Comments for the Editor

Participants Edit

Aris Mumpuni (arismumpuni)

Messages

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Add Message

DNA Barcoding of Coprophilous microfungi from Banyumas, Central Java, Indonesia

xxx xxx

Abstract. Coprophilous fungi are a group of fungi that are ecologically interesting in relation to herbivores. These fungi are cosmopolitan in distribution and ~~spread cosmopolitely~~ found wherever herbivorous animals are present. ~~and~~ They play a predominant role in the decomposition of organic matter. ~~They are broken down by a~~ in which organic matter passes through series of events involving physical processes such as leaching and mechanical degradation; as well as through biological processes such as degradation by microbes ~~which involve~~ by secreting several exoenzymes. The role of coprophilous fungi is very important as the main decomposers of the lignocellulosic material of herbivorous animal waste which is widespread in nature. Previous research on the inventory and identification of coprophilous fungi in the Banyumas region was still limited to the macroscopic genera, so the results have not been able to provide a comprehensive picture of the presence of coprophilous fungi, especially in the region. Identifying the types of microscopic coprophilous fungi that live in herbivorous animal waste such as lignocellulosic material is necessary to reveal its taxonomy. This study aimed to invent and identify microscopic coprophilous fungi obtained in the Banyumas Regency area. Based on the purposive random sampling method, the obtained fungi were analyzed using molecular methods ~~molecularly~~ through the stages of DNA isolation, barcoding analysis, DNA sequencing and phylogeny analysis of fungal cultures. The results of this study obtained species: *Emmia lacerate*, *Ceriporia lacerate*, *Trichosporon insectorium* and *Lentinus squarosulus*; and genera: *Fusarium* sp., *Aspergillus* sp., and *Trichosporon* sp.

Keywords: coprophilous fungi, inventory, molecular identification

Running title: DNA barcoding and bioprospecting of coprophilous microfungi

INTRODUCTION

Coprophilous fungi are saprophytic fungi that live in animal dung. These fungi utilize the feces of various animals, especially herbivores as their substrates (Melo et al., 2012). Masunga et al. (2006) stated that coprophilous fungi are a group of Zygomycota, Ascomycota and Basidiomycota. According to Krug et al. (2004) most of the coprophilous fungi are known to inhabit in the dung of herbivorous livestock such as sheep and cows. According to Sinsabaugh et al. (1981), these fungi spread cosmopolitely wherever herbivorous animals are present and play a predominant role in the decomposition of organic matter. The ~~y~~ organic matter ~~are~~ is broken down by a series of events involving physical processes such as leaching and mechanical degradation; as well as through biological processes such as degradation by microbes involving several exoenzymes.

Several researches on coprophilous fungi in Indonesia have been carried out, among others, by Mumpuni and Wahyono (2016). The research found 4 genera of macroscopic coprophilous fungi, namely *Coprinopsis*, *Panaeolus*, *Mycena*, and *Stropharia* in the coastal tourism area of Parangtritis, Yogyakarta. Furthermore, Mumpuni et al. (2020) also reported that in the former Banyumas Residency (District: Banjarnegara, Purbalingga, Banyumas, and Cilacap) there were 12 genera of macroscopic coprophilous fungi, namely *Panaeolus*, *Coprinopsis*, *Stropharia*, *Tricholoma*, *Lycoperdon*, *Ascobolus*, *Rhodocybe*, *Conocybe*, *Bolbitius*, *Leucocoprinus*, *Mycena*, and *Hypholoma*. The coprophilous fungi obtained from these studies were limited to the macroscopic fungi that were found at the time of sampling. To obtain more comprehensive results, a broader research is needed through the isolation of microscopic coprophilous fungi from the substrate of herbivorous animal waste.

Zuber et al. (2011) stated that the standard method for identifying fungal species is morphological analysis, which consists of macroscopic and microscopic observations. Macroscopic analysis consists of the determination

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Commented [UR2]: Though mentioned several researches but a single reference is cited, please add few more references or rephrase the sentence as: According to Mumupuni and Wahyono (2016) several ……

of the color, size and ~~structure~~ structural characteristics of the fruiting body. Further analysis of microscopic characteristics was mainly carried out on comparisons of spore appearance. An alternative to morphological analysis is the identification of fungal species based on their genetic studies. The molecular analysis method that has been used is the DNA forensic method (Herbert et al., 2004), using polymorphism against 2 (two) non-encoding polymorphic Internal Transcriber Spacers (ITS), namely ITS1 and ITS2. According to Nilson et al. (2008), DNA sequence analysis of these fragments has been successfully used for taxonomic studies on fungi. Lee et al. (2000) revealed that ITS1 and ITS2 regions are common markers used in the identification of fungal species. ITS fragments are extremely useful in species identification because of their long, sequential polymorphisms. Studies that have been carried out have proven that the ITS region provides excellent results in molecular systematics down to the species level as well as in the determination of geographical variations between species. These fragments are present in multiple copies, so they can be amplified even on damaged marking material, which still gives significant results in studies carried out for forensic purposes. The effectiveness of the ITS region polymorphism analysis for forensic purposes has been tested, among others, on the differentiation of the psychotropic fungi of the genera *Panaeolus* and *Psilocybe* based on the length of the polymorphisms in the amplification products of this region.

Molecular tools complementing with morphological ~~ones~~ characteristics ~~are very~~ is a promising approach in ~~identifying~~ rapid identification of species ~~and can be used to rapidly and~~ for ~~reliably~~ reliable ~~evaluate~~ evaluation of biological diversity. These markers have been ~~applied~~ effectively and successfully used ~~to~~ in the identification of fungal species since the 1990s (White et al., 1991; Burns et al., 1991)~~.~~; ~~however~~However, the strategy based on the sequencing of standardized genomic fragments (DNA barcoding~~–~~) was recognized afterwards (Hollingsworth, 2007). The primary difference between molecular identification tools and the "DNA barcode" approach is that the latter involves the use of a standard DNA region that is specific for a taxonomic group. Badotti et al., (2017) stated that one advantage of using the ITS region as a standard marker is that most fungal species have been identified based on this genomic region.

In order to reveal the ~~taxonomy~~ taxonomic identity and bioprospection of coprophilous fungi, ~~this~~ present study has been elaborated to invent and identify microscopic coprophilous fungi obtained in the Banyumas Regency area.

