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Phylogenetic placement of *Akanthomyces muscarius*, a new endophyte record from *Nypa fruticans* in Thailand

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Abstract

A species of *Akanthomyces* (*Cordycipitaceae*, Hypocreales) was isolated as an endophyte from healthy leaves of *Nypa fruticans* collected in Krabi Province, Thailand. The species was identified as *Akanthomyces muscarius* based on phylogenetic analyses of the ITS gene region, as well as a combined LSU, SSU, TEF1 and RPB2 sequence dataset. Previously reported descriptions for *A. muscarius* are brief and based on few observations. In the present study, detailed descriptions of cultural and morphological characters of the new isolate are given. Phylogenies based on maximum likelihood and Bayesian analyses indicate that our new isolate clusters with extant strains of *A. muscarius* with good support and is sister to the genus *Lecanicillium*. Descriptions of the isolate match well with previously published data and our phylogeny supports the species identification. The asexual fungus *A. muscarius* is a new record for Thailand.

Key words – new record – *Akanthomyces* – *Cordycipitaceae* – mangrove

Introduction

Mangrove plants, especially leaves, are generally colonized by a variety of microfungi (Raghukumar et al. 1995, Swe et al. 2008, Sivakumar 2013, Doilom et al. 2017, Li et al. 2018, Devadatha et al. 2018). *Nypa fruticans* is a palm that develops in the upper regions of mangroves stretching from the brackish water zone at river mouths to almost inland freshwater (Rozainah & Aslezaeim 2010). Various terrestrial fungi have been recorded on the aerial parts of *N. fruticans*, e.g. *Fasciatispora petrakii, Astrosphaeriella nipicola, Oxydothis nypicola* (Hyde & Alias 1999, 2000, Poonyth et al. 2000), and some have been reported to be intertidal and endophytic (Hyde & Alias 1999).

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Akanthomyces belongs to the family *Cordycipitaceae* (Sung et al. 2007a, Kepler et al. 2012, Maharachchikumbura et al. 2015, 2016), Hypocreales (Lindau 1897). Species in *Cordycipitaceae* mostly occur on arthropods (e.g. *Cordyceps militaris*), plants (e.g. *Torrubiella alba*), other fungi (e.g. *Lecanicillium uredinophilum*) and in soil (e.g. *Lecanicillium primulinum*) (Link 1833, Vuillemin 1912, Petch 1932, Zare & Gams 2001, Sung et al. 2007a, Kouvelis et al. 2008, Kaifuchi et al. 2013, Park et al. 2015, Wijayawardene et al. 2017a, Zhang et al. 2006). The asexual genera placed in this family are *Akanthomyces*, *Amphichorda*, *Beauveria*, *Engyodontium*, *Gibellula*, *Granulomanus*, *Isaria*, *Lecanicillium*, *Microhilum*, and *Simplicillium* (Sung et al. 2007a, Nonaka et al. 2013, Wijayawardene et al. 2017b).

The genus *Akanthomyces* as proposed by Lebert (1858), including the type *A. aculeatus*, primarily infects Lepidoptera (Mains 1950, Sung et al. 2007b, Vincent et al. 1988). *Akanthomyces* species are characterized by their white, cream or flesh-colored, cylindrical, attenuated synnemata, covered with a hymenium of phialides (Hsieh et al. 1997). *Akanthomyces* is reported to be phylogenetically distinct from *Beauveria* and *Cordyceps* (Kepler et al. 2017). Some species of *Akanthomyces* are associated with *Torrubiella* and are pathogens of spiders (e.g. *Akanthomyces novoguineensis*). Besides its association with *Torrubiella*, *Akanthomyces* has been listed as an asexual morph of some *Cordyceps* species, e.g. *Cordyceps tuberculata* and *C. confragosa*, thus having a wider host range (Kepler et al. 2017, Sung et al. 2007b, Wijayawardene et al. 2017b). While revising the phylogenetic affinities of members within the family *Cordycipitaceae*, Kepler et al. (2017) reported that *Lecanicillium lecanii*, as well as some other species of *Lecanicillium*, namely *L. attenuatum, L. muscarium,* and *L. sabanense*, fall within *Akanthomyces* and proposed to reject *Lecanicillium,* as the former has priority over *Lecanicillium* (Kepler et al. 2017). There is some evidence that suggests fungal endophytes may help plants to withstand and endure unfavorable ecological conditions (de Bary 1884, Bills 1996, Dingle & McGee 2003, Porras-Alfaro et al. 2008, Wilson 1995) and furthermore promote plant development (Ernst et al. 2003). These inhabitants may deliver the same or similar secondary metabolites (Kusari et al. 2008, Stierle et al. 2003, Shu et al. 2014) as their host and may play a crucial part *in vivo*, e.g. signaling, defense, and regulation of the symbiosis (Schulz & Boyle 1998). In studies such as plant ecology and evolutionary relationships, fungal endophytes are very important as they can be latent pathogens, saprobes or beneficial symbionts (Sung et al. 2008, Doilom et al. 2017).

