Sequence-based identification and characterisation of cultivated filamentous fungi in the Alan Bunga peat ecosystems of Sarawak, Malaysia

Z. Ayob, N.A. Kusai and S.R.A. Ali

Applied Entomology and Microbiology, Biology Research Division, Malaysian Palm Oil Board, Selangor, Malaysia

SUMMARY

The identification of cultivated fungal isolates using multiple primer set approaches reveals a lot of information on how soil fungi change as land use changes in tropical peat swamp forest ecosystems. This study aimed to identify the cultivated species of fungi from three different Alan Bunga peat swamp forest ecosystems: secondary forest, logged-over secondary forest, and 3.5 years after oil palm planting. A total of 45 fungal isolates were cultivated on two different types of media (potato dextrose agar [PDA] and malt extract agar [MEA]), and subsequently amplified using two different primer sets, namely ITS (ITS1/ITS4) and 18S rDNA (EF4/fung5). The morphological characteristics of fungal isolates were examined under a light microscope. Forty-two fungal isolates were successfully verified by PCR and DNA sequencing. The majority of the cultivated fungal species belonged to the phylum Ascomycota; one was from the Basidiomycota. *Trichoderma asperellum* (34 %) was the commonest fungal species detected in secondary forest using ITS primer whereas *Aspergillus fumigatus* (22 %) was the commonest using 18S rDNA primer. In conclusion, the ITS1/ITS4 primer set showed greater accuracy than the EF4/fung5 primer set in identification of the fungal taxonomic groups to species level.

KEY WORDS: ITS region, logged-over secondary forest, oil palm plantation, peat swamp forest, 18S rDNA.

INTRODUCTION

Peatlands cover an estimated area of 400 Mha, equivalent to 3 % of Earth's land surface, and tropical peatlands constitute over 8 % (33–49 Mha) of the global peat area (Veloo *et al.* 2015). Malaysian peatlands cover an area of about 2.6 Mha of which Sarawak has the largest share, amounting to over 1.6 Mha, equivalent to 70 % of tropical peatlands (Veloo *et al.* 2014, Melling 2016). The majority of peat swamp forests in Sarawak have been extensively converted for agricultural purposes, mainly into oil palm plantations, causing concern in terms of the sustainability of oil palm cultivation on peatlands (Melling *et al.* 2013). Indonesia and Malaysia are the world's largest suppliers of palm oil, accounting for 87 % of global production (Vaidyanathan 2011).

Fungi are ubiquitous in peat, as are bacteria and archaea. Fungi participate in vital ecological functions, for example, as decomposers, mutualists and pathogens (Liu *et al.* 2015, Ramlah Ali *et al.* 2016). They are involved in control of the organic carbon turnover which contributes to global carbon cycling (Treseder & Holden 2013, Tveit *et al.* 2013) and greenhouse gas emissions (Toma *et al*. 2011). Fungi are also considered to be most active in the oxic surface layers across all peatland types (Sun *et al.* 2014).

An older approach to the identification of peatland fungi is based on the morphological characteristics of isolates. But this can be challenging, especially when non-experts are dealing with the cultures of fungi, since a limited number of morphological characters are used for identification (Raja *et al*. 2017). Thus, identification of fungi from cultures requires high proficiency and expertise, which comes only with a lot of experience. A further drawback to this approach is that some slow-growing species may be overlooked, while the abundance of species with high sporulating rates may be over-estimated (Jeewon & Hyde 2007).

Molecular aids to identification of peatland fungi are more recent but have been little used in the Alan Bunga peat swamp ecosystem. The most common ribosomal target sequences for identification are the internal transcribed spacers (ITS) region and the 18S ribosomal DNA (18S rDNA) (Romanelli *et al.* 2010). The ITS region is most commonly used in fungal identification and is recognised as the standard DNA barcoding marker for species-level identification of fungi (Raja *et al*. 2017). Meanwhile, the 18S rDNA gene is a universal marker that is able to amplify a wide range of fungi with variations at the intraspecific level (Liu *et al*. 2015, Kusai *et al*. 2018). However, the amplification of the fungal ITS region and 18S rDNA may be biased towards specific fungal

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taxonomic groups (Singh *et al*. 2012b). A multiple primer approach, which targets the ITS region (fungal-specific) and 18S rDNA (universal) primer sets, reduces this bias.

Based on previous studies, the phylogeny and taxonomy of ITS sequences should be suitable for analysis of high-throughput sequencing datasets and should be more taxonomically informative in the GenBank database than 18S rDNA sequences in DNA-based studies (Anderson & Parkin 2007, Kittelmann *et al.* 2012, Liu *et al.* 2015). For instance, Nurulita *et al.* (2016b) have analysed the fungal diversity of tropical peat swamp forest ecosystems in Indonesia by ITS primer using a culture-independent approach (Denaturing Gradient Gel Electrophoresis; DGGE). In addition, comprehensive studies have been made using both primers, ITS region and 18S rDNA gene, to discover the fungal diversity in aquatic and soil environmental samples (Singh *et al.* 2012b, Liu *et al.* 2015, Xu *et al.* 2016, Lv *et al.* 2017).

In this article we describe the application of these methods to verify and compare the cultivated fungal species from three different Alan Bunga peat swamp forest ecosystems (secondary forest, logged-over secondary forest and oil palm 3.5 years after planting), using morphological characters and fungal-specific (ITS) and universal (18S rDNA) primer sets. This is the first study that compares cultivated fungal species using multiple primer sets, and should improve the accuracy of fungal identification at species level in the three tropical peat swamp forest ecosystems.

METHODS

Peat sampling

Peat samples were collected from ten sites in each of three Alan Bunga peat swamp forest ecosystems, namely secondary forest (SF), logged-over secondary forest (LF) and 3.5 YAP (years after planting) oil palm (OP) in the vicinity of Sibu, Sarawak, Malaysia (Figure 1). The sites were distributed across an area of approximately 192 km^2 centred on 2º 33' N, 111º 59' E, to the north-east of the state capital Kuching. The geographic coordinates of the 30 sites are given in Table A1 (Appendix). The one-hectare area of logged-over secondary forest is used for experimental purposes and kept free from vegetation by regular spraying with herbicide and manual

Figure 1. Example sampling sites in the three Alan Bunga peat swamp forest ecosystems: A) secondary forest (SF); B) logged-over secondary forest (LF); C) 3.5 YAP (years after planting) oil palm (OP); and D) map showing the location of the study near Sibu, Sarawak, Malaysia.

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weeding. Herbicide applications are commonly used in oil palm plantations during the immature phase to control weeds, in order to avoid suppression of growth and yield loss of oil palm (Mohamad *et al*. 2010). These herbicide applications cause short-term impacts on the fungal communities in the soil (Zain *et al*. 2013). Meanwhile, the development of oil palm cultivation was started in 2009 and $ZnSO_4$ (0.15 kg/palm) was applied. Rakib *et al*. (2017) have reported that the application of zinc sulphate is readily absorbed and utilised in growth by young oil palm plants, and there are positive effects on the soil fungal communities of plantations treated in this way (Li *et al*. 2013, Kerfahi *et al*. 2014, McGuire *et al*. 2015, Tripathi *et al*. 2016, Kusai *et al*. 2018).

The soil types survey at each site was undertaken by Paramananthan Agricultural Soil Surveys (M) Sdn Bhd. The peat types of secondary forest and oil palm plantation were classified as a mixture of Naman and Kenyana series which consisted of sapric peat with either no wood or undecomposed wood (Paramanathan 2016). Peat in the logged-over secondary forest was classified as Kenyana series; sapric with undecomposed wood (Paramanathan 2016). The study area receives an annual rainfall of 2460 mm, with most precipitation occurring between September and December.

Soil samples were collected from pre-assigned GPS points (Table A1) and chilled in an ice box during transport from the field to the laboratory, then stored at 4 °C until analysed. Total carbon (C) and nitrogen (N) were measured using a Primacs SNC (Skalar Analytical B.V., Lab Science Solution, Shah Alam, Selangor, Malaysia). In this machine, the soil samples were combusted at 1040 °C, total C was measured using an infrared detector and total N was determined by a thermal conductivity detector (Nurulita *et al.* 2016a). Meanwhile, the inorganic elements were analysed using a Perkin Elmer ELAN DRC-e ICP-Mass Spectrometer (Perkin Elmer Sdn Bhd, Petaling Jaya, Selangor, Malaysia). The soil samples were dried to constant weight in an oven at 60 °C. Approximately 0.3 g of dried sample was soaked in 95 % nitric acid, filtered and digested using a Titan MPS Microwave Sample Preparation System (Perkin Elmer Sdn Bhd, Petaling Jaya, Selangor, Malaysia). The digested soil extracts were serially diluted with deionised water prior to use for determination of inorganic elements.