MATERIALS AND METHODS

Study area

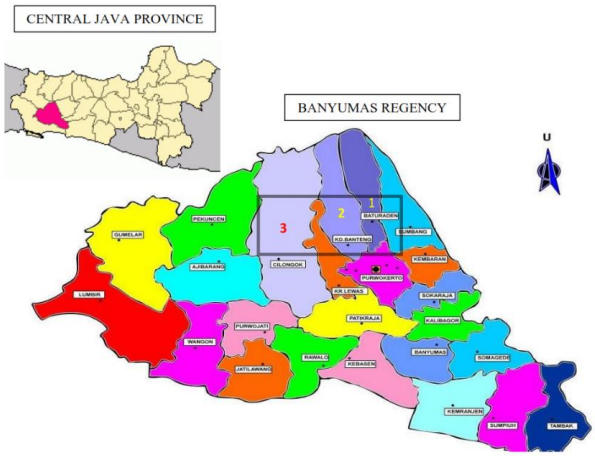

Figure 1. Sampling location in Regency of Banyumas at districts : Baturraden (1); Kedungbanteng (2); and Cilongok (3).

Procedures

Sampling, isolation and purification ~~of the obtained~~ coprophilous fungi~~.~~

~~This~~ Present study was ~~held~~ carried out ~~by~~ for elaborating the survey ~~method~~on coprophilous fungi from Baturraden, Kedungbanteng, and Cilongok districts. Samples in the form of cow dung were taken from these ~~3 (three)~~ three Districts ~~in~~ of Banyumas Regency~~: Baturraden District; Kedungbanteng District; and Cilongok District~~. Cow dung samples were taken from a predetermined location using a pry tool from a maximum depth of 10 cm below the surface of a 1-month-old dung pile that is already in the landfill. The isolation of coprophilous fungi was carried out by making a 10^{-3} - 10^{-5} dilution series.~~,~~ ~~grew a~~ A drop of the ~~dilution~~ diluted extract was grown in Soil Extract Agar (SEA) media with the addition of Chloramphenicol. Incubation was carried out at room temperature for 3-7 days. The fungus that ~~grows~~ grown on this media ~~is~~ was then purified on Potato Dextrose Agar (PDA) media by serial culture method until pure culture obtained. Subsequently, the purified fungi were then inoculated into Malt Extract Broth (MEB) media. The cultures were incubated at room temperature for 15 days until the mycelia filled the Erlenmeyer flask. Mycelia are harvested using filtration and washed twice with distilled water. Wet mycelia are then directly used for DNA isolation, or freeze-dry and stored at -20ºC for DNA isolation.

~~**Coprophilous fungal mycelium culture**~~

Isolation of DNA from culture mycelium

Isolation of DNA ~~isolation of~~ from the pure coprophilous fungal isolates were carried out with PrestoTM Mini gDNA for yeast (Geneaid) until 100 µl of the DNA solution was obtained as follows:

a. Pure cultures of coprophilous fungi (up to 2 x 108) from agar plates were inserted into 1.5 mL microsentrifugation tubes and centrifuged 5,000 x g, 10 minutes. ~~Then, t~~The pellets were further processed and the supernatant is removed.
b. Nearly 50-200 mg of pellet (up to 2 x 108) were added with 600 µl of GT Buffer and resuspended with a vortex or pipette to become lysate.
c. The lysate was transferred to a Beadbeating tube and added 5 µl of RNase A (50 mg/ml). Furthermore, the lysate was pulverized for 10 minutes, at 37° C and incubated at 70° C for 10 minutes. During the incubation process, the tubes were turned every 3 minutes.
d. The sample mixture in the Beadbeating tube was added with 100 µl of PR Buffer homogenized with vortex to avoid the formation of foam (detergent) on ice for 5 minutes and centrifuged at 11,000x g for 3 minutes at room temperature (25° C). Next, 450 µl of the supernatant was transferred to a new 1.5 ml microcentrifugation tube.
e. 450 µl of GB Buffer and 450 µl of absolute ethanol were added to the sample mixture above and homogenized by shaking the tube carefully for 10 seconds.
f. GD Column is attached to 2 ml collection tube. 700 µl of sample mixture was inserted into the GD column and centrifuged at a speed of 16,000x g for 1 minute at room temperature (25° C). Next, the solution that passes through the GD column in the collection tube was discarded and the GD column was put back into the 2 ml collection tube. The remaining sample mixture in the GD column was again centrifuged at a rate of 16,000x g for 1 minute at room temperature (25° C) and the solution passing through the GD column in the collection tube was discarded again.
g. 400 µl of W1 Buffer was inserted into the GD Column and centrifuged at 16,000x g for 30 seconds at room temperature (25° C). Next, the solution that passes through the GD column in the collection tube was discarded and the GD column was put back in the 2 ml collection tube. 600 µl of Wash Buffer was inserted into the GD Column and centrifuged at a speed of 16,000x g for 30 seconds at room temperature (25° C). The solution that passes through the GD column in the collection tube was discarded and the GD column was put back in the 2 ml collection tube. The remainder of the sample mixture in GD column was again centrifuged at a speed of 16,000x g for 1 minute and dried in a collum matrix.
h. The dried GD Column was paired with 1.5 ml of a new microcentrifugation tube and 100 µl of preheated Elution Buffer1 (TE2 or water3) was added to the column matrix. Subsequently incubated for at least 2 minutes until the Elution Buffer was absorbed in the DNA and centrifuged at a speed of 16,000 x g for 2 minutes at room temperature (25° C) until a (pure) DNA solution was obtained. DNA solutions can be directly used for PCR analysis or stored at -80° C (for a long time).

Barcoding analysis of the coprophilous fungi

Amplification of the ITS locus was carried out using ITS-1 primers with the base sequence DNA: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS-4 with the base sequence DNA: 5'-TCCTCCGCTTATTGATGC-3'. The volume of the PCR mixture used was 25 µ~~u~~l consisting of: 1 µ~~u~~l genomic DNA template, 12.5 µ~~u~~l 2x MyTaq

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Red Mix (Bioline), 1 ul primer ITS-1 and ITS-4 (20 uM / ul) and 9.5. ul ddH2O. Amplification was carried out for 35 cycles on an Applied Biosystems 96-Well GeneAmp 9700 thermal cycler machine with the following conditions: pre-denaturation stage at 95ºC for 3 minutes, denaturation stage at 95ºC for 10 seconds, the primary attachment stage (annealing) at 52ºC for 30 seconds, and the extension stage at 72ºC for 45 seconds. DNA amplicon visualization using 1-2% agarose gel electrophoresis. PCR products were purified using ZymocleanTM Gel DNA Recovery Kit (Zymo Research).