The present study reports on a new record of *Akanthomyces muscarius* as an endophyte on *Nypa fruticans* in Thailand. The species is characterized based on morphology and molecular support, the latter based on analyses of multigenes.

Materials & Methods

Isolates and specimens

Living (fresh) plant samples of *Nypa fruticans* were collected from Krabi Province in Thailand. At the site, asymptomatic leaf samples were collected from the aerial parts, and placed in separate paper bags. The samples were brought to the laboratory in an ice box and were refrigerated at 4 °C. Fungal isolation was performed the next day, following the method described in Doilom et al. (2017). Asymptomatic leaves were rinsed in tap water to remove debris and dirt. Samples were cut using a sterilized hole puncher (5 mm diam). Plant tissue surfaces were sterilized by shaking for 1 minute in 4.5% sodium hypochlorite (NaOCl) followed by a minute in sterile water. The samples were then disinfected in 70% ethanol for 1 minute, followed by three rinses with sterile distilled water. The tissues were dried by blotting on sterile filter paper. Four to five segments were placed in each Petri-dish on potato dextrose agar (PDA) supplemented with 100 mg/ml streptomycin and 50% of sea water. The dishes were sealed with parafilm and incubated at 27 °C ± 2 °C for one or two weeks until the onset of fungal growth. Fungal colonies were selected and purified by transferring single hyphal tips daily onto PDA plates throughout a 2-week period. Pure cultures were maintained on PDA for further studies. Living cultures of isolates were deposited in the Mae Fah Luang Culture Collection, Thailand (MFLUCC). Specimens have been deposited in the Mae

Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Representative isolates and specimens have been deposited at the BIOTEC Bangkok Herbarium (BBH). Faces of Fungi number is provided as outlined in Jayasiri et al. (2015). Comparison of DNA sequences across species are based upon recommendations proposed by Jeewon & Hyde (2016).

Morphological observations

Discs of five mm diameter were cut from the edge of actively growing colonies and placed onto fresh PDA plates. Three replicates were made and incubated at 27 °C. The average colony diameter (mm) was measured after ten days of incubation. Colony characteristics such as colour (above and below), elevation, margin, shape and surface were observed after 14 days of incubation (Lacap et al. 2003). Details of colony colour was defined with the Methuen Handbook of Color (Kornerup & Wanscher 1967) and documented. Morphological characters were examined from cultures which sporulated on PDA after 3–4 weeks. Morphological observations were made using a Motic SMZ 168 Series stereomicroscope and photographed by an OLYMPUS BX51 microscope imaging system. The fungal structures, such as phialides, conidiogenous cells and conidia were measured using Image frame work software.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted with modified method of CTAB as outlined by Jeewon et al. (2002). DNA was extracted from freshly harvested mycelium (500 mg), scraped from the margin of a colony on a PDA plate incubated at 27 °C ± 2 °C for 7–10 days and the quality of DNA samples extracted was examined in 1.5% resolution agarose gel. The ITS regions were amplified and sequenced with the primers ITS5/ITS4 (White et al. 1990), the LSU and SSU were amplified using LR0R & LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994) and NS1 & NS4 (White et al. 1990) primers, respectively, the TEF1 and RPB2 gene regions were amplified using EF1-983F & EF1-2218R (Rehner & Buckley 2005) and RPB22-5f & FRPB2-7cr (Liu et al. 1999) primers, respectively.

The amplification PCR reactions were performed in a total volume of 25 µl. PCR mixtures contained TaKaRa Ex-Taq DNA polymerase 0.3 µl, 12.5 µl of 2 × PCR buffer with 2.5 µl of dNTPs, 1 µl of each primer, 9.2 µl of double-distilled water and 100–500 ng of DNA template. PCR reactions were run on a BIORAD 1000 Thermal Cycler (Applied Biosystems, Foster City, CA, U.S.A.) under the following conditions: initial denaturation of 5 minutes at 95 °C, followed by 35 cycles of 30 seconds at 94 °C, 45 seconds, annealing at 55 °C, 1 minute at 72 °C, and a final extension of 10 minutes at 72 °C, for TEF1 and RPB2. For rDNA genes such as LSU, SSU and ITS we used initial denaturation of 5 minutes at 95 °C, followed by 35 cycles of 30 seconds at 94 °C, 45 seconds annealing at 58 °C, 1 minute at 72 °C, and a final extension of 10 minutes at 72 °C. Positive amplicons were visualized on 1% agarose gel under UV light using a Gel DocTM XR+ Molecular Imager (BIO-RAD, USA). Sequencing of the positive amplicons with primers mentioned above was carried by Sun-biotech Company Sequencer (Beijing, China).