Moisture proportion

Approximately 5 g of peat was weighed accurately, dried to constant weight in an oven at 105 °C for 48 hours, and weighed again. Percentage of soil moisture was expressed (Nurulita *et al.* 2016a, Krishnan *et al*. 2017) as

$$\text{Moisture \%} = 100 * (w1 - w2) / w2 \quad [1]$$

where w1 = weight of peat before oven drying and w2 = weight of soil after oven drying.

Peat pH

The peat was dried to constant weight in an oven incubator at 105 °C. Approximately 10 g of dried soil was suspended in 25 mL of deionised water and vortexed for 5 minutes at 120 rpm. Soil pH was measured using a pH meter with glass electrode (Nurulita *et al.* 2016a, Krishnan *et al.* 2017).

Isolation of fungi

Approximately one gram of peat was suspended in 9 mL of sterile deionised water and mixed thoroughly by shaking at 150 rpm for 2 hr. Subsequently, serial 10-fold dilutions were performed and 100 µL aliquots of 10^{-4} and 10^{-5} dilutions of each sample were plated in five replicates onto two culture media, namely potato dextrose agar (PDA) and malt extract agar (MEA) for isolation of fungi. All plates were incubated at 25 °C and fungal colonies were observed after 3 days to isolate the fast-growing and fast-sporulating fungi. Fungal colonies were identified on the basis of colony features and pigmentation. The colonies were subsequently sub-cultured on new PDA and MEA to obtain pure cultures. The pure cultures were sub-cultured for 3 days at 25 °C and used for molecular identification. All pure cultures were maintained in glycerol stock at -80 °C.

Morphological characteristics

The macroscopic and microscopic features of fungal isolates on PDA or MEA plates were acquired using the slide culture techniques documented in Fischer & Dott (2002). The prepared slides were incubated at 25 °C for 3 days before examination. Macroscopic characteristics were based on colony appearance and pigmentation; microscopic features such as conidia shape, size and conidiophore were observed using light microscopy (Kusai *et al.* 2015).

Fungal genomic DNA extraction

Fungal DNA was extracted from fresh fungal mycelium cultured on PDA and MEA plates after 3 days' incubation at 25 °C. The fungal mycelium was scraped using a sterile blade and the cells were broken with 500 µL lysis buffer in a 2.0 mL 'beads tube', for effective homogenisation in fungal DNA extraction. Fungal genomic DNA was extracted using

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a fungus-DNA isolation kit (Norgen Biotek Corp., Nanogene Solutions Sdn Bhd, Batu Caves, Selangor, Malaysia) according to the manufacturer's protocol. The DNA was stored at -20 °C prior to use as a template for DNA amplification.

Fungal DNA amplification

The ITS region and 18S rDNA gene were amplified using two primer sets, namely ITS1-F 5'-TCCGTAG GTGAACCTGCGG-3' / ITS4-R 5'-TCCTCCGCTT ATTGATATGC-3' (White *et al.* 1990) and EF4-F 5'-GGAAGGG[G/A]TGTATTTATTAG-3' / fung5-R5'-GTAAAAGTCCTGGTTCCCC-3' (Smit *et al.* 1999). The polymerase chain reaction (PCR) for fungal ITS and 18S rDNA amplification was performed in a 25 µL reaction mixture according to the standard procedures by Go Taq DNA polymerase, Promega (Interscience Sdn Bhd, Shah Alam, Selangor, Malaysia). The PCR mixtures comprised 1.25 units of PCR buffer, 2.5 mM $MgCl_2$, 0.3 % BSA, 0.5 mM of each deoxynucleotide triphosphate (dNTP), 0.4 µM of each primer, 1.25 units of Taq polymerase, 1.0 µl of each DNA template and sterile deionised water, which was used to make up the total volume of reaction mixture to 25 µl. The PCR amplification was performed with an Eppendorf Mastercycler® nexus (Medigene Sdn Bhd, Puchong, Selangor, Malaysia) using the following cycling protocol: initial denaturation at 94 °C for 3 minutes, followed by 30 cycles [denaturation at 94 °C for 1 minute, annealing at 54 °C (for ITS) or 48 °C (for 18S rDNA) for 1 minute], extension at 72 °C for 1 minute and final extension at 72 °C for 5 minutes. For the negative control, the PCR template was replaced with sterile deionised water. The PCR products were loaded and run on 1 % agarose gel electrophoresis in 1 × Tris-acetate-ethylenediaminetetraacetic acid (TAE) buffer. Gels were stained with 0.1% (v/v) SYBR DNA gel stain and visualised with an Alpha Imager HP system (Alpha Innotech, San Leandro, CA, USA).

Purification of DNA and sequence analysis

The positive gel-bands of the PCR products were excised and purified using a QIAquick® Gel Extraction Kit (Qiagen Biotechnology Malaysia Sdn Bhd, Bangsar, Kuala Lumpur, Malaysia) according to the manufacturer's instructions. All purified PCR products were sent to First Base Laboratories for sequencing. The sequences were analysed by using the basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/) to identify the closest match to fungal isolates in the National Centre for Biotechnology Information (NCBI) GenBank database.

Statistical analyses

The analyses of physico-chemical characteristics of the peat samples were conducted in triplicate and the data obtained were analysed using SPSS for Windows software (SPSS 16.0 for Windows Evaluation Version software, SPSS Inc., USA). The normality of data was assessed using the Shapiro-Wilk test. All data were analysed using the Kruskal-Wallis test for non-parametric data ($n = 3$). Differences were considered to be significant if the probability $P < 0.05$ (Pallant 2007).

RESULTS

Physico-chemical characteristics of peat

In Table 1, moisture proportion, peat pH, peat depth and total C differed significantly ($P < 0.05$), while total N and C/N quotient were not significantly different ($P > 0.05$) across the three peat ecosystems. The moisture proportion was highest in the secondary forest followed by the 3.5 YAP oil palm plantation and the logged-over secondary forest. Peat pH ranged from 3.5 to 3.8. The peat was a few centimetres deeper in the logged-over secondary forest than in the other two ecosystems. Total C was highest in the oil palm plantation followed by secondary forest and

Table 1. Physico-chemical characteristics of soil in the three Alan Bunga peat swamp forest ecosystems. Data are presented as mean ± standard error of mean (SEM). Asterisks indicate variables that differed significantly between ecosystems (non-parametric Kruskal-Wallis ANOVA; $n = 3$, $P < 0.05$). YAP = years after planting.

Ecosystem	Moisture proportion (%)*	pH*	Peat depth (cm)*	Total C (%)*	Total N (%)	C/N quotient (%)
Secondary forest (SF)	77 ± 4	3.6 ± 0.01	108 ± 5	50 ± 3	1.4 ± 0.1	21
Logged-over secondary forest (LF)	65 ± 0.7	3.8 ± 0.03	128 ± 4	45 ± 2	1.3 ± 0.1	20
3.5 YAP oil palm (OP)	69 ± 1	3.5 ± 0.04	113 ± 4	51 ± 2	1.3 ± 0.1	22

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logged-over secondary forest, but the C/N quotients did not differ. The concentrations in soil (Table 2) of elements such as boron (B), chromium (Cr), and zinc (Zn) differed significantly ($P < 0.05$) between ecosystems, whereas the levels of other elements did not vary. The highest concentrations of Zinc (Zn), nickel (Ni), chromium (Cr), calcium (Ca), molybdenum (Mo) and magnesium (Mg) were found in the oil palm plantation. Potassium (K) and Iron (Fe) accumulated most in the secondary forest but their concentrations did not differ significantly between the three ecosystems, whilst phosphorus (P) and manganese (Mn) concentrations were highest in the logged-over forest. The concentrations of other trace elements, namely lead (Pb), boron (B) and cadmium (Cd), were also highest in the oil palm plantation.

Morphological fungal characteristics

On the basis of macroscopic and microscopic characteristics, fungal isolates were identified as *Aspergillus fumigatus* (Figures 2a–c), *A. flavus* (Figures 2d–f), *A. niger* (Figures 2g–i), *A. tubingensis* (Figures 2j–l), *Aspergillus* sp. (Figures 2m–o), *Trichoderma asperellum* (Figures 2p–r), *Hypocrea muroiana* (Figures 2s–u), *Penicillium* sp. (Figures 2v–x) and *Phanerochaete chrysosporium* (Figures 2y–z).

Aspergillus fumigatus on PDA medium was velvety dark green in the centre with white aerial mycelia at the border (Figure 2a). *A. fumigatus* produced uniseriate phialides on the vesicle, and greenish columnar conidial heads composed of catenulate conidia. The conidia were phialosporous and globose (Figures 2b and c).

Aspergillus flavus on PDA medium was yellow-green in the centre and had white aerial mycelia towards the edge. The texture of the colony was flattened and granular (Figure 2d). *A. flavus* produced rough-walled conidiophore stipes, uniseriate phialides on the vesicle, and greenish conidial heads composed of catenulate conidia. Conidia were phialosporous and globose (Figures 2e and f).

Aspergillus niger on PDA medium was dark brown, with aerial mycelia towards the edge of the Petri dish (Figure 2g). *A. niger* produced biseriate conidiophores with septate metulae and phialides, and radiate conidial heads composed of catenulate conidia on dark brown globose vesicles. Conidia were phialosporous, globose, brown, with rough-walled surfaces, and found in clusters (Figures 2h and i).