DNA sequencing

The purified PCR products ~~are~~ were then sequenced using the bi-directional method using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit, v. 3.0 (Applied Biosystems) and the product reactions were separated and ~~analyzed~~ analysed using the ABI Prism 310 and/or the ABI Prism 3100 Genetic ~~Analyzers~~Analysers. Sequencing is carried out by a third party (PT Genetika Science Indonesia). Data was submitted to GenBank (http://www.ncbi.nlm.nih.gov/) for data analysis.

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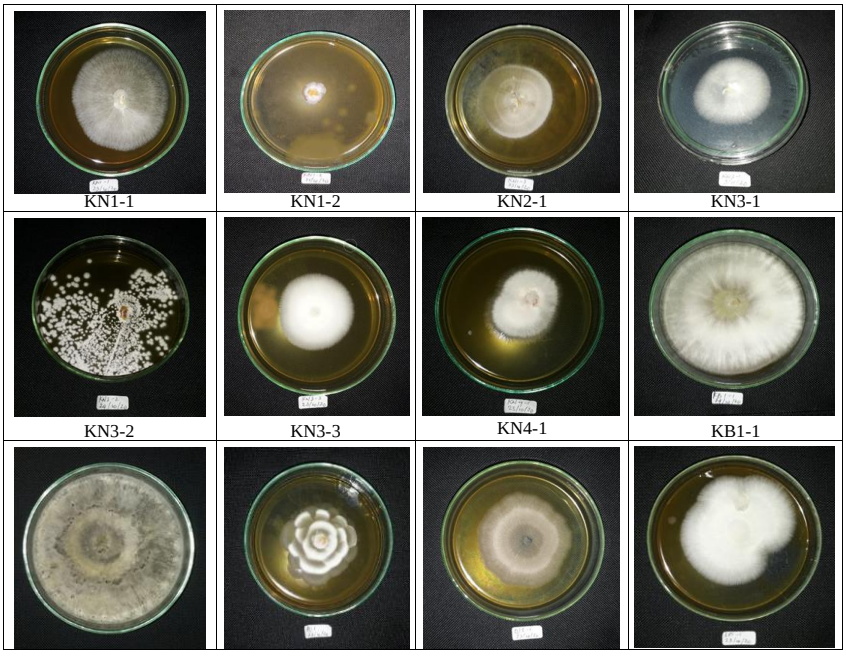
Data analysis

Electropherograms were edited manually, contigs were merged and multiple alignments were made for all data sequences using Genetool software (Biotools Inc). The Neighbor-Joining (N-J) distance algorithm uses the Kimura2 parameter model as used by PAUP (v.4.0b10) (Swofford, 2000) used for phylogenetic analysis. Heuristic analysis using parsinomy was also undertaken.

RESULTS AND DISCUSSION

Result

By elaborating purposive random sampling technique ~~in~~ of the coprophilous fungal isolates from ~~3 (three)~~ three Districts (*viz.* Kedungbanteng, Baturraden, and Cilongok) of Banyumas Regency~~.~~, ~~this research obtained~~ During this investigation, 16 samples of coprophilous fungal isolates were isolated with different somatic phase characteristics (Fig.1). The fungal genomic DNA quantification was done following the fungal isolates purification ~~follow~~ as mentioned in ~~(~~Table 1.~~)~~



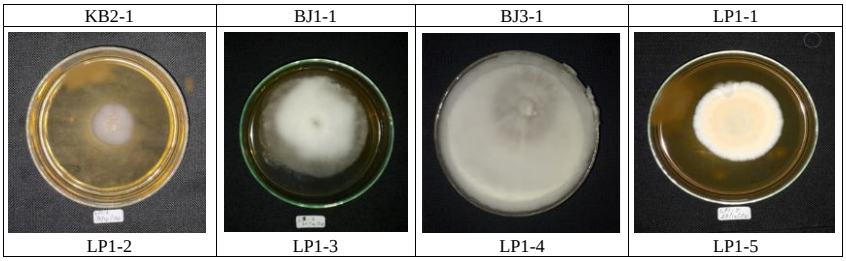


Figure 1. C~~h~~oprophilous fungal isolates from Regency of Banyumas, 5 days old culture.

Table 1. Fungal genomic DNA quantification

No.	Nama Sample	Conc. (ng/µl)	$A_{260/280}$	$A_{260/230}$	Volume (µl)
1	KN1-1	14.2	1.98	0.30	40
2	KN1-2	31.6	1.98	0.14	40
3	KN2-1	29.0	1.93	0.41	40
4	KN3-1	9.3	2.02	0.14	40
5	KN3-2	22.3	1.90	0.17	40
6	KN3-3	9.6	1.65	0.39	40
7	KN4-1	22.3	1.90	0.17	40
8	KB1-1	18.0	2.01	0.19	40
9	KB2-1	96.9	1.89	0.82	40
10	BJ1-1	18.0	1.94	0.12	40
11	BJ3-1	26.7	1.94	0.11	40
12	LP1-1	23.1	1.89	0.04	40
13	LP1-2	11.7	1.98	0.11	40
14	LP1-3	24.5	1.92	0.28	40
15	LP1-4	21.1	1.86	0.27	40
16	LP1-5	55.5	1.93	0.58	40

The data in Table 1. show~~s~~ the results of genomic DNA quantification of several coprophilous fungal isolates that had concentrations far below 20 ng/µl, namely KN1-1, KN3-1, KN3-3, and LP1-2. ~~Actually a~~An isolate with a DNA concentration below 20 ng/µl ~~is~~ was not optimal for molecular analysis~~,~~; however, in general the DNA of the identified isolates have a fairly good purity. The results of measuring the absorbance value of OD (λ) 260/280 by spectrophotometric analysis that has a value of more than 1.8, ~~namely~~ *viz.* KN1-1, KN1-2, KN2-1, KN3-1, KN3-2, KN4-1, KB1-1, BJ1-1, BJ3-1, LP1-2, LP1- 3 and LP1-5 indicate possible RNA contamination; whereas the results of spectrophotometric analysis with a value of less than 1.8, namely KN3-3, indicate the possibility of protein contamination. A good value of DNA purity is 1.8, which indicates that the DNA sample is free from RNA or protein contamination, namely samples KB2-1, LP1-1 and LP4-1.