Molecular phylogenetic analyses

Combined matrices constructed by concatenation of LSU, SSU, TEF and RPB2 sequences utilizing BioEdit software v7.0.5.2 (Hall 1999) to guarantee the sequence integrity. BLAST search (http://blast.ncbi.nlm.nih.gov/) was performed to aid in selection of most homologous sequences to be used in the dataset. GenBank accession numbers and culture collection numbers of DNA sequence data used to construct the phylogenetic tree are listed in Table 1 and Supplementary Table 1. In the combined analysis, 42 taxa, including *Simplicillium lanosoniveum* (CBS 101267 and CBS 704.86) as the outgroup taxon, were utilized. Datasets of different individual gene regions were also analyzed. Phylogenetic analyses were performed under different optimality criteria with maximum likelihood (ML) analysis and Bayesian inference (BI).

The evolutionary models for Bayesian analysis and maximum likelihood were selected independently for each locus using MrModeltest v. 2.3 (Nylander 2004) under the Akaike

Information Criterion (AIC) implemented in both PAUP v. 4.0b10. GTR + I + G model was the best-fit model of each locus for Bayesian analysis and maximum likelihood as determined by AIC in MrModeltest.

ML was generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) with 1000 separate runs. Bayesian inference was conducted using the Markov Chain Monte Carlo (MCMC) method with MrBayes v. 3.2.2 (Ronquist et al. 2011). 1 M generations were selected with sampling frequency every 1000 generations. Phylogenetic trees were visualized using Figtree software and edited using Microsoft PowerPoint 2016. Sequence data in this study are deposited in GenBank. Accession numbers for the species are provided in Table 1.

Table 1 Culture collection and GenBank accession numbers used in the multi-gene phylogenetic analysis. Sequences for the new isolate and the type species are shown in **red bold** and **black bold**, respectively.

Species	GenBank Accessions				
	Strain	TEF	LSU	SSU	RPB2
Akanthomyces aculeatus	**TS772**	**KC519366**	**KC519370**	**KC519368***	**NA**
Akanthomyces muscarius	**MFLU 181145**	**MH511807**	**MH497224**	**MH497222**	**MH511806**
Akanthomyces attenuatus	CBS 402.78	EF468782	AF339565	AF339614	EF468935
Akanthomyces attenuatus	KACC 42493	KM283804	KM283780	KM283756	KM283846
Akanthomyces dipterigenus	CBS 102072	KM283819	KM283796	KM283772	KM283861
Akanthomyces dipterigenus	CBS 126.27	KM283820	KM283797	KM283773	KM283862
Akanthomyces lecanii	CBS 102067	KM283818	KM283795	KM283771	KM283860
Akanthomyces lecanii	CBS 101247	DQ522359	KM283794	KM283770	KM283859
Akanthomyces muscarius	CBS 143.62	KM283821	KM283798	KM283774	KM283863
Akanthomyces sabanensis	JChA5	KC633266	KC875225	KC633251	KC633250
Akanthomyces tuberculatus	OSC 111002	DQ522338	DQ518767	DQ522553	DQ522435
Cordyceps bifusispora	EFCC 5690	EF468746	EF468806	EF468952	EF468909
Cordyceps bifusispora	EFCC 8260	EF468747	EF468807	EF468953	EF468910
Cordyceps cardinalis	OSC 93610	EF469059	AY184963	AY184974	EF469106
Cordyceps cf. *ochraceostromata*	ARSEF 5691	EF468759	EF468819	EF468964	EF468921
Cordyceps cf. *pruinosa*	NHJ 10684	EF468761	EF468823	EF468968	NA
Cordyceps cf. *takaomontana*	NHJ 12623	EF468778	EF468838	EF468984	EF468932
Cordyceps exasperate	MCA 2155	MF416486	MF416542	MF416596	NA
Cordyceps kyusyuensis	EFCC 5886	EF468754	EF468813	EF468960	EF468917
Cordyceps militaris	OSC 93623	DQ522332	AY184966	AY184977	NA
Cordyceps polyarthra	MCA 1009	MF416488	MF416544	MF416598	NA
Cordyceps pruinosa	ARSEF 5413	DQ522351	AY184968	AY184979	DQ522451
Cordyceps pseudomilitaris	NBRC 101409	NA	JN941393	JN941748	NA
Cordyceps roseostromata	ARSEF 4871	NA	AF339523	AF339573	NA
Cordyceps scarabaeicola	ARSEF 5689	DQ522335	AF339524	AF339574	HQ880954
Cordyceps staphylinidicola	ARSEF 5718	EF468776	EF468836	EF468981	NA
Lecanicillium acerosum	CBS 418.81	KM283810	KM283786	KM283762	NA
Lecanicillium antillanum	CBS 350.85	DQ522350	AF339536	AF339585	DQ522450
Lecanicillium aphanocladii	CBS 797.84	KM283811	KM283787	KM283763	NA
Lecanicillium aranearum	CBS 726.73a	EF468781	AF339537	AF339586	EF468934
Lecanicillium dimorphum	CBS 345.37	KM283812	KM283788	KM283764	KM283854
Lecanicillium flavidum	CBS 300.70D	KM283813	KM283789	KM283765	KM283855
Lecanicillium fungicola var. *fungicola*	CBS 992.69	KM283816	KM283792	KM283768	KM283857

Table 1 Continued.