Table 2. Element concentrations (mg/kg) in the three Alan Bunga peat swamp forest ecosystems. The values are means ± standard error of mean (SEM). '*0*' digits in italics are not significant but indicate the position of the decimal point. Asterisks indicate variables that were shown to differ significantly ($n = 3$; $P < 0.05$) between the three peat ecosystems using the non-parametric Kruskal-Wallis ANOVA.

Element	Site		
	(SF)	(LF)	(OP)
Magnesium (Mg)	6*0* ± 5	7*0* ± 4	7*0* ± 1*0*
Iron (Fe)	5*0* ± 6	3*0* ± 6	4*0* ± 1*0*
Boron (B)*	0.14 ± 0.04	0.02 ± 0.01	2 ± 0.4
Calcium (Ca)	1*00* ± 2*0*	1*00* ± 2*0*	2*00* ± 7*0*
Phosphorus (P)	2*00* ± 4*0*	2*00* ± 4*0*	1*00* ± 3*0*
Chromium (Cr)*	4*00* ± 6*0*	2*00* ± 3*0*	7*00* ± 1*00*
Manganese (Mn)	6*0* ± 1*0*	1*00* ± 2*0*	7*0* ± 3*0*
Nickel (Ni)	2*00* ± 3*0*	9*0* ± 1*0*	1*000* ± 7*00*
Copper (Cu)	2*0* ± 3	2*0* ± 4	5*0* ± 3*0*
Zinc (Zn)*	8*00* ± 4*00*	6*0* ± 2*0*	5*000* ± 4*000*
Molybdenum (Mo)	2*0* ± 4	9 ± 1	1*00* ± 8*0*
Cadmium (Cd)	0.8 ± 0.1	0.5 ± 0.1	0.9 ± 0.4
Lead (Pb)	4 ± 1	4 ± 1	8 ± 5
Potassium (K)	2*00* ± 5*0*	2*00* ± 3*0*	1*00* ± 3*0*

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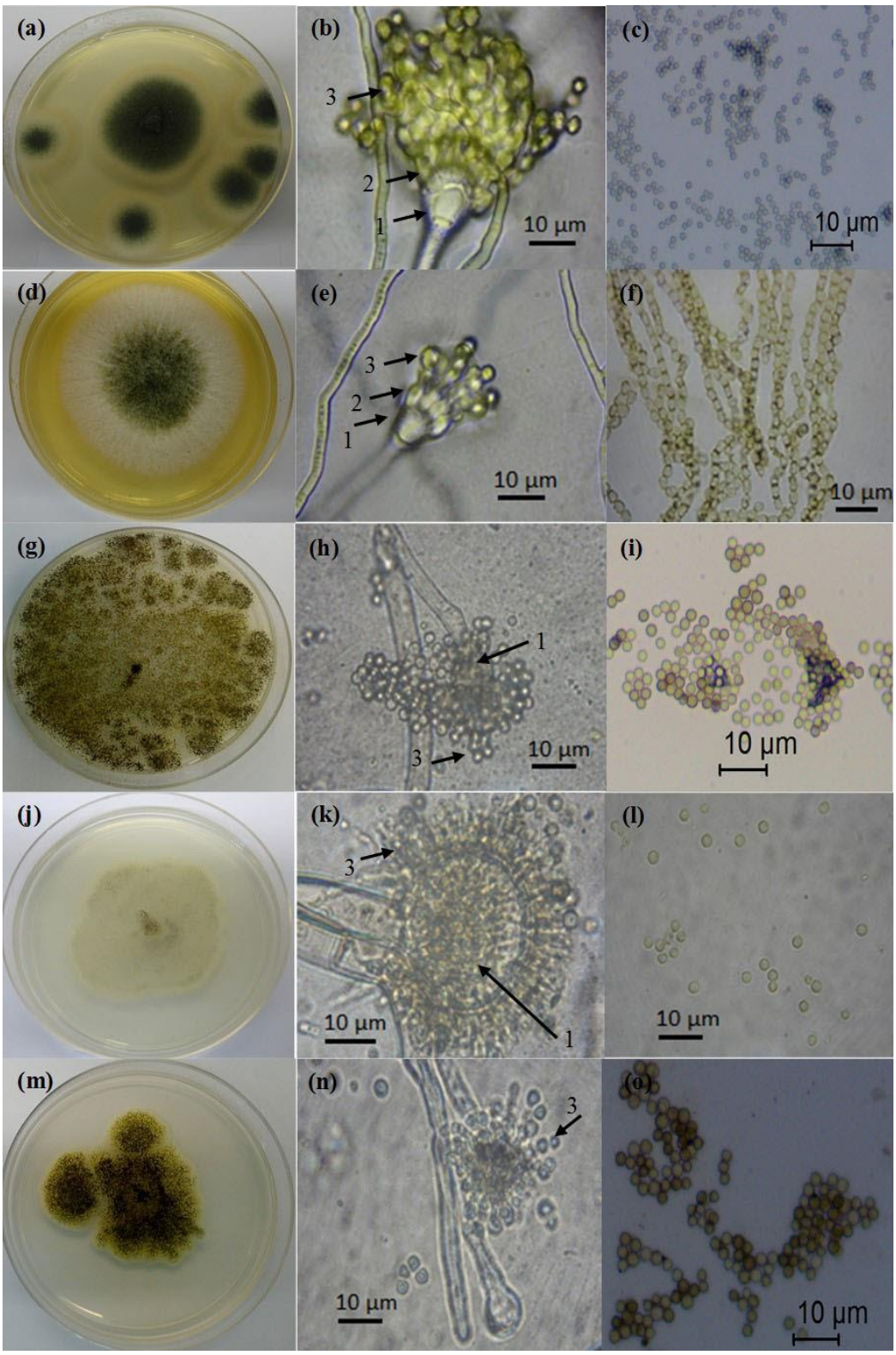

Figure 2 (Part 1). Macroscopic and microscopic characteristics of isolated fungi from Alan Bunga peat swamp forest ecosystems. *Aspergillus fumigatus*:- a) Colony appearance on potato dextrose agar (PDA); b) Conidia formed at the tip of conidiophore, vesicle, phialides; c) Conidia; *Aspergillus flavus*:- d) Colony appearance on PDA; e) Conidiophore, vesicle, phialides; f) Conidia; *Aspergillus niger*:- g) Colony appearance on PDA; h) Conidia formed at the tip of conidiophore, spore mass, vesicle, phialides; i) Conidia; *Aspergillus tubingensis*:- j) Colony appearance on PDA; k) Conidiophore, vesicle, phialides; l) Conidia; *Aspergillus* sp.:- m) Colony appearance on PDA; n) Conidia formed at the tip of conidiophore, spore mass, vesicle, phialides; o) Conidia. Arrows indicate: 1) vesicle; 2) phialides; 3) conidia chain.

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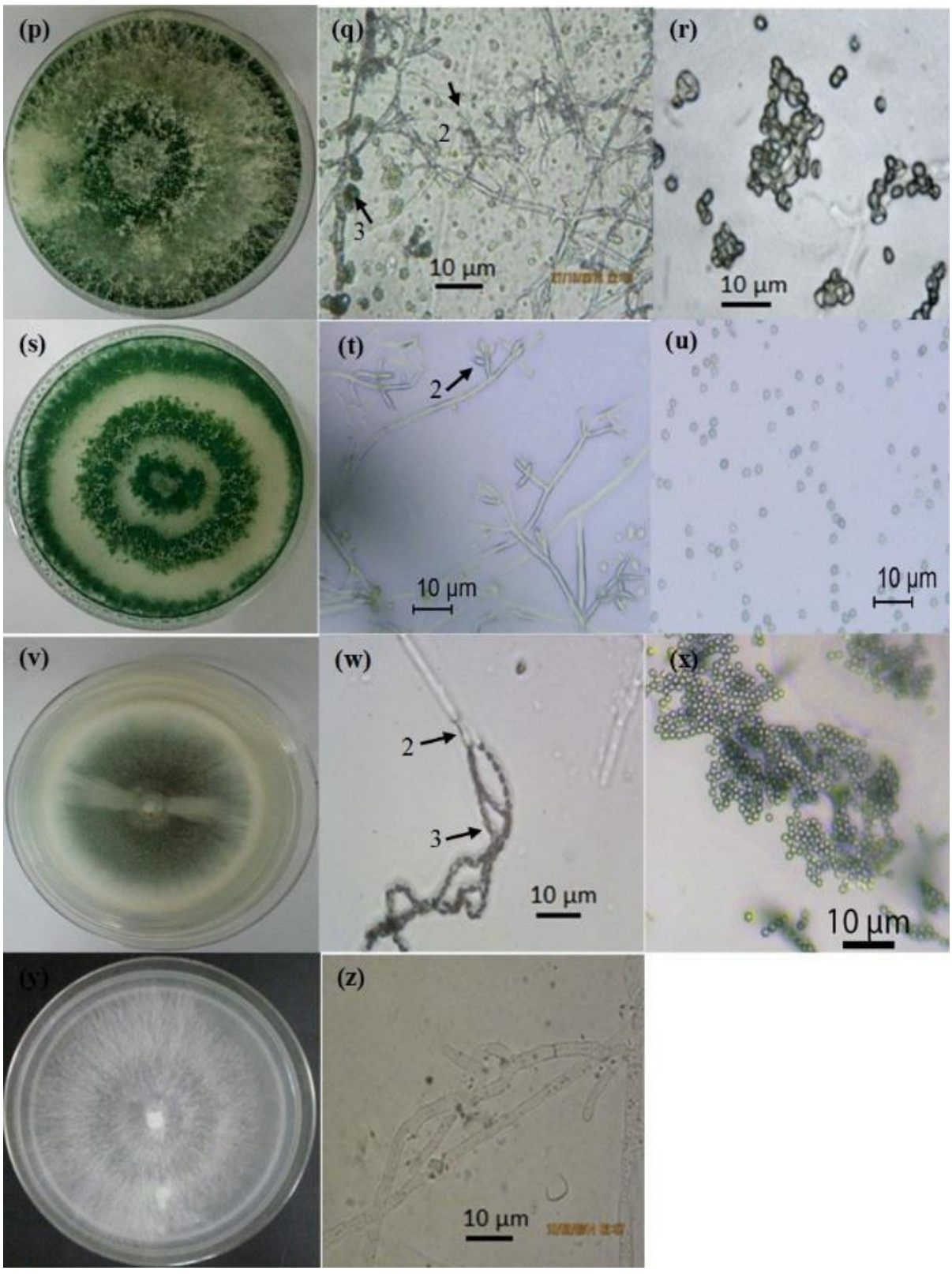