~~However, in~~ In all samples of coprophilous fungi are based on the measurement results ~~the~~ i.e., absorbance value of OD 260/230 that had a value of less than 1.8 ~~which~~ indicates the possibility of polysaccharide contamination. According to Boyer, (2005) DNA that is free of polysaccharide contamination has an OD value of 260/230 more than 2.0. The concentration of DNA samples was measured for the absorbance value at the OD 260 nm wavelength, while the DNA purity was measured the absorbance value at the OD 260/280 nm wavelength and the optimal DNA purity if the absorbance value at λ260 / 280 nm ranged from 1.8. An absorbance value lower than 1.8 indicates that the DNA sample is contaminated with protein, while an absorbance value higher than 1.8 indicates that the DNA sample is contaminated with RNA (Sambrook & Russel, 2001). DNA purity based on the ratio of absorbance values OD 260/230 nm ~~that~~ of DNA with good purity has a value ranging from 2.0-2.2. If the absorbance value of the OD 260/230 is lower than 2, the DNA is contaminated with carbohydrates, organic matter, or other chemicals.

Figure 2. shows the results of DNA amplification at the ITS gene locus from coprophilous fungal samples using ITS-1 and ITS-4 primers. Of the 16 samples of coprophilic fungi isolated from the cow dung, only 9 samples (KN1-1, KN1-2, KN3-1, KN3-2, KN3-3, KN4-1, KB1-1, BJ3-1, and LP1-3) showed optimal DNA amplification as indicated by specific DNA amplicons i.e. single and thick DNA bands. Furthermore, the nine samples were continued with the DNA sequencing stage. According to Agrawal (2008) the purity of DNA samples can also affect PCR results, so that to obtain optimal PCR results, pure DNA is needed. ~~Meanwhile, according~~ According to Sambrook & Russel (2001) the appearance of a specific DNA band which is marked by a single band and is thick enough indicates that the extraction of genomic DNA has optimal quantity and purity.

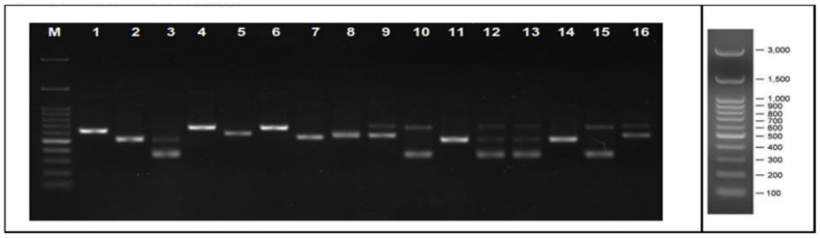


Figure 2. The results of the amplification of ITS gene loci from coprophilous fungi samples using ITS-1 and ITS-4 primers. Description: well M: DNA Ladder 100 pb, wells no. 1-16 samples of coprophilous fungal DNA.

The results of DNA sequencing in nine selected samples (Table 2.), showed the presence of good purity (except one sample (i.e., KB1-1) for which blast nucleotide analysis could not be carried out from the NCBI database because in this sample the DNA base sequence could not be determined due to noise from the sample as DNA purity is not good enough). According to Bruce et al. (2002) some of the factors that can affect the optimal DNA sequencing results are: first, temperatures of denaturation, annealing and extension at the cycle sequencing stage; second,and the separation of DNA molecules during the purification and precipitation stages.

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Table 2. Sequence assembly result – PCR products