Species	GenBank Accessions				
	Strain	**TEF**	**LSU**	**SSU**	**RPB2**
Lecanicillium fungicola var. *aleophilum*	CBS 357.80	KM283815	KM283791	KM283767	KM283856
Lecanicillium fusisporum	CBS 164.70	KM283817	KM283793	KM283769	KM283858
Lecanicillium psalliota	CBS 532.81	EF469067	AF339560	AF339609	EF469112
Lecanicillium psalliotae	CBS 363.86	EF468784	AF339559	AF339608	KM283854
Lecanicillium uredinophilum	KACC 44066	KM283808	KM283784	KM283760	KM283850
Lecanicillium uredinophilum	KACC 44082	KM283806	KM283782	KM283758	KM283848
Lecanicillium wallacei	CBS 101237	EF469073	AY184967	AY184978	EF469119
Simplicillium lanosoniveum	CBS 101267	DQ522357	AF339554	AF339603	DQ522463
Simplicillium lanosoniveum	CBS 704.86	DQ522358	AF339553	AF339602	DQ522464

NA: Not available, * = Not used in the analysis.

Results

Phylogenetic analyses

The combined gene dataset comprises 42 taxa, including 14 *Lecanicillium*, 11 *Akanthomyces* and 15 *Cordyceps* strains and two *Simplicillium lanosoniveum* (CBS 101267 and CBS 704.86) as outgroup taxon. The combined dataset comprised of 4083 characters. The RAxML analysis for the combined dataset provided a best scoring tree (Fig. 1) with a final ML optimization likelihood value of -20863.821421. The matrix had 1146 distinct alignment patterns, with 18.21% of undetermined characters or gaps. Parameters for the GTR + I + G model of the combined LSU, SSU, TEF and RPB2 were as follows: estimated base frequencies; A = 0.243474, C = 0.256785, G = 0.281390, T = 0.218351; substitution rates AC = 1.128485, AG = 3.646164, AT = 0.987760, CG = 0.949120, CT = 9.123589, GT = 1.000000; proportion of invariable sites I = 0.615674; gamma distribution shape parameter α = 0.549124. The Bayesian analysis resulted in 1000 trees after 1 M generations. The sample frequency was taken at every 1000 generations and the burnin frequency was set for 0.25. The outcome of the analyses resulted in two strongly supported monophyletic clades with *Cordyceps* (Clade A) and *Akanthomyces* (Clade B). Our isolate forms a well-supported subclade with *Akanthomyces muscarius* (CBS 143.62) and shows a close association with *Akanthomyces attenuatus* (CBS 402.78), (Clade B1, supported by 98% BS and 1.00 PP), Fig. 1.

In a separate phylogenetic analysis of the ITS dataset (45 taxa), consisting of 10 *Akanthomyes*, 3 *Cordyceps*, 31 *Lecanicillium* strains and one *Simplicillium lanosoniveum* (CBS 704.86) as an out group, Fig. 3 (supplementary table 1), *Akanthomyces muscarius* MFLUXXX, clusters with other strains of *Akanthomyces muscarius* (CBS 143.62, IMI 179173, ZG39), in the ML analysis, supported by 63% BS (Clade A1, Fig. 3) support. Phylogeny resulted in similar topologies as those derived from the concatenated dataset (Fig. 3).

Taxonomy

Akanthomyces muscarius (Petch 1932), Spatafora, Kepler & B. Shrestha (2017). Fig. 2

Index Fungorum number: 484535, Facesoffungi number: FoF04377

Synonyms, see (Zare & Gams 2001).

Endophyte on leaves of *Nypa fruticans* Wurmb. Asexual morph: *Colonies* reaching 2–2.5 cm diam. after 10 days at 26 °C on PDA, circular, compact, with reverse cream to pale yellow (rarely yellow). *Mycelium* composed of septate, branched, hyaline, smooth hyphae. *Conidiophores* macronematous, mononematous, erect, flexuous, septate, branched, smooth, 13.6–28.4 × 1.8–2.5

μm ($\bar{x}$ = 22.6 × 2.3 μm, n = 9). *Conidiogenous cells* 10–28 × 1.5–2.8 μm ($\bar{x}$ = 15 × 1.6 μm, n = 11), phialidic, with a minute collarette, developing at the tip of prostrate or on secondary branches of conidiophores, singly or in verticels, swollen at the base, tapered towards the tip, smooth. *Conidia* solitary, 2.4–7.2 × 1.7–2.6 μm ($\bar{x}$ = 4.5 × 1.8 μm, n = 25), variable in size, slimy, ellipsoidal, subcylindrical to cylindrical, base attenuate, apex rounded, 1-celled, smooth, hyaline to subhyaline. Sexual morph: Undetermined (Zare & Gams 2001).