Figure 2 (Part 2). Macroscopic and microscopic characteristics of isolated fungi from Alan Bunga peat swamp forest ecosystems. *Trichoderma asperellum*:- p) Colony appearance on malt extract agar (MEA); q) Conidia formed at the tip of conidiophore, spore mass, phialides; r) Conidia; *Hypocrea muroiana*:- s) Colony appearance on MEA; t) Conidiophore, phialides; u) Conidia; *Penicillium* sp.:- v) Colony appearance on MEA; w) Conidia formed at the tip of conidiophore, spore mass, metula, phialides; x) Conidia; *Phanerochaete chrysosporium*:- y) Colony appearance on MEA; z) septate hyphae. Arrows indicate: 2) phialides; 3) conidia chain.

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Aspergillus tubingensis on PDA media was white to cream in the centre with white aerial mycelia at the border (Figure 2j). *A. tubingensis* produced biseriate conidiophores with septate metulae and phialides. Conidial heads were dark brown, radiate, composed of catenulate conidia and globose vesicles. The conidia were globose and phialosporous with rough-walled surface (Figures 2k and l).

Aspergillus sp. on PDA medium was velvety dark brown with shades of green at the edge of the colony (Figure 2m). *Aspergillus* sp. produced uniseriate phialides on the vesicle, and had pale brown radiate conidial heads composed of catenulate conidia. Conidia were phialosporous, pale brown and globose (Figures 2n and o).

Trichoderma asperellum on MEA medium formed a dark green colony with compact turfs of white mycelium in concentric ring-like zones (Figure 2p). *T. asperellum* produced branched conidiophores and spore masses at irregularly verticillate phialides. Conidia were formed in conidial heads clustered at the tips of the phialides, phialosporous and bearing ellipsoidal or ovale clusters (Figures 2q and r).

Hypocrea muroiana on MEA medium formed a flat greenish colony with white mycelium in concentric ring-like zones (Figure 2s). *H. muroiana* produced branched conidiophores and spore masses at irregularly verticillate phialides. The conidia were phialosporous with a globose shape and clustered at the ends of conidiophores (Figures 2t and u).

Penicillium sp. on MEA medium formed a flattened, greyish-green coloured colony with cottony white at the edge of the colony (Figure 2v). *Penicillium* sp. produced conidia formed in globose shaped clusters, brush-like in appearance at the tips of the phialides, and catenulate conidia on each phialide (Figures 2w and x).

Phanerochaete chrysosporium on MEA medium formed a colony of sparse white aerial mycelium (Figure 2y). The fungus pattern was created by septate hyphae with branching (Figure 2z).

Comparison of fungal isolates identified by ITS and 18S rDNA primer sets

The results of the BLAST analysis of ITS region and 18S rDNA gene sequences are given in Table A2. For each of the three ecosystems, the fungal isolates from this study are listed along with the closest match isolates (*i.e*. closest genetic similarity) amongst the sequences deposited (max identity) in GenBank, with NCBI accession numbers. Out of 42 fungal isolates successfully identified using ITS primer, 29 isolates originated from secondary forest, six from logged-over secondary forest, and seven from oil palm plantation. Using 18S rDNA primer, a total of 29, seven and six isolates were identified in the corresponding ecosystems. However, three (ND in Table A2) out of 42 isolates could not be identified with ITS and 18S rDNA primers, perhaps due to errors during PCR amplification and sequencing (Bachy *et al.* 2013). Across the three peat ecosystems, Ascomycota were the commonest phyla detected using ITS and 18Sr DNA primers, followed by Basidiomycota. Isolates for the three peat ecosystems identified using ITS primer fell into three orders, namely Eurotiales (64 %), Hypocreales (33 %) and Polyporales (3 %); whereas five orders, namely Eurotiales (64 %), Hypocreales (22 %), Polyporales (3 %), Mytilinidiales (3 %) and Saccharomycetales (3 %) were detected using 18S rDNA primer. At the genus level, six genera were observed in total and, of these, five belonged to the Ascomycota and one to the Basidiomycota, accounting for 98 % and 2 % of the diversity of the isolated fungi, respectively. Similarly, *Aspergillus* was abundant and accounted for 62 % and 65 % of cultivable fungi identified using ITS and 18S primers, respectively.

At genus level for the fungal ITS region, four genera were identified across the three peat ecosystems: *Aspergillus* (62 %), *Trichoderma* (32 %), *Penicillium* (3 %) and *Phanerochaete* (3 %), while a total of six genera were identified using 18S rDNA primer: *Aspergillus* (65 %), *Trichoderma* (27 %), *Meyerozyma* (2 %), *Mytilinidion* (2 %), *Phanerochaete* (2 %) and *Penicillium* (2 %). As shown in Tables 3 and A2, 26 isolates identified using ITS primer were in the dominant genus (*Aspergillus*, 62 %) and comprised five isolates: *Aspergillus fumigatus* (13, 32 %), *Aspergillus flavus* (10, 24 %), *Aspergillus niger* (1, 2 %), *Aspergillus oryzae* (1, 2 %) and *Aspergillus tubingensis* (1, 2 %), followed by *Trichoderma asperellum* (14, 34 %), *Penicillium dalea* (1, 2 %) and *Phanerochaete chrysosporium* (1, 2 %).

In contrast to the 18S rDNA primer results, on the basis of the fungal ITS region, a total of 27 isolates from the three peat ecosystems were categorised into the commonest genus *Aspergillus* (66 %) but only two isolates were identified, namely *Aspergillus fumigatus* (17, 42 %) and *Aspergillus* sp. (10, 24 %). Six isolates were in the genus *Trichoderma* (26 %) with four isolates identified as *Trichocomaceae* sp. (1, 2 %), *Trichoderma* sp. (1, 2 %), *Hypocrea atroviridis* (7, 17 %) and *Hypocrea muroiana* (2, 5 %). In addition, four genera with one isolate each were identified as *Phanerochaete sanguinea* (1, 2 %), *Mytilinidion tortile* (1, 2 %), *Meyerozyma guilliermondii* (1, 2 %) and *Penicillium* sp. (1, 2 %) (Tables 3 and A2).

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The fungal ITS sequences showed closest matches with NCBI accessions across all three peat ecosystems, the total of 29 isolates from secondary forest comprising 14 different isolates of *Trichoderma* accounting for 34 %, 13 different isolates of *Aspergillus* accounting for 32 % and single isolates of *Penicillium* and *Phanerochaete* accounting for 2 % each. The commonest genus detected in the logged-over secondary forest and oil palm plantation was *Aspergillus*, which comprised six and seven different isolates accounting for 14 % and 16 %, respectively (Table A2). The 18S rDNA sequences showed the closest match to the GenBank database for 29 fungal isolates from secondary forest which comprised six genera with 14 different isolates of *Aspergillus* accounting for 34 %, 11 different isolates of *Trichoderma* accounting for 28 % and single fungal isolates in the genera *Phanerochaete*, *Mytilinidion*, *Meyerozyma* and *Penicillium* each accounting for 2 %. The indication of commonest genus for logged-over secondary forest and oil palm plantation was consistent with the ITS primer results. Seven identified isolates were collected from logged-over secondary forest accounting for 16 %, while the six different isolates found in oil palm plantation accounted for 14 %. *Aspergillus* was the commonest fungal isolate across the three peat ecosystems.