No	Sample Name	Sequences
1.	KN1-1	**Sequence Assembly 636bp** 1 TGAACCTGCG GAAGGATCAT TATCGAGTTT TGAACGGGTT GTAGCTGGCC TTTAACGAGG 61 TATGTGCACG CCTGGCTCAT CCACTCTCAA CCTCTGTGCA CTTTATGTAA GAAACGGTGT 121 AAGCCAGCTA TTTAATAGTC GGTAATAAGC CTTTCTTATG TTTACTACAA ACGCTTCAGT 181 TATAGAATGT TTACTGTGTA TAACACAATT ATATACAACT TTCAGCAACG GATCTCTTGG 241 CTCTCGCATC GATGAAGAAC GCAGCGAAAT GCGATAAGTA ATGTGAATTG CAGAATTCAG 301 TGAATCATCG AATCTTTGAA CGCACCTTGC ACTCCTTGGT ATTCCGAGGA GTATGCCTGT 361 TTGAGTCTCA TGGAATTCTC AACCCCTAAA TTTTGTAATG AAGTTTAGTG GGCTTGGACT 421 TGGAGGTTGT GTCGGCTTCT AGTCGACTCC TCTGAAATGT ATTAGCGTGA ATCTTACGGA 481 TCGCCTTCAG TGTGATAATT ATCTGCGCTG TGGTGTTGAA GTATTTATTA GTTCATGCTT 541 ATAGTCGTCT CTTACCGAGA CAATTTATGA CAATCTGAGC TCAAATCAGG TAGGACTACC 601 CGCTGAACTT AAGCATATCA ATAAGCCGGA GGAAGG
2.	KN1-2	**Sequence Assembly 533bp** 1 TAGGTGAACC TGCGGAAGGA TCATTAGTGA TTGCCTTTAT AGGCTTATAA CTATATCCAC 61 TTACACCTGT GAACTGTTCT ACTACTTGAC GCAAGTCGAG TATTTTTACA AACAATGTGT 121 AATGAACGTC GTTTTATTAT AACAAAATAA AACTTTCAAC AACGGATCTC TTGGCTCTCG 181 CAGCGATGAA GAACGCAGCG AATTGCGATA AGTAATGTGA ATTGCAGAAT TCAGTGAATC 241 ATCGAATCTT TGAACGCAGC TTGCGCTCTC TGGTATTCCG GAGAGCATGC CTGTTTCAGT 301 GTCATGAAAT CTCAACCACT AGGGTTTCCT AATGGATTGG ATTTGGGCGT CTGCGATTTC 361 TGATCGCTCG CCTTAAAAGA GTTAGCAAGT TTGACATTAA TGTCTGGTGT AATAAGTTTC 421 ACTGGGTCCA TTGTGTTGAA GCGTGCTTCT AATCGTCCGC AAGGACAATT ACTTTGACTC 481 TGGCCTGAAA TCAGGTAGGA CTACCCGCTG AACTTAAGCA TATCAATAAG CGG
3.	KN3-1	**Sequence Assembly 647bp** 1 AGGATCATTA TCGAGTTTTG AAACGGGTTG TAGCTGGCCT TCCGAGGCAT GTGCACGCCC 61 TGCTCATCCA CTCTACACCT GTGCACTTAC TGTGGGTTTC AGGAGCTTCG AAAGCGAGAA 121 AAGGGGCCTT CACGGGCTTT TTTCTTGCCT AGTTGTTACT GGGCCTACGT TTCACTACAA 181 ACACTTATAA AGTATCAGAA TGTGTATTGC GATGTAACGC ATCTATATAC AACTTTCAGC 241 AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA 301 ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC TTGCGCTCCT TGGTATTCCG 361 AGGAGCATGC CTGTTTGAGT GTCATGAAAT TCTCAACCTA ACGGGTTCTT AACGGGACTT 421 GCTTTAGGCT TGGACTTGGA GGTTCTTGTC GGCTTGCTTC AATGTCAAGT CGGCTCCTCT 481 TAAATGCATT AGCTTGGTTC CTGTGCGGAT CGGCTCACGG TGTGATAATT CGCTACGCCG 541 CGACCGTTGA AGCGTTTTTA TAGGCCAGCT TCTAGTCGTC TCTTTACGAG ACAATAATCA 601 TCGAACTCTG ACCTCAAATC AGGTAGGACT ACCCGCTGAA CTTAAGC
4.	KN3-2	**Sequence Assembly 584bp** 1 AGGTGAACCT GCGGAAGGAT CATTACCGAG TGCGGGTCCG CGTGGCCCAA CCTCCCACCC 61 GTGCCTATTG TACCCTGTTG CTTCGGCGGG CCCGCCAGCC TTCGGGCTGG CCGCCGGGGG 121 GCGTCTCGCC CCCGGGCCCG TGCCCGCCGG AGACCCCAAC ATGAACCCTG TTCTGAAAGC 181 TTGGTGTCTG AGTGTGATTG TTTGCAATCA GTTAAAACTT TCAACAATGG ATCTCTTGGT 241 TCCGGCATCG ATGAAGAACG CAGCGAAATG CGATAACTAA TGTGAATTGC AGAATTCAGT 301 GAATCATCGA GTCTTTGAAC GCACATTGCG CCCCCTGGTA TTCCGGGGGG CATGCCTGTC 361 CGAGCGTCAT TGCTGCCCTC AAGCCCGGCT TGTGTGTTGG GCCCTCGTCC CCCGGCTCCC 421 GGGGGACGGG CCCGAAAGGC AGCGGCGGCA CCGCGTCCGG TCCTCGAGCG TATGGGGCTT 481 TGTCTTCCGC TCTGCAGGCC CGGCCGGCGC CCGCCGACGC ATAACAACTT TTTTCCAGG 541 TTGACCTCGG ATCAGGTAGG GATACCCGCT GAACTTAAGC ATAT
5.	KN3-3	**Sequence Assembly 670bp** 1 AACCTGCGGA AGGATCATTA TCGAGTTTTG AAACGGGTTG TAGCTGGCCT TCCGAGGCAT 61 GTGCACGCCC TGCTCATCCA CTCTACACCT GTGCACTTAC TGTGGGTTTC AGGAGCTTCG 121 AAAGCGAGAA AAGGGGCCTT CACGGGCTTT TTTCTTGCCT AGTTGTTACT GGGCCTACGT 181 TTCACTACAA ACACTTATAA AGTATCAGAA TGTGTATTGC GATGTAACGC ATCTATATAC 241 AACTTTCAGC AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGATA 301 AGTAATGTGA ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC TTGCGCTCCT 361 TGGTATTCCG AGGAGCATGC CTGTTTGAGT GTCATGAAAT TCTCAACCTA ACGGGTTCTT 421 AACGGGACTT GCTTTAGGCT TGGACTTGGA GGTTCTTGTC GGCTTGCTTC AATGTCAAGT 481 CGGCTCCTCT TAAATGCATT AGCTTGGTTC CTGTGCGGAT CGGCTCACGG TGTGATAATT 541 GTCTACGCCG CGACCGTTGA AGCGTTTTTA TAGGCCAGCT TCTAGTCGTC TCTTTACGAG 601 ACAATAATCA TCGAACTCTG ACCTCAAATC AGGTAGGACT ACCCGCTGAA CTTAAGCATA 661 TCAATAAGGC
6.	KN4-1	**Sequence Assembly 522bp** 1 AGGGATCATT ACCGAGTTTA CAACTCCCAA ACCCCTGTGA ACATACCAAT TGTTGCCTCG 61 GCGGATCAGC CCGCTCCCGG TAAAACGGGA CGGCCCGCCA GAGGACCCCT AAACTCTGTT 121 TCTATATGTA ACTTCTGAGT AAAACCATAA ATAAATCAAA ACTTTCAACA ACGGATCTCT 181 TGGTTCTGGC ATCGATGAAG AACGCAGCAA AATGCGATAA GTAATGTGAA TTGCAGAATT 241 CAGTGAATCA TCGAATCTTT GAACGCACAT TGCGCCCGCC AGTATTCTGG CGGGCATGCC 301 TGTTCGAGCG TCATTTCAAC CCTCAAGCCC CCGGGTTTGG TGTTGGGGAT CGGCGAGCCC 361 TTGCGGCAAG CCGCCCCGA AATCTAGTGG CGGTCTCGCT GCAGCTTCCA TTGCGTAGTA 421 GTAAAACCCT CCCAACTGGT ACGCGGCGCG GCCAAGCCGT TAAACCCCCA ACTTCTGAAT 481 GTTGACCTCG GATCAGGTAG GAATACCCGC TGAACTTAAG CA
7.	KB1-1	**Repeat Sequencing Process**
8.	BJ3-1	**Sequence Assembly 516bp** 1 TGATATGCTT AAGTTCAGCG GGTAGTCCTA CCTGATTTCA GGCCAGAGTC AAAGTAATTG 61 TCCTTGCGGA CGATTAGAAG CACGCTTCAA CACAATGGAC CCAGTGAAAC TTATTACACC 121 AGACATTAAT GTCAAAGTTG CTAACTCTTT TAAGGCGAGC GATCAGAGAT CGCAGACGCC 181 CAAATCCAAT CCATTAGGAA ACCCTAGTGG TTGAGATTTC ATGACACTGA AACAGGCATG 241 CTCTCCGGAA TACCAGAGAG CGCAAGCTGC GTTCAAAGAT TCGATGATTC ACTGAATTCT 301 GCAATTCACA TTACTTATCG CAATTCGCTG CGTTCTTCAT CGATGCGAGA GCCAAGAGAT 361 CCGTTGTTGA AAGTTTTATT TTGTTATAAT AAAACGACGT TCATTACACA TTGTTTGTAA 421 AAATACTCGA CTTGCGTCAA GTAGTAGAAC AGTTCACAGG TGTAAGTGGA TATAGTTATA 481 AGCCTATAAA GGCAATCACT AATGATCCTT CCGCAG
9.	LP1-3	**Sequence Assembly 539bp** 1 TCCGTAGGTG AACCTGCGGA AGGATCATTA GTGATTGCCT TTATAGGCTT ATAACTATAT 61 CCACTTACAC CTGTGAACTG TTCTACTCCT TGACGCAAGT CGAGTATTTT TACAAACAAT 121 GTGTAATGAA CGTCGTTTTA TTATAACAAA ATAAAACTTT CAACAACGGA TCTCTTGGCT 181 CTCGCATCGA TGAAGAACGC AGCGAATTGC GATAAGTAAT GTGAATTGCA GAATTCAGTG 241 AATCATCGAA TCTTTGAACG CAGCTTGCGC TCTCTGGTAT TCCGGAGAGC ATGCCTGTTT 301 CAGTGTCATG AAATCTCAAC CACTAGGGTT TCCTAATGGA TTGGATTTGG GCGTCTGCGA 361 TCTCTGATCG CTCGCCTTAA AAGAGTTAGC AAGTTTGACA TTAATGTCTG GTGTAATAAG 421 TTTCACTGGG TCCATTGTGT TGAAGCGTGC TTCTAATCGT CCGCAAGGAC AATTACTTTG 481 ACTCTGGCCT GAAATCAGGT AGGACTACCC GCTGAACTTA AGCATATCAA TAAGCGGAG