Culture characteristics – Colony on PDA, white above, fluffy and regular, bottom yellowish, hyphae, septate, branched, and smooth, after 10 days.

Material examined – THAILAND, Krabi Province, isolated from leaves of *Nypa fruticans* (*Arecaceae*), 11 Sept. 2017, E. Vinit E104 (MFLU 18-1145, culture: MFLUCC 17-2540).

Notes – *Akanthomyces muscarius* is cosmopolitan with a wide range of hosts and has been synonymized several times (see Zare & Gams 2001). Phylogenetically this species clearly differs from all other species used in this analysis such as *A. lecanii, A. tuberculatus, L. antillanum, L. aphanocladii, L. aranearum,* and *A. attenuatus.*

Our isolate is very similar to *A. muscarius*, and hence we have added it as a new host record. The basic morphological comparison between similar taxa and their distribution are listed in Table 2. Conidiogenous cells and conidia were selected to compare between mentioned taxa (Zare & Gams 2001, 2008).

Discussion

Akanthomyces muscarius was isolated as an endophyte from leaf tissues of *Nypa fruticans*. Previously, *Astrosphaeriella nipicola*, *Fasciatispora petrakii*, *Neolinocarpon nypicola*, *Oxydothis nypicola* (from rachides) and *Rhipidocarpon javanicum* (from leaves) have been isolated from the aerial parts of *Nypa fruticans* (Cooke 1888, Hyde 1994, Hyde & Alias 1999, Hyde & Frohlich 1998, Patouillard 1897). It is interesting that an entomopathogenic species was isolated from *Nypa fruticans*. Previously, *A. lecanii* has been reported as a natural endophyte in bearberry (*Ericaceae*) (Askary et al. 1998, Benhamou & Brodeur 2001, Widler & Muller 1984) and ironwood (Bills & Polishook 1991). Several other genera containing known entomopathogenic fungi, including *Acremonium, Cladosporium, Clonostachys,* and *Paecilomyces*, have been isolated as naturally occurring endophytes from various tissues of the coffee plant in several countries (Clement et al. 1994, Breen 1994, Vega et al. 2008, West et al. 1988, Wicklow et al. 2005).

In the multigene phylogenetic analysis, *Akanthomyces muscarius* MFLU 18-1145 and a known isolate of *A. muscarius* (strain CBS 143.62) cluster together with a close affinity to *A. attenuatus* CBS 402.78 (Clade B1, Fig. 1). A similar phylogenetic scenario was obtained from single gene phylogenetic analyses where our isolate clustered together with other strains of *A. muscarius* (Clade A1, Fig. 3). In our multigene phylogenetic analysis, SSU (KC519368) DNA sequence of the type species (*A. aculeatus*) was not included as the alignment was rather ambiguous. We suspect that there is a need to verify the DNA sequence data deposited for this fungus.

Morphologically, *Akanthomyces* is indistinguishable from *Lecanicillium*, as conidiophores arise from aerial hyphae, bearing one or two whorls of phialides, in prostrate conidiophores numbers of phialides are multiple or solitary; conidia adhere in slimy heads or fascicles. However, conidia of *Lecanicillium* appear to be ellipsoidal to oblong-oval and oval, whereas in *Akanthomyces,* most of the conidia are ellipsoidal to sub-cylindrical (Chen et al. 2017, Chiriví-Salomón et al. 2015, Sung et al. 2001, Zare & Gams 2001, 2003).

Phylogenetic analysis of isolates and available sequences indicates that *Akanthomyces* has a broad geographic and host range, *Akanthomyces* occurs in Antarctica, Australia, Ecuador, Estonia, Finland, India, Indonesia, Italy, Jamaica, Japan, Mexico, New Zealand, Poland, Peru, Sri Lanka, Thailand, Turkey, UK, USA, and West Indies and is associated with plants such as *Arctium* sp., *Coffea* sp., *Dianthus* sp., *Heliotropium peruvianum, Sargassum* sp. and Arachnida and Lepidoptera species (Sukarno et al. 2009, Vincent et al. 1988).