Table 3. Taxonomic affiliations of the sequenced fungal species.

Phylum	Class Order	Family *Genus*	Species (% ITS, % 18S rDNA)
Ascomycota	Eurotiomycetes Eurotiales	Aspergillaceae *Aspergillus*	*Aspergillus flavus* (24,0)
			Aspergillus fumigatus (32,42)
			Aspergillus niger (2,0)
			Aspergillus oryzae (2,0)
			Aspergillus sp. (0,24)
			Aspergillus tubingensis (2,0)
		Aspergillaceae *Penicillium*	*Penicillium dalea* (2,0)
			Penicillium sp. (0,2)
	Sordariomycetes Hypocreales	Hypocreaceae *Trichoderma*	*Hypocrea atroviridis* (0,17)
			Hypocrea muroiana (0,5)
			Trichocomaceae sp. (0,2)
			Trichoderma asperellum (34,0)
			Trichoderma sp. (0,2)
	Dothideomycetes Mytilinidiales	Mytilinidiaceae *Mytilinidion*	*Mytilinidion tortile* (0,2)
	Saccharomycetes Saccharomycetales	Debaryomycetaceae *Meyerozyma*	*Meyerozyma guilliermondii* (0,2)
Basidiomycota	Agaricomycetes Polyporales	Phanerochaetaceae *Phanerochaete*	*Phanerochaete chrysosporium* (2,0)
			Phanerochaete sanguinea (0,2)

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DISCUSSION

Physico-chemical characteristics of peat

The soil properties and conditions of the three peat ecosystems differed significantly (Table 1). The Alan Bunga peat forest had the highest soil moisture proportion and exhibited swampy conditions, reflecting high water table (at the peat surface) and abundance of spongy peat (Tripathi *et al*. 2016). As a result, soil pH differed somewhat in all peat ecosystems and may have depended partly on the differences in organic substances in the soil (Anshari *et al.* 2010). In our case, soil pH was probably lower (more acidic) in oil palm plantation on peat than in secondary forest and logged-over secondary forest because it was subjected to fertiliser application, and especially due to the addition of zinc sulphate and subsequent release of sulphate when Zn was taken up by the oil palm plants (Metwally *et al*. 1993, Colombo *et al*. 2016). In addition, total C and C/N quotient were consistently highest in the oil palm plantation, followed by secondary forest and logged-over secondary forest. High total C and low total N indicated that decomposition and mineralisation rates were low (Nurulita *et al.* 2016a).

Morphological characteristics of fungi

The morphological characteristics of each culture are based on the colour and appearance of colonies, and conidia shape and formation. The number of isolated fungal species was significantly related to the isolation method and culture medium (Zhou *et al.* 2014). The present study did not recover any yeast species. This was in agreement with Amha *et al.* (2015), who reported that yeast was less abundant and accounted for only 10 % of fungal isolates in peatlands. The anamorphic ascomycetes were distributed across all three peat ecosystems and were the most-identified fungi on the basis of molecular identifications supported by morphological characterisations. These findings agree with previous studies from aquatic and soil environmental samples (Thormann 2006, Singh *et al.* 2012a, b; Kim *et al.* 2014, Zhou *et al.* 2014, Liu *et al.* 2015, Xu *et al.* 2016, Lv *et al.* 2017). Fungal isolates from peatlands, such as *Trichoderma*, *Aspergillus*, *Penicillium*, *Verticillium*, *Mortierella* and *Cladosporium* genera, were prolific sporulators with fast growth rates on standard culture media (Thormann 2006).

It is interesting that the fungi isolated can tolerate extreme conditions such as high temperature, acidity, and salinity, and have been classified by their different characteristics, *i.e.* saprophytic or parasitic, depending on their ecological roles (Thormann 2006). For instance, *Aspergillus* spp. were reported to play an important role in the denitrification process under anoxic conditions (Singh *et al.* 2012a). Furthermore, Zhou *et al.* (2014) reported that fungal isolates such as *Aspergillus* spp., *Penicillium* spp., *Acremonium* spp. and *Trichoderma* spp. play important roles as fungal biological agents controlling or preventing soil-borne fungal diseases. Recently, a study was conducted on *Trichoderma asperellum* as an efficient antagonistic plant pathogen against *Fusarium oxysporum* (Patel & Saraf 2017). In addition, one of the important fungi identified in this study was an uncommon basidiomycete. This fungus seemed to have a slow growth rate and required special culture media and growing conditions (Thormann 2006). Basidiomycete fungi play an important role as degraders of recalcitrant polymers. They are capable of utilising complex polymers including the lignin, tannin and other polyphenols in peat soils (Grum-Grzhimaylo *et al.* 2016). Thus, it has been suggested that individual fungal species may have specific ecological roles in different environments (Classen *et al.* 2015). In the present work, there were no phylotypes of Zygomycota and Chytridiomycota, which are the most frequently reported fungi in boreal peatlands (Thormann *et al*. 2001, Thormann 2006, Thormann & Rice 2007, Lin *et al.* 2012, Grum-Grzhimaylo *et al*. 2016). However, our results are consistent with those of Kerfahi *et al.* (2014), who used a culture-independent approach (454-pyrosequencing) and reported the absence of other fungal groups in the forest and agricultural soils of Sabah (Malaysia); and of Kusai *et al*. (2018), who found low abundance (10 %) of fungal isolates (of *Mortierella chlamydospora* and *Umbelopsis isabellina* from phylum Zygomycota) in Maludam Natural Park, where both Cermat Ceria logged-over forest and Durafarm oil palm plantation accounted for 1 %. Yet, the evidence suggesting that these fungal groups were not abundant (Singh *et al*. 2012a, b) in our study sites is limited. Longer isolation periods and specialised culture media may be necessary to reveal their presence, as they may be slow-growing and thus covered by fast-growing fungi (Ortega-Morales *et al*. 2016).

Comparison of fungal isolates identified by ITS and 18S rDNA gene primer sets

The isolated fungal species could be distinguished through ITS and 18S rDNA sequencing. The most abundant phylum identified was Ascomycota and less frequent was Basidiomycota; these phyla were distributed across all three peat ecosystems. The results obtained in the present study were similar to those in previous reports where ascomycetes were found to be dominant in various peatland ecosystems

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(Anderson *et al.* 2003, Thormann 2006, Winsborough & Basiliko 2010, Andersen *et al.* 2013, Man *et al.* 2015, Grum-Grzhimaylo *et al.* 2016, Ramlah Ali *et al.* 2016). It was also consistent with Xu *et al.* (2016) who studied the phylotypes of fungal communities in a deep-sea sediment of the Pacific Ocean and concluded that they were dominated by Ascomycota and Basidiomycota.

On the other hand, *Hypocrea atroviridis*, the teleomorph of *Trichoderma atroviride*, was recorded by 18S rDNA sequencing in the secondary forest. The isolated fungal samples of 'SF 2C-4 PDA F1' and 'SF 3A-5 PDA F1' from the secondary forest were identified as *T. asperellum* from ITS sequencing but categorised as *H. atroviridis* by 18S rDNA sequencing. These observations appeared when the anamorph and teleomorph nomenclatures of the deposited sequences in the GenBank database were improperly or inconsistently named (Romanelli *et al.* 2010). The inconsistency revealed that the ITS sequence gave an accurate identification of fungal species; it is also supported by the morphological features. A previous study recorded a few fungal species from the same area (Ramlah Ali *et al.* 2016). This contradiction might be due to primer bias towards specific taxonomic groups and the limited number of primers used (Singh *et al.* 2012a). We used multiple primer sets (ITS and 18S rDNA) to enable the recovery of more fungal species and minimise erroneous naming by submitting additional information about our fungal species to the GenBank database. A similar approach was adopted by Anderson *et al.* (2003), who recorded a high diversity of fungi from grassland soil samples by using four ITS and 18S rDNA primer sets. Majid *et al.* (2015) used a combination of phenotypic (morphological and colony appearance) and molecular analysis by DNA sequencing to accurately identify their fungal isolates.

The results obtained from 18S rDNA sequencing included one isolated fungus ('SF 1C-4 MEA F1') from the secondary forest that was identified only to family level (*Trichocomaceae* sp.). This might be due to a high genetic homology of sequences within the family (Lv *et al.* 2017). Nevertheless, *Trichoderma asperellum* was successfully identified using ITS sequencing, with high similarity (99 %) to GenBank data, showing that the ITS region can be used to identify isolated fungi to the species level (Romanelli *et al.* 2010). All of the fungal species that we isolated could be identified to species level using ITS primer, across all three peat ecosystems.