The results of blast nucleotide analysis can be seen in Table 3., on referring to the NCBI database proved that the samples KN1-1, KN1-2. KN3-1, KN3-2, KN3-3, BJ3-1, and LP1-3 are quite convincing, because these samples show consistent blast nucleotide yields in one or two specific species, if there is a difference, it only shows homotypic synonym, taxon synonym or obligate synonym of the current name of the species.

Table 3. Results of blast nucleotide analysis from the NCBI database

No.	Samples	Result Links					
		Description	Max Score	Total score	Query cover	E value	Per Ident
1	KN1-1	*Emmia lacerata* isolate A01	1136	1136	99%	0.0	99.84%
		Ceriporia lecerata isolate A1S5-D23	1135	1135	100%	0.0	99.69%
		Ceriporia lacerata isolate BPEF81	1123	1123	99%	0.0	99.52%
		Ceriporia lacerata isolate WS1JB14	1121	1121	97%	0.0	100.00%
		Ceriporia lacerata isolate X12	1118	1118	99%	0.0	99.21%
		Emmia lacerata MYA 12S07	1116	1116	99%	0.0	99.21%
		Emmia sp strain Cef 13	1116	1116	99%	0.0	99.21%
		Ceriporia lacerata isolate CIFE 29	1116	1116	98%	0.0	99.52%
		Basidiomycota sp SYBC-L17	1116	1116	99%	0.0	99.21%
		Ceriporia lacerata genes for 18S	1116	1116	99%	0.0	99.21%
		http://www.ncbi.nlm.nih.gov/nuccore/MH734799.1,KJ780757.1,KF151851.1,KT844687.1,KF850375.1,LC431580.1,MK775821.1,KM388611.1,HQ891300.1,LC312413.1					
2	KN1-2	*Trichosporon asahii* strain CU12015 6	962	962	100%	0.0	100%
		Trichosporon asahii isolate M15	962	962	100%	0.0	100%
		Trichosporon sp isolate EE(19)-CHc	962	962	100%	0.0	100%
		Trichosporon asahii isolate E22922	962	962	100%	0.0	100%
		Trichosporon asahii strain DMic 165073	962	962	100%	0.0	100%
		Trichosporon asahii culture CBS 2497	962	962	100%	0.0	100%
		Trichosporon asahii strain V9	962	962	100%	0.0	100%
		Trichosporon asahii strain 18S	962	962	100%	0.0	100%
		Trichosporon asahii strain APMSU6	962	962	100%	0.0	100%
		Trichosporon asahii strain YCH116	962	962	100%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/MT482659.1,MT136544.1,MK267768.1,MG241533.1,KY105711.1,KT900123.1,KT900118.1,KT282395.1,KM982986.1					
3	KN3-1	*Lentinus squarrosulus* isolate TAM1004	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher WARRIPt	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher WARRI34	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher UNIP13	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher Odi26	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher IBD43	1168	1168	100%	0.0	100%
		Lentinus sp BAB5060	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher BORH0009	1162	1162	99%	0.0	100%