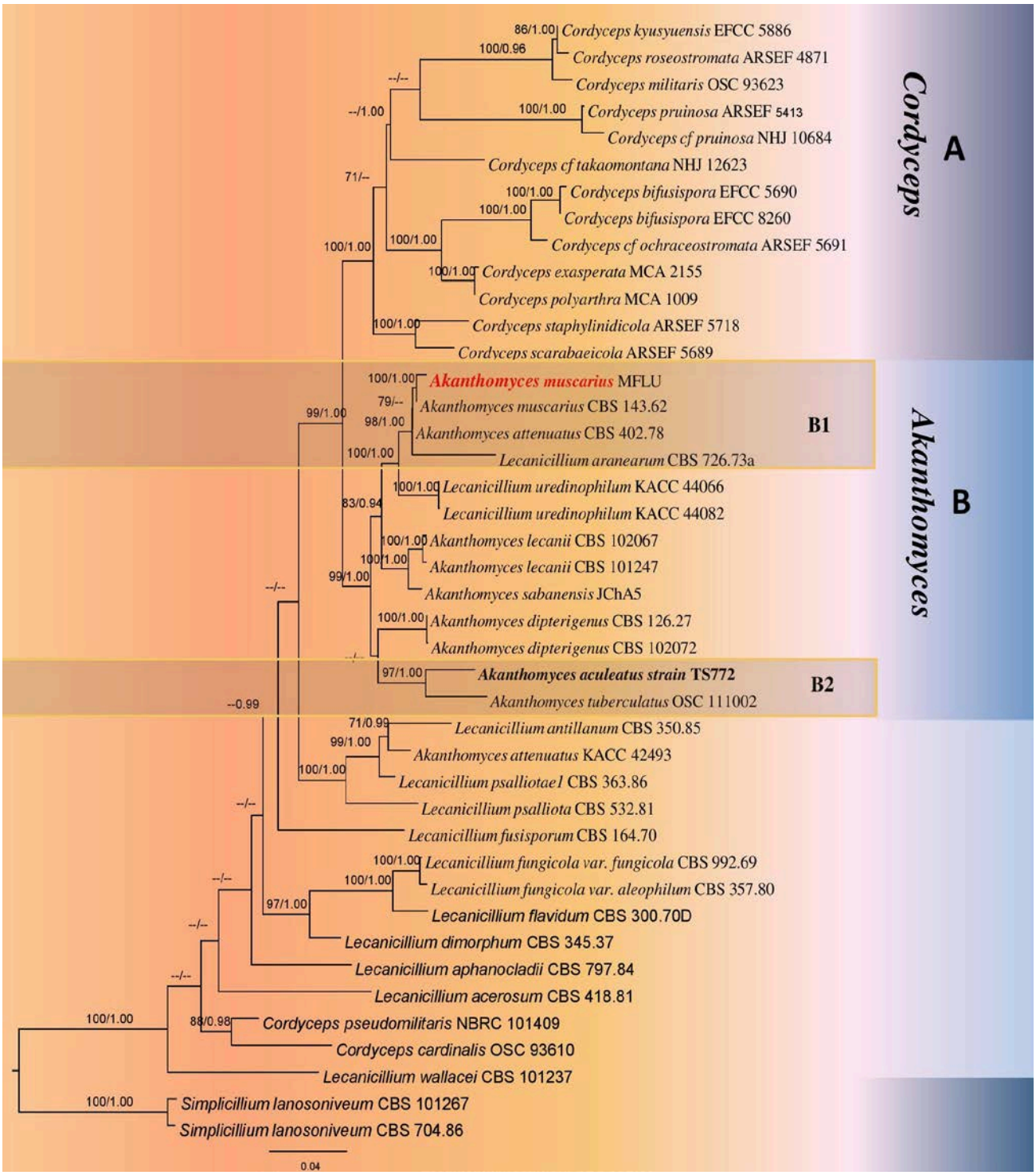

Fig. 1 – Maximum likelihood majority rule consensus tree for the isolates based on a dataset of combined LSU, SSU, TEF and RPB2 sequence data. Bootstrap support values for maximum likelihood (ML) greater than 70% and Bayesian posterior probabilities greater than 0.90 are indicated above the nodes as MLBS/PP. The scale bar represents the expected number of changes per site. The tree is rooted with *Simplicillium lanosoniveum* CBS101267 and CBS704.86. The strain numbers are noted after the species names. The new strain is in **red bold**.

Despite the wide geographic and host range of this species, there appears to be low sequence variation within nuclear gene markers examined as postulated by Jeewon & Hyde (2016). We speculate herein that this low level of genetic differentiation is because this species underwent rapid expansion with a wide range host.