The identification of the fungal isolate 'SF 7C-4 MEA F1' by ITS and 18S rDNA sequences was contradictory. The ITS sequence identified the fungal isolate as *Trichoderma asperellum* with 98 % similarity and 515/535 identities in the GenBank database, while 18S rDNA sequence analysis identified it as the ascomycetous yeast, *Meyerozyma guilliermondii*, with lower similarity (83 %) and identities (439/445). Likewise, 18S rDNA sequence analysis indicated low similarity (80 %) for the 'SF 5C-5 MEA F1' isolate, which was predicted to be *Mytilinidion tortile* with 336/418 identities; whereas the result based on ITS sequence analysis was *Aspergillus flavus* with 99 % similarity and 556/557 identities. This brings to mind the suggestion of Bachy *et al.* (2013), that PCR and sequencing errors might be inflating the estimation of microbial diversity by creating false taxa. In mycology, the ITS region has been the most commonly sequenced region for taxonomic identification at species level (Xu *et al.* 2016), and the 18S rDNA sequences do not always provide sufficient taxonomic resolution for the identification of fungi to genus or species level (Liu *et al.* 2015). Therefore, we propose that the primer set for the ITS region is the most suitable primer set for identification of the fungal community in Sarawak peat swamp ecosystems.

Multiple primer sets would give different types of biases for fungal taxonomy such as primer specificity, mismatches and sequence length, and the findings suggest that the use of multiple primers will generate a more accurate diagnosis of fungal diversity in peat ecosystems. However, the ITS primer was an efficient target region for identifying the closely related fungal isolates in peat swamp ecosystems. Taken together, these observations suggest that further investigation using a next-generation sequencing (NGS) approach would greatly contribute to our knowledge of the functional diversity, and to developing our understanding, of fungal communities adapted to the conversion of tropical peat swamp forest to oil palm plantation.

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REFERENCES

Amha, Y., Bohne, H. & Alsanius, B. (2015) Patterns of fungal and bacterial carbon mineralization across Northern European peatlands.

 DOI: 10.19189/MaP.2018.OMB.331

Geomicrobiology Journal, 32, 914–923.

Andersen, R., Chapman, S.J. & Artz, R.R.E. (2013) Microbial communities in natural and disturbed peatlands: A review. *Soil Biology and Biochemistry*, 57, 979–994.

Anderson, I.C. & Parkin, P.I. (2007) Detection of active soil fungi by RT-PCR amplification of precursor rRNA molecules. *Journal of Microbiological Methods*, 68, 248–253.

Anderson, I.C., Campbell, C.D. & Prosser, J.I. (2003) Potential bias of fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. *Environmental Microbiology*, 5, 36–47.

Anshari, G.Z., Afifudin, M., Nuriman, M., Gusmayanti, E., Arianie, L., Susana, R. & Nusantara, R.W. (2010) Drainage and land use impacts on changes in selected peat properties and peat degradation in West Kalimantan Province, Indonesia. *Biogeosciences*, 7, 3403–3419.

Bachy, C., Dolan, J.R., López-García, P., Deschamps, P. & Moreira, D. (2013) Accuracy of protist diversity assessments: morphology compared with cloning and direct pyrosequencing of 18S rRNA genes and ITS regions using the conspicuous tintinnid ciliates as a case study. *The ISME Journal*, 7, 244–255.

Classen, A.T., Sundqvist, M.K., Henning, J.A., Newman, G.S., Moore, J.A.M., Cregger, M.A., Moorhead, L.C. & Patterson, C.M. (2015) Direct and indirect effects of climate change on soil microbial and soil microbial plant interactions: What lies ahead? *Ecosphere*, 6, 130–151.

Colombo, F., Macdonald, C.A., Jeffries, T.C., Powell, J.R. & Singh, B.K. (2016) Impact of forest management practices on soil bacterial diversity and consequences for soil processes. *Soil Biology and Biochemistry*, 94, 200–210.

Fischer, G. & Dott, W. (2002) Quality assurance and good laboratory practice in the mycological laboratory—compilation of basic techniques for the identification of fungi. *International Journal of Hygiene and Environmental Health*, 205, 433–442.

Grum-Grzhimaylo, O.A., Debets, A.J.M. & Bilanenko, E.N. (2016) The diversity of microfungi in peatlands originated from the White Sea. *Mycologia*, 108, 233–254.

Jeewon, R. & Hyde, K.D. (2007) Detection and diversity of fungi from environmental samples: Traditional *versus* molecular approaches. In: Varma A. (ed.) *Advanced Techniques in Soil Microbiology*, Vol. 11, Springer, Berlin, Heidelberg, 1–15.

Kerfahi, D., Tripathi, B.M., Lee, J., Edwards, D.P. & Adams, J.M. (2014) The impact of selective-logging and forest clearance for oil palm on fungal communities in Borneo. *PLoS ONE*, 9(11), 1–8.

Kim, H., You, Y-H., Yoon, H., Seo, Y., Kim, Y-E., Choo, Y-S., Lee, I-J., Shin, J-H. & Kim, J-G. (2014) Culturable fungal endophytes isolated from the roots of coastal plants inhabiting Korean east coast. *Mycobiology*, 42, 100–108.

Kittelmann, S., Naylor, G.E., Koolaard, J.P. & Janssen, P.H. (2012) A proposed taxonomy of anaerobic fungi (Class Neocallimastigomycetes) suitable for large-scale sequence-based community structure analysis. *PLoS One*, 7, 1–13.

Krishnan, Y., Bong, C.P.C., Azman, N.F., Zakaria, Z., Othman, N.A., Abdullah, N., Ho, C.S., Lee, C.T., Hansen, S.B. & Hara, H. (2017) Co-composting of palm empty fruit bunch and palm oil mill effluent: Microbial diversity and potential mitigation of greenhouse gas emission. *Journal of Cleaner Production*, 146, 94–100.

Kusai, N.A., Azmi, M.M.Z., Zulkifli, S., Yusof, M.T. & Zainudin, N.A.I.M. (2015) Morphological and molecular characterization of *Curvularia* and related species associated with leaf spot disease of rice in Peninsular Malaysia. *Rendiconti Lincei. Scienze Fisiche e Naturali*, 27(2), 205–214.

Kusai, N.A., Ayob, Z., Maidin, M.S.T. Safari, S. & Ali, S.R.A. (2018) Characterization of fungi from different ecosystems of tropical peat in Sarawak, Malaysia. *Rendiconti Lincei. Scienze Fisiche e Naturali*, 29(2), 469–482.

Li, J., Zheng, Y., Yan, J., Li, H., Wang, X., He, J. & Ding, G. (2013) Effects of different regeneration scenarios and fertilizer treatments on soil microbial ecology in reclaimed opencast mining areas on the Loess Plateau, China. *PLoS ONE*, 8(10), 1–11.

Lin, X., Green, S., Tfaily, M.M., Prakash, O., Konstantinidis, K.T., Corbett, J.E., Chanton, J.P., Cooper, W.T. & Kostka, K.E. (2012) Microbial community structure and activity linked to contrasting biogeochemical gradients in Bog and Fen environments of the Glacial Lake Agassiz peatland. *Applied and Environmental Microbiology*, 78(19), 7023–7031.

Liu, J., Yu, Y., Cai, Z., Bartlam, M. & Wang, Y. (2015) Comparison of ITS and 18S rDNA for estimating fungal diversity using PCR-DGGE. *World Journal of Microbiology and Biotechnology*, 31, 1387–1395.

Lv, X-C., Jiang, Y-J., Liu, J., Guo, W-L., Liu, Z-B., Zhang, W., Rao, P-F. & Ni, L. (2017) Evaluation of different PCR primers for denaturing gradient gel electrophoresis (DGGE) analysis of fungal community structure in traditional fermentation

 DOI: 10.19189/MaP.2018.OMB.331

starters used for Hong Qu glutinous rice wine. *International Journal of Food Microbiology*, 255, 58–65.

Majid, A.H.A., Zahran, Z., Rahim, A.H.A., Ismail, N.A., Rahman, W.A., Zubairi, K.S.M., Dieng, H. & Satho, T. (2015) Morphological and molecular characterization of fungus isolated from tropical bed bugs in Northern Peninsular Malaysia, *Cimex hemipterus* (Hemiptera: Cimicidae). *Asian Pacific Journal of Tropical Biomedicine*, 5, 707–713.

Man, B., Wang, H., Xiang, X., Ruicheng, W, Yun, Y. & Gong, L. (2015). Phylogenetic diversity of culturable fungi in the Heshang Cave, central China. *Frontiers in Microbiology*, 6, 1–11.

McGuire, K.L., D'Angelo, H., Brearley, F.Q., Gedallovich, S.M., Babar, N., Yang, N., Gillikin, C.M., Gradoville, R., Bateman, C., Turner, B.L., Mansor, P., Leff, J.W. & Fierer, N. (2015) Responses of soil fungi to logging and oil palm agriculture in Southeast Asian tropical forests. *Microbial Ecology*, 69(4), 733–747.

Melling, L. (2016) Peatland in Malaysia. In: Osaki, M. & Tsuji, N. (eds.) *Tropical Peatland Ecosystems*, Springer, Japan, 59–73.