		Lentinus squarrosulus small subunit ribosomal *Lentinus squarrosulus* strain WCR1201	1159 1155	1159 1155	100% 100%	0.0 0.0	99.85% 99.69%
		http://www.ncbi.nlm.nih.gov/nuccore/MH172168.1,KT273380.1,KT273379.1,KT273370.1,KT273364.1,KR155105.1,MH053154.1,KT956127.1					
4	KN3-2	*Aspergillus allahabadii* strain CGMC 3 03920	1054	1054	100%	0.0	100%
		Aspergillus allahabadii strain CGMC 3 02584	1054	1054	100%	0.0	100%
		Aspergillus allahabadii genes for 18S rRNA	1054	1054	100%	0.0	100%
		Aspergillus candidus isolate CY104	1054	1054	100%	0.0	100%
		Aspergillus allahabadii strain CMV004E2	1049	1049	100%	0.0	99.83%
		Aspergillus allahabadii strain CGMCC 3 01332	1049	1049	100%	0.0	99.83%
		Aspergillus niveus strain URM7046	1048	1048	99%	0.0	99.83%
		Aspergillus niveus strain CBS 132162	1045	1045	100%	0.0	99.66%
		Aspergillus allahabadii strain NN046949	1043	1043	98%	0.0	100%
		Aspergillus niveus strain NN043511	1043	1043	98%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/MH292843.1,MH292842.1,LC152416.1,HQ607958.1,MK450628.1,MH292844.1,KM613137.1,MH865978.1,KX443215.1,KX443211.1					
5	KN3-3	*Lentinus* sp BAB-5060	1205	1205	99%	0.0	100%
		Lentinus squarrosulus voucher WARRIPt	1196	1196	98%	0.0	100%
		Lentinus squarrosulus voucher Odi26	1196	1196	98%	0.0	100%
		Lentinus squarrosulus strain WCR1201	1193	1193	99%	0.0	99.70%
		Lentinus squarrosulus voucher UNIP13	1191	1191	98%	0.0	100%
		Lentinus squarrosulus voucher WARRI34	1189	1189	98%	0.0	100%
		Lentinus squarrosulus IBD43	1189	1189	98%	0.0	100%
		Lentinus sp S5	1188	1188	99%	0.0	99.55%
		Lentinus squarrosulus small subunit	1185	1185	98%	0.0	99.85%
		Lentinus squarrosulus voucher BORH0009	1180	1180	97%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/KR155105.1,KT273380.1,KT273370.1,KT956127.1,KT273373.1,KT273379.1,KT273364.1,JN253598.1,MH053154.1,KP283484.1					
6	KN4-1	*Fusarium proliferatum* strain CBB-4	942	942	100%	0.0	100%
		Fusarium fujikuroi strain S106	942	942	100%	0.0	100%
		Fusarium proliferatum strain 4156	942	942	100%	0.0	100%
		Fusarium proliferatum strain 4054	942	942	100%	0.0	100%
		Fusarium fujikuroi strainYT-4	942	942	100%	0.0	100%
		Fusarium diaminii strain YT-2	942	942	100%	0.0	100%
		Fusarium proliferatum strain BL4	942	942	100%	0.0	100%
		Fusarium proliferatum strain GFR39	942	942	100%	0.0	100%
		Fusarium annulatum strain F-6	942	942	100%	0.0	100%
		Fusarium proliferatum strain HYC1410080401	942	942	100%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/MT560212.1,MT549849.1,MN817705.1,MN817704.1,MT477707.1,MT477704.1,MT466521.1,MT447544.1,MT434005.1,MT378328.1					
7	BJ3-1	*Trichosporon asahii* isolate SY4-1 clone SY4-1B	931	931	100%	0.0	100%
		Trichosporon insectorium culture CBS 10422	931	931	100%	0.0	100%
		Trichosporon insectorium culture CBS 10421	931	931	100%	0.0	100%
		Trichosporon faecale culture CBS 4828	931	931	100%	0.0	100%
		Trichosporon insectorium strain ATCC 20506	931	931	100%	0.0	100%
		Trichosporon insectorium ATCCMYA-4361	931	931	100%	0.0	100%
		Trichosporon faecale strain DH545	931	931	100%	0.0	100%
		Trichosporon faecale CBS 4828	927	927	100%	0.0	99.81%
		Trichosporon asahii strain CU12015 6 *Trichosporon asahii* strain CU12015 21	927	927	100%	0.0	99.81%
		http://www.ncbi.nlm.nih.gov/nuccore/KY963115.1,KY105746.1,KY105745.1,KY105736.1,HM802133.1,NR 111353.1,EF153624.1,NR 073242.1,MT482659.1,MT482658.1					
8	LP1-3	*Trichosporon asahii* isolate SY4-1 clone SY4	973	973	100%	0.0	100%
		Trichosporon faecale culture CBS 4826	973	973	100%	0.0	100%
		Trichosporon insectorium strain ATCC 20506	973	973	100%	0.0	100%
		Trichosporon insectorium ATCC MYA-4361	973	973	100%	0.0	100%

		Trichosporon faecale CBS 4828	073	073	100%	0.0	100%
		Trichosporon insectorium culture CBS 10422	971	971	99%	0.0	100%
		Trichosporon asahii strain CU12015 6	968	968	100%	0.0	99.81%
		Trichosporon asahii isolate M15	968	968	100%	0.0	99.81%
		Trichosporon sp isolate EE(19)-CHc	968	968	100%	0.0	99.81%
		Trichosporon asahii isolate E22922	968	968	100%	0.0	99.81%
		http://www.ncbi.nlm.nih.gov/nuccore/KY963115.1,KY105736.1,HM802133.1,NR 111353.1,NR073242.1,KY105746.1,MT482659.1,MT136544.1,MK605936.1,MK267768.1					

The results of the blast nucleotide analysis from the NCBI database are then displayed in the form of a phylogenetic tree which can be seen in Fig. 2 to Fig. 9 as follows:

