such as ground, leaves and plant cavities, secreting sticky honeydew like other insects [15,23]. In this case, the ant could be able to utilize the fungal fruiting body as the nest. Because of the hard and tough structure of the fungus, the ants live inside the fungal fruiting body as a safe house to protect themselves from harsh environments. Furthermore, the ants secrete feces or honeydew which could benefit the fungus' growth and development.

Symbiosis is likely an appropriate term for the fungus-to-ant interaction under this circumstance. The role of the fungus towards the ant nest is to stabilize the carton and the role of the ants associating with fungi is to provide some secretions [4,24]. Fungi in Capnodiaceae isolated from ant carton according to the report are common in *Crematogaster* nest with hard and tough texture [4]. The clear ecological role of the fungus with *Crematogaster* ants has not been developed as there is no supportive evidence regarding to the association of ant and fungus. However, in this case, it is apparent that the tough fruiting body of *M. formicartus* is surrounded by the *D. thoracicus* population and it exhibits a cavity structure for the ants to reside inside the fruiting body rather than inside the plant tissue. This evidence proves that the ant uses the fungal fruiting body as a place to live. The fungi in the Capnodiaceae family are also related to substances secreted by insects including ants for their growth and development [25]. Certain chemicals derived from the black ants could be essentially beneficial to the development of this fungus. This interaction infers a symbiotic association of the fungus and the ant.

The phylogenetic trees derived from the use of ribosomal rDNA sequences, 18s and 28s suggested that the fungus was in Capnodiaceae because it was clustered in the same branch with *Microxyphium citri* with supportive statistical scores as shown in Figure 3 and 4. One of characteristics of the fungi in this family is that they are saprophytic on insect honeydew [22]. *M. formicartus* is thus likely to use this secretion from the ants for its development of strong fruiting body which latterly becomes the residence for the ants. In ITS trees, the clade *of M. formicartus* was closely situated with unnamed Capnodiales spp. [4]. The isolates of these Capnodiales spp. were obtained from nests of an ant species, *Crematogaster* collected from tropical countries, Cameroon, Malaysia and Thailand. This led to a question of whether *M. formicartus* was one of these reported Capnodiales spp. The sexual morph of the fungus was however not reported, only mycelium isolated from the ant nests from a plant, *Barteria nigritana* and unidentified sources. The necklace-like feature of mycelium of the capnodiaceaous fungi was similar to *M. formicartus* but no conidium was produced. Because of the differences where it was found and conidial formation, *M. formicartus* was able to be claimed as another species apart from them. Thus, a new genus and species exclusive to the fungus have been described and established in this paper.

5. Conclusions

As the consequence of the phylogenetic trees, morphological features and ecological aspect of this capnodiaceaous fungus, it is convincing that the fungus is a new genus in Capnodiaceae and a new species which is symbiotically associated with *D. thoracicus* ant i.e. the fungus feeds on the ant secretion for its development and the ants live in the fungal fruiting body as their nest. Due to the relationship with the ant and function of the fungus, *Mycodomus formicartus* as the official name has therefore been established.

6. Acknowledgements

This study was supported by Applied Taxonomic center, Khon Kaen University and International College, Khon Kaen University. Also, Agricultural Research Center for Sustainable Economy, Khon Kaen University and Center of excellence on Agricultural Biotechnology, Khon Kaen University (AG-BIO/PERDO-CHE) was to be thanked for assisting the laboratory and molecular equipment and special thanks to Associate Professor Dr. Decha Wiwatwitaya from Ants Museum, Kasetsart University for identifying the ant and Mr. Kyle Daniel Craven for proofreading the manuscript.

7. References

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