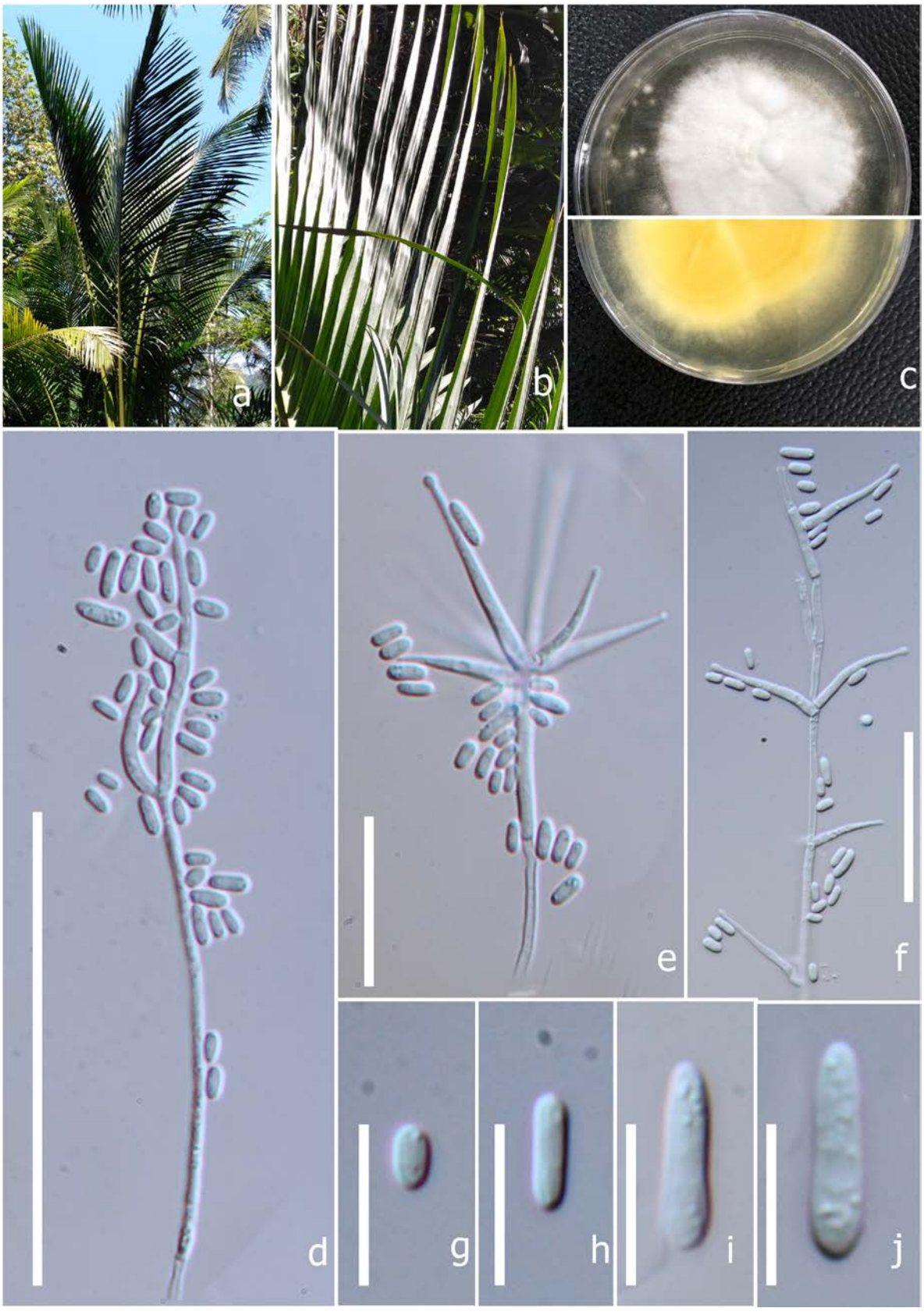

Fig. 2 – *Akanthomyces muscarius* (MFLU-CC 17-2540). a, b Fresh leaf of *Nypa fruticans*. c Culture plate on PDA (upper). d Conidiogenesis (1st and 2nd arrows indicate conidia and conidiogenous cell, respectively). e, f Conidiophores (arrowed), conidiogenous cells and conidia. g–j Conidia. Scale bars = d = 50 µm, e,f = 20 µm, g,h = 5 µm. Arrows = d = Conidia, f = Conidiogenous cell.