Melling, L., Tan, C.S.Y.T., Goh, K.J. & Hatano, R. (2013) Soil microbial and root respirations from three ecosystems in tropical peatland of Sarawak, Malaysia. *Journal of Oil Palm Research*, 25, 44–57.

Metwally, A.I., Mashhady, A.S., Falatah, A.M. & Reda, M. (1993) Effect of pH on zinc adsorption and solubility in suspensions of different clays and soils. *Journal of Plant Nutrition and Soil Science*, 156, 131–135.

Mohamad, R.B., Wibawa, W., Mohayidin, M.G., Puteh, A.B., Juraimi, A.S., Awang, Y. & Lassim, M.B.M. (2010) Management of mixed weeds in young oil-palm plantation with selected broad-spectrum herbicides. *Pertanika Journal of Tropical Agricultural Science*, 33(2), 193–203.

Nurulita, Y., Adetutu, E.M., Gunawan, H., Zul, D. & Ball, A.S. (2016a) Restoration of tropical peat soils: The application of soil microbiology for monitoring the success of the restoration process. *Agriculture, Ecosystems and Environment*, 216, 293–303.

Nurulita, Y., Adetutu, E.M., Kadali, K.K., Shahsavari, E., Zul, D., Taha, M. & Ball, A.S. (2016b) Assessment of the influence of oil palm and rubber plantations in tropical peat swamp soils using microbial diversity and activity analysis. *Journal of Agricultural Chemistry and Environment*, 5, 53–65.

Ortega-Morales, B.O., Narváez-Zapata, J., Reyes-Estebanez, M., Quintana, P., De la Rosa-García, S.d.C., Bullen, H., Gómez-Cornelio, S. & Chan-Bacab, M.J. (2016) Bioweathering potential of cultivable fungi associated with semi-arid surface microhabitats of Mayan buildings. *Frontiers in Microbiology*, 7, 1–12.

Pallant, J. (2007) *SPSS Survival Manual*. Open University Press, McGraw-Hill, New York, USA, 226–228.

Paramananthan, S. (2016) *Organic Soils of Malaysia*: *Their Characteristics, Mapping, Classification and Management for Oil Palm Cultivation*. Malaysian Palm Oil Council, Selangor, Malaysia, 106–107.

Patel, S. & Saraf, M. (2017) Biocontrol efficacy of *Trichoderma asperellum* MSST against tomato wilting by *Fusarium oxysporum* f. sp. *lycopersici*. *Archives of Phytopathology and Plant Protection*, 50, 228–238.

Raja, H.A., Miller, A.N., Pearce, C.J. & Oberlies, N.H. (2017) Fungal identification using molecular tools: A primer for the natural products research community. *Journal of Natural Products*, 80(3), 756–770.

Rakib, M.R.M., Bong, C-F.J., Khairulmazmi, A., Idris, A.S., Jalloh, M.B. & Ahmed, O.H. (2017) Association of copper and zinc levels in oil palm (*Elaeis guineensis*) to the spatial distribution of *Ganoderma* species in the plantations on peat. *Journal of Phytopathology*, 165(4), 276–282.

Ramlah Ali, S.A., Safari, S., Thakib, M.S., Bakeri, S.A. & Ghani, N.A.A. (2016) Soil fungal community associated with peat in Sarawak identified using 18S rDNA marker. *Journal of Oil Palm Research*, 28, 161–171.

Romanelli, A.M., Sutton, D.A., Thompson, E.H., Rinaldi, M.G. & Wickes, B.L. (2010) Sequence-based identification of filamentous Basidiomycetous fungi from clinical specimens: a cautionary note. *Journal of Clinical Microbiology*, 48, 741–752.

Singh, P., Raghukumar, C., Meena, R.M., Verma, P. & Shouche, Y. (2012a) Fungal diversity in deep-sea sediments revealed by culture-dependent and culture-independent approaches. *Fungal Ecology*, 5, 543–553.

Singh, P., Raghukumar, C., Verma, P. & Shouche, Y. (2012b) Assessment of fungal diversity in deep-sea sediments by multiple primer approach. *World Journal of Microbiology and Biotechnology*, 28, 659–667.

Smit, E., Leeflang, P., Glandorf, B., van Elsas, J.D. & Wernars, K. (1999) Analysis of fungal diversity in the wheat rhizosphere by sequencing of cloned PCR-amplified genes encoding 18S rRNA and temperature gradient gel electrophoresis. *Applied*

 DOI: 10.19189/MaP.2018.OMB.331

and Environmental Microbiology, 65, 2614–2621.
Sun, H., Terhonen, E., Koskinen, K., Paulin, L., Kasanen, R. & Asiegbu, F.O. (2014) Bacterial diversity and community structure along different peat soils in boreal forest. *Applied Soil Ecology*, 74, 37–45.
Thormann, M.N. (2006) Diversity and function of fungi in peatlands: A carbon cycling perspective. *Canadian Journal of Soil Science*, 5, 281–293.
Thormann, M.N. & Rice, A.V. (2007) Fungi from peatlands. *Fungal Diversity*, 24, 241–299.
Thormann, M.N., Currah, E.S. & Bayley, S.E. (2001) Microfungi isolated from *Sphagnum fuscum* from a Southern Boreal Bog in Alberta, Canada. *The Bryologist*, 104(4), 548–559.
Toma, Y., Takakai, F., Darung, U., Kuramochi, K., Limin, S.H., Dohong, S. & Hatano, R. (2011) Nitrous oxide emission derived from soil organic matter decomposition from tropical agricultural peat soil in central Kalimantan, Indonesia. *Soil Science and Plant Nutrition*, 57(3), 436–451.
Treseder, K.K. & Holden, S.R. (2013) Fungal carbon sequestration. *Science*, 339(6127), 1528–1529.
Tripathi, B.M., Song, W., Slik, J.W.F., Sukri, R.S., Jaafar, S., Dong, K. & Adams, J.M. (2016) Distinctive tropical forest variants have unique soil microbial communities, but not always low microbial diversity. *Frontiers in Microbiology*, 7, 1–11.
Tveit, A., Schwacke, R., Svenning, M.M. & Urich, T. (2013) Organic carbon transformations in high-Arctic peat soils: key functions and micro-organisms. *The ISME Journal*, 7(2), 299–311.
Vaidyanathan, G. (2011) Counting the carbon cost of peatland conversion. *Nature News*, 7 March 2011. Online at: http://www.nature.com/news/2011/110307/full/news.2011.139.html, accessed 22 Jun 2017.
Veloo, R., Paramananthan, S. & Van Ranst, E. (2014) Classification of tropical lowland peats revisited: The case of Sarawak. *Catena*, 118, 179–185.
Veloo, R., van Ranst, E. & Selliah, P. (2015) Peat characteristics and its impact on oil palm yield. *NJAS - Wageningen Journal of Life Sciences*, 72–73, 33–40.
White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.) *PCR Protocols: a Guide to Methods and Applications*, Academic Press, San Diego, 315–322.
Winsborough, C. & Basiliko, N. (2010) Fungal and bacterial activity in northern peatlands. *Geomicrobiology Journal*, 27, 315–320.
Xu, W., Luo, Z., Guo, S. & Pang, K. (2016) Fungal community analysis in the deep-sea sediments of the Pacific Ocean assessed by comparison of ITS, 18S and 28S ribosomal DNA regions. *Deep Sea Research Part I: Oceanographic Research Papers*, 109, 51–60.
Zain, N.M.M., Mohamad, R.B., Sijam, K., Morshed, M.M. & Awang, Y. (2013) Effects of selected herbicides on soil microbial populations in oil palm plantation of Malaysia: A microcosm experiment. *African Journal of Microbiology Research*, 7(5), 367–374.
Zhou, Y., He, S., Gong, G., Zhang, S., Chang, X., Liu, N., Sun, X., Qi, X., Ye, K. & Wang, Y. (2014) Soil fungal diversity in three nature reserves of Jiuzhaigou County, Sichuan Province, China. *Annals of Microbiology*, 64, 1275–1290.

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Author for correspondence:
Dr Zahidah Ayob, Soil Microbial Biodiversity and Function Group, Applied Entomology and Microbiology Unit, Biological Research Division, Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia. Tel: +603 87693842; Email: zahidahayob@mpob.gov.my

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Appendix

Table A1. The geographic coordinates (latitude, longitude) of sampling sites in the three Alan Bunga peat swamp forest ecosystems (Sarawak, Malaysia). YAP = years after planting.