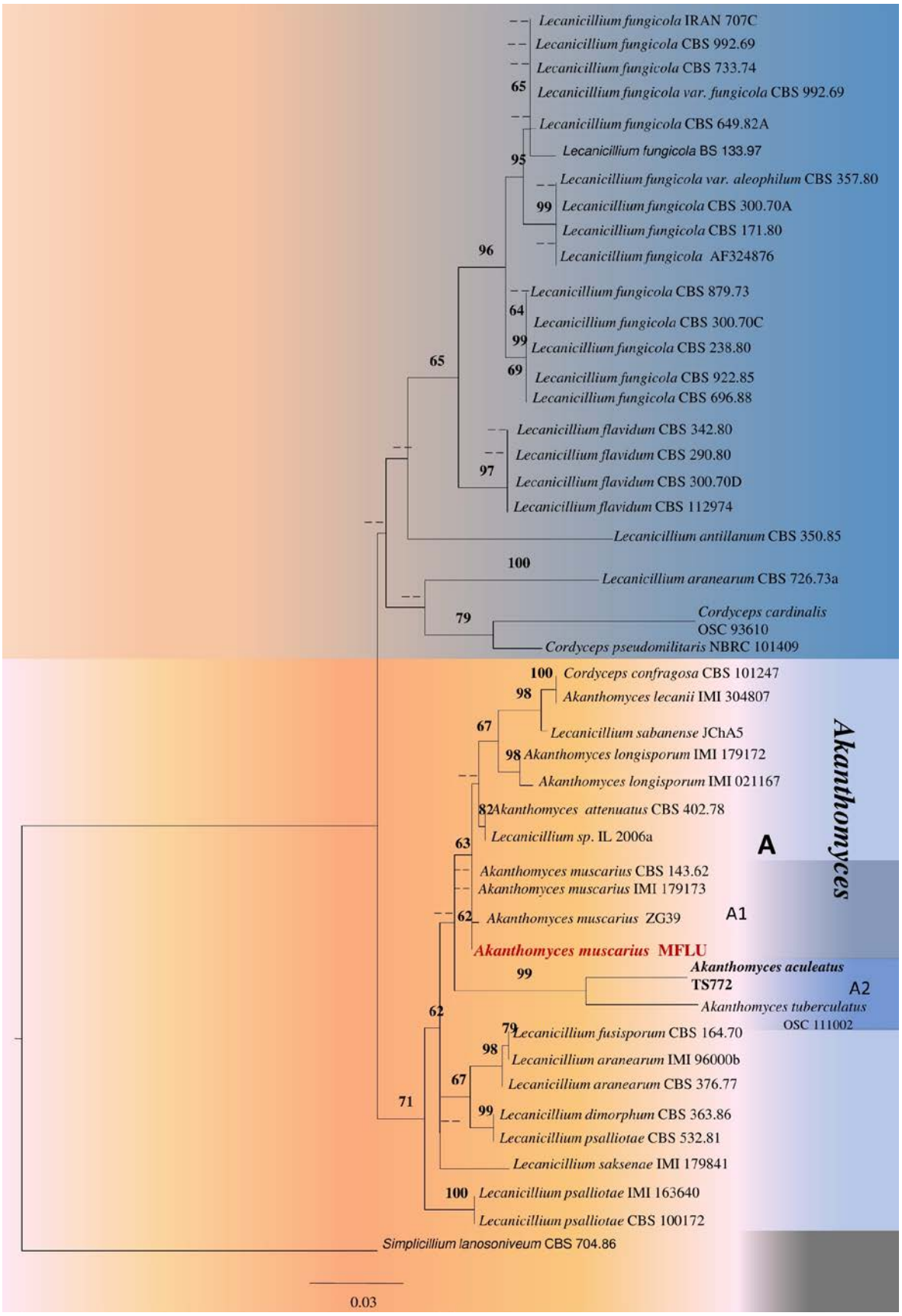

Fig. 3 – Maximum likelihood majority rule consensus tree for the isolates based on ITS sequence data. Bootstrap support values for maximum likelihood (ML) greater than 50% are indicated above the nodes as MLBS. The scale bar represents the expected number of changes per site. The tree is rooted with *Simplicillium lanosoniveum* CBS704.86. The strain numbers are noted after the species names. The new strain is in red bold.

Table 2 Morphological comparison of *Akanthomyces muscarius* and closely related species.

Species	Strain no.	Distribution	Conidia (µm)	Phialides (µm)
Akanthomyces attenuatus	CBS 402.78	New Zealand, USA, Poland, Japan, India, Estonia	4.5–6.5 × 1.5–2.0	9–5.5 × 1–2
Lecanicillium aranearum	CBS 726.73a	Ghana, India	5-8 × 0.7–1.5	20–30 × 1.2–1·5
Akanthomyces aculeatus	**TS772** (Type)	USA, UK, Estonia, Ecuador	1-3 × 0.5-1 µm	5–8 × 2–3
Akanthomyces lecanii	CBS 101247	West Indies, Jamaica, Peru, UK, Italy, Finland, Turkey, Mexico, New Zealand, Sri Lanka	2.5–4.2 (3.5) × 1–1.5	11–30 (20) × 1.4–1.8
Akanthomyces muscarius	CBS 143.62	UK, Italy, Antarctica	(2–)2.5–5.5 (–6) × 1–1.7	15–35 (20) × 1–1.7
Akanthomyces muscarius	**MFLU 181145**	Thailand	2.5–6.8 (4.6) × 1.7–2.6 (1·8)	10.5–28.0 (15.0) × 1.5–2.8

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