Ecosystem	Coordinates
Secondary forest (SF)	2° 33' 49.70" N, 111° 59' 20.82" E
	2° 33' 49.74" N, 111° 59' 21.60" E
	2° 33' 49.74" N, 111° 59' 22.52" E
	2° 33' 50.40" N, 111° 59' 21.39" E
	2° 33' 50.69" N, 111° 59' 21.41" E
	2° 33' 52.56" N, 111° 59' 20.76" E
	2° 33' 52.84" N, 111° 59' 20.94" E
	2° 33' 53.45" N, 111° 59' 20.38" E
	2° 33' 54.16" N, 111° 59' 20.26" E
	2° 33' 54.91" N, 111° 59' 20.63" E
Logged-over secondary forest (LF)	2° 33' 58.80" N, 111° 58' 3.83" E
	2° 33' 58.90" N, 111° 58' 5.51" E
	2° 33' 59.34" N, 111° 58' 3.83" E
	2° 33' 59.31" N, 111° 58' 5.44" E
	2° 33' 58.39" N, 111° 58' 5.48" E
	2° 33' 58.30" N, 111° 58' 3.87" E
	2° 33' 58.08" N, 111° 58' 3.89" E
	2° 33' 58.11" N, 111° 58' 5.46" E
	2° 33' 57.77" N, 111° 58' 5.45" E
	2° 33' 57.87" N, 111° 58' 3.84" E
3.5 YAP oil palm (OP)	2°33' 25.16" N, 111° 59' 12.67" E
	2°33' 33.95" N, 111° 59' 8.88" E
	2°33' 40.21" N, 111° 59' 11.43" E
	2°33' 44.03" N, 111° 59' 13.60" E
	2°33' 45.16" N, 111° 59' 6.26" E
	2°33' 48.77" N, 111° 59' 6.26" E
	2°33' 54.61" N, 111° 59' 8.52" E
	2°33' 26.08" N, 111° 59' 8.56" E
	2°33' 26.21" N, 111° 59' 10.65" E
	2°33' 25.11" N, 111° 59' 8.88" E

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Table A2. List of fungal isolates identified from the three ecosystems and amplified using ITS and 18S rDNA primers, in each case showing the species/strain and NCBI accession number of the closest match in the GenBank database. MEA = malt extract agar; PDA = potato dextrose agar; ND = not detected (*i.e*. no GenBank record corresponding to the sequence); YAP = years after planting.

		ITS			18S rDNA		
Site	Isolates	Fungal species	Similarity	Accession No.	Fungal species	Similarity	Accession No.
Secondary forest (SF)	1B -5 MEA F1	*Aspergillus flavus* strain UOA/HCPF 5774	99%	FJ878681	*Aspergillus* sp. strain ZAS6	99%	KX431149
	1B -5 PDA F1	*Aspergillus fumigatus* strain WBS001	97%	KU350716	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	1C -4 MEA F1	*Trichoderma asperellum* isolate Tasp27	99%	KU170994	*Trichocomaceae* sp. LM192	96%	EF060538
	1C -4 MEA F2	*Trichoderma asperellum* isolate Tasp29	99%	KU170996	*Trichoderma* sp. 49	99%	KU350745
	1C -4 MEA F3	*Trichoderma asperellum* strain HNZZ1006	92%	JQ040317	ND	ND	ND
	1D -4 MEA F1	*Aspergillus fumigatus* strain FS160	98%	FJ844610	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	1D -5 PDA F1	*Aspergillus oryzae* isolate A	95%	KU565735	*Aspergillus* sp. strain ZAS6	99%	KX431149
	2A -4 MEA F1	*Aspergillus flavus* isolate B19-1	100%	JN676112	*Aspergillus* sp. strain ZAS6	99%	KX431149
	2A -4 PDA F1	ND	ND	ND	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	2B -4 PDA F1	*Trichoderma asperellum* strain CHI9	98%	KR868311	*Hypocrea atroviridis* strain SMF-H08	99%	JX242484

		ITS			18S rDNA		
Site	Isolates	Fungal species	Similarity	Accession No.	Fungal species	Similarity	Accession No.
Secondary forest (SF)	2B -5 PDA F1	ND	ND	ND	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	2C -4 PDA F1	*Trichoderma asperellum* strain Pt9	99%	KU198280	*Hypocrea atroviridis* strain SMF-H08	99%	JX242484
	3A -4 MEA F1	*Aspergillus flavus* isolate pkm2	96%	KP418782	*Aspergillus* sp. strain ZAS6	99%	KX431149
	3A -5 PDA F1	*Trichoderma asperellum* strain Pt9	99%	KU198280	*Hypocrea atroviridis* strain SMF-H08	99%	JX242484
	3D -4 MEA F1	*Trichoderma asperellum* isolate Tasp28	99%	KU170995	*Hypocrea atroviridis* strain SMF-H08	99%	JX242484
	4B -4 MEA F1	*Phanerochaete chrysosporium* strain H008	98%	EU872426	*Phanerochaete sanguinea*	99%	AB084597
	5C -5 MEA F1	*Aspergillus flavus* strain UOA/HCPF 5774	99%	FJ878681	*Mytilinidion tortile* strain EB 0377	80%	GU323189
	5D -5 MEA F1	*Aspergillus flavus* strain ZJ4-A	88%	FJ487932	*Hypocrea muroiana* strain DsppF6	99%	KT958527
	5D -5 PDA F1	*Aspergillus fumigatus* strain FS160	99%	FJ844610	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	6B -4 MEA F1	*Trichoderma asperellum* isolate Tasp29	100%	KU170996	*Aspergillus* sp. strain ZAS6	100%	KX431149
	6B -5 PDA F1	*Trichoderma asperellum* isolate TGD-1	99%	KX538809	*Hypocrea atroviridis* strain SMF-H08	99%	JX242484
	7B -5 MEA F1	*Aspergillus flavus* strain IM21	99%	KX011593	*Aspergillus fumigatus* strain YA-14	99%	FJ560718

		ITS			18S rDNA		
Site	Isolates	Fungal species	Similarity	Accession No.	Fungal species	Similarity	Accession No.
Secondary forest (SF)	7B -5 PDA F1	*Trichoderma asperellum* isolate Tasp33	99%	KU171000	*Hypocrea atroviridis* strain SMF-H08	99%	JX242484
	7C -4 MEA F1	*Aspergillus fumigatus* strain 2101	97%	KJ194123	*Aspergillus fumigatus* strain YA-14	97%	FJ560718
	7C -4 MEA F2	*Aspergillus fumigatus* strain A0625	87%	KF494830	*Hypocrea atroviridis* strain SMF-H08	99%	JX242484
	7C -4 MEA F3	*Trichoderma asperellum* isolate Tasp27	96%	KU170994	*Hypocrea muroiana* strain DsppF6	99%	KT958527
	8C -5 PDA F1	*Aspergillus fumigatus* strain A0611	99%	KF577886	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	9A -4 MEA F1	*Trichoderma asperellum* isolate Tasp8	98%	KU170975	*Meyerozyma guilliermondii* strain Nc49HB-1	83%	KR336835
	9C -4 MEA F1	*Trichoderma asperellum* isolate TV-3	100%	KX538814	ND	ND	ND
	9A -5 PDA F1	*Penicillium dalea* strain SGE48	99%	JQ776541	*Penicillium* sp. isolate52	99%	KU350746
	10C -5 MEA F1	*Trichoderma asperellum* strain AF14	100%	JX677934	*Aspergillus fumigatus* strain YA-14 1	99%	FJ560718
Logged- over secondary forest (LF)	2B -5 PDA F1	ND	ND	ND	*Aspergillus* sp. isolate MJ1-3	99%	HM590651
	3B -4 MEA F1	*Aspergillus flavus* isolate L7	92%	KF738806	*Aspergillus* sp. strain ZAS6	99%	KX431149
	4B -5 PDA F1	*Aspergillus flavus* strain IM21	100%	KX011593	*Aspergillus* sp. strain ZAS6	99%	KX431149

		ITS			18S rDNA		
Site	Isolates	Fungal species	Similarity	Accession No.	Fungal species	Similarity	Accession No.
Logged- over secondary forest (LF)	5C -5 MEA F1	*Aspergillus fumigatus* strain A0611	100%	KF577886	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	5D -5 PDA F1	*Aspergillus fumigatus* isolate ITA9F5	98%	KP122751	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	8C -4 PDA F1	*Aspergillus fumigatus* isolate 24L15T1	100%	KP724998	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	10C -4 PDA F1	*Aspergillus flavus* isolate L7	99%	KF738806	*Aspergillus* sp. strain ZAS6	99%	KX431149
3.5 YAP oil palm (OP)	3B -5 PDA F1	*Aspergillus fumigatus* isolate TN-281	99%	KX610742	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	5C -4 PDA F1	*Aspergillus niger* isolate A	100%	KU565727	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	6D -4 PDA F1	*Aspergillus tubingensis* isolate AT01	94%	GQ118987	ND	ND	ND
	7C -4 PDA F1	*Aspergillus fumigatus* strain R1	100%	KJ001801	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	9D -5 PDA F1	*Aspergillus fumigatus* strain SGE57	99%	JQ776545	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	9B -5 PDA F3	*Aspergillus fumigatus* strain R1	99%	KJ001801	*Aspergillus fumigatus* strain YA-14	99%	FJ560718
	10B -5 PDA F1	*Aspergillus flavus* strain IM21	99%	KX011593	*Aspergillus* sp. strain ZAS6	99%	KX431149