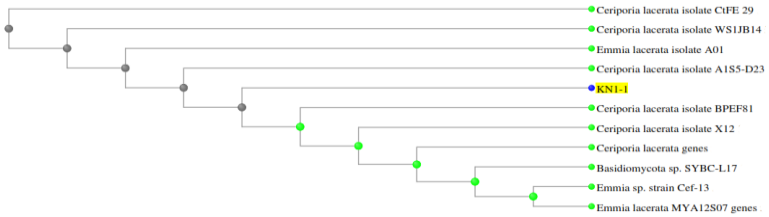

Figure 2. Phylogenetic tree of KN1-1 chaoprophilous fungal isolate

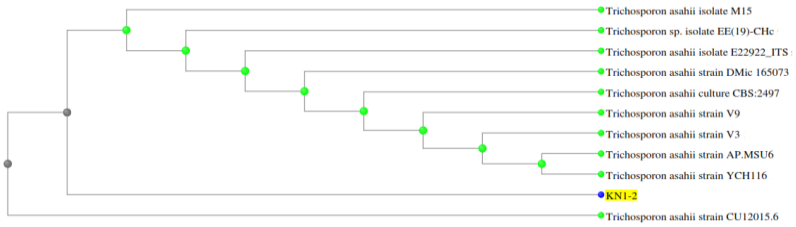

Figure 3. Phylogenetic tree of KN1-2 chaoprophilous fungal isolate

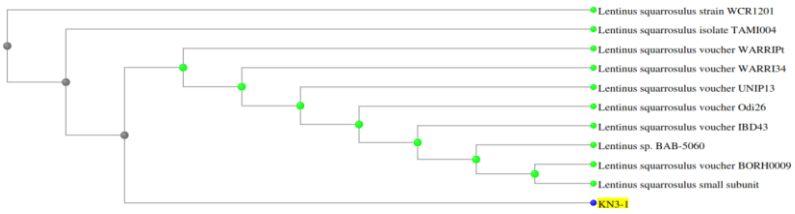

Figure 4. Phylogenetic tree of KN3-1 chaoprophilous fungal isolate

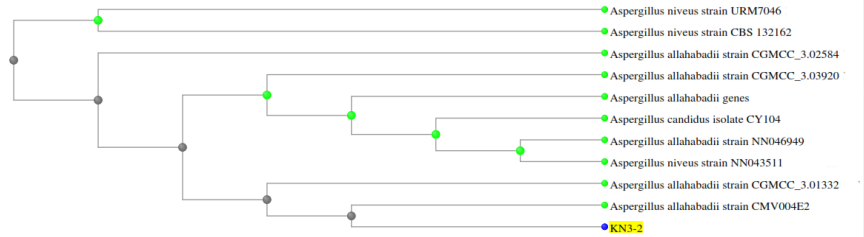

Figure 5. Phylogenetic tree of KN3-2 chaoprophilous fungal isolate

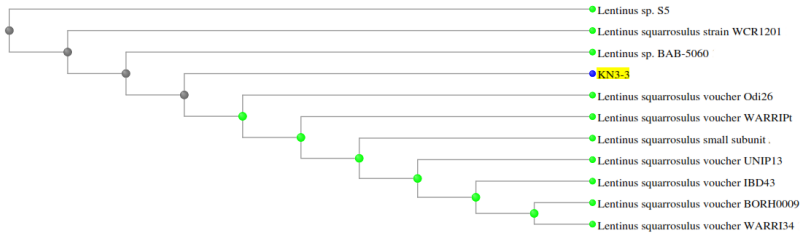

Figure 6. Phylogenetic tree of KN3-3 chaoprophilous fungal isolate

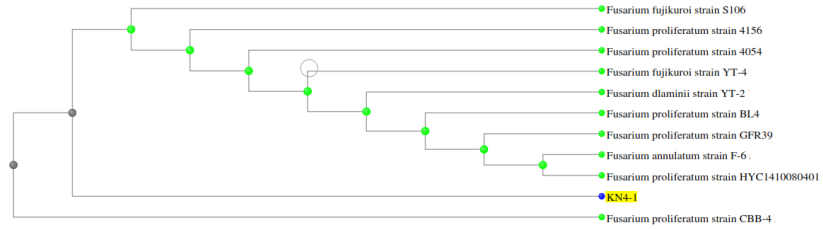

Figure 7. Phylogenetic tree of KN4-1 chaoprophilous fungal isolate

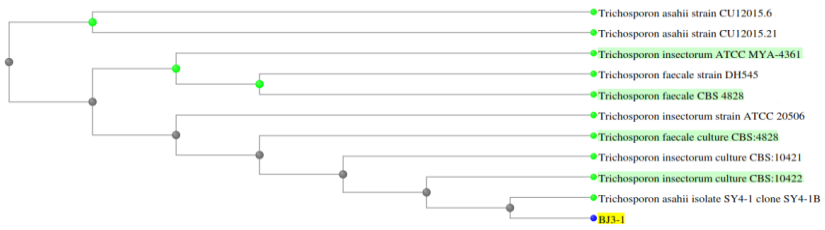

Figure 8. Phylogenetic tree of BJ3-11coprophilous fungal isolate

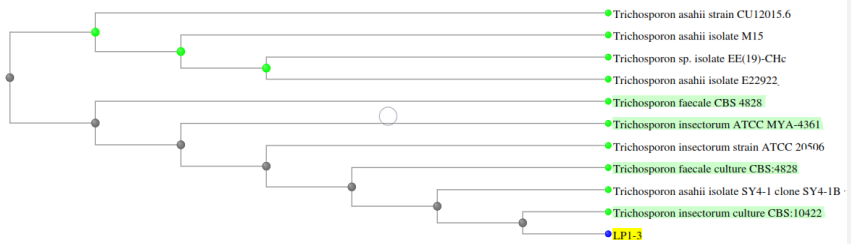

Figure 9. Phylogenetic tree of LP1-3 ~~c~~hoprophilous fungal isolate

Discussion

Based on the results of blast nucleotide analysis from the NCBI database (Table 3) and the resulting phylogenetic trees (Fig, 2 to 9.), several samples that can be determined to the species level are as follows: (1) KN1-1 sample shows identical to *Emmia lacerata* and *Ceriporia lacerata*; (2) Sample KN1-2 shows identical to *Trichosporon asahii*; (3) KN3-1 and KN3-3 samples showed identical *Lentinus squarolusus*. However, some samples that have not yet been determined to the species level are as follows: (1) the KN4-1 sample is thought to be the genus *Fusarium*. KN4-1 cannot be determined at the species level because it has similarities with several species. If necessary, the KN4-1 sample needs to be subjected to further blast nucleotide analysis using a more specific database to determine the genus *Fusarium*, such as *Fusarium* ID; (2) The KN3-2 sample is thought to be the genus *Aspergillus*, this sample cannot be determined at the species level because it has similarities with several species; (3) Samples BJ3-1 and LP3-1 are thought to be of the genus *Trichosporon*, but they cannot be determined at the species level because they are also similar to several species. Thus, if necessary, morphological analysis can also be carried out, so that this morphological data can be used to complement the obtained molecular data. The use of 16S rRNA markers in blast nucleotides from the NCBI database on samples of microorganisms such as fungi is identical (similar) at the species level, namely if the percentage identity value is above 97.5%, while at the genus level, namely if the percentage identity value is above 95% (Stackebrandt and Goebel, 1994).

Commented [UR7]: If possible, try to use morpho taxonomic key so that the authors can reach up to species level. Work carried out by the authors is really appreciable.

The presence of these coprophilous fungi on the substrate of cow dung, both at the species and genus levels, shows its adaptability to the environment with complex lignocellulosic materials. Cow dung provides a habitat for various types of organisms, including coprophilous fungi, which break down the nutrient content in it for recycling. Nutrients contained in cow dung, among others, were mentioned by Melsasail et al. (2019) namely that the contents of C, N, P and K in cow dung are: C-Organic ranging from 8.69% - 10.42%; Nitrogen (N-total) 0.68% - 0.88%; Phosphorus (P)/P_2O_5 value 0.22% - 0.34%; and potassium (K-total)/K_2O value 0.36% -0.56%.

Several genera of the fungi that can be isolated and identified molecularly in this study have never been reported as coprophilic fungi in previous studies, except for *Trichosporon* spp which was found in chicken manure (Obire et al., 2008); buffalo dung (Lorliam et al., 2013); and rhino dung (Makhuvele et al., 2017). ~~Meanwhile, other~~ Other fungi (*Fusarium fujikuroi*) were reported to be obtained from the soil as plant pathogens (Al-Ansari, 2018; Cen et al., 2020). On wood (*Ceriporia lacerata*) that was reported by Wulandari et al. (2018), ~~who~~ found that two resupinate fungal isolates in East Kalimantan which were classified as the *Ceriporia* species. *C. inflata* and *C. lacerata* were identified based on morphological characteristics and analysis of the internal transcribed spacer (ITS) and nuclear ribosomal large subunit (nL SU) data sequences; *Lentinus squarrosulus*, which is an edible fungus commonly found growing in the wild on decaying tree trunks during the rainy season. Similar to other macro fungal species, this fungus can grow in a wide variety of substrates and habitats. Many *Lentinus* species have been reported to grow in nature on special substrates and can grow on pasteurized substrates (Morais et al., 2000, Philippousis et al., 2001). Hu et al. (2013) reported the discovery of *A. allahabadii* on the rock face of Angkor Thom Cambodia temples. Microbial biofilm on the surface of the temple stone destroys the integrity of the substrate material and is a biodeteriogent that is responsible for the destruction of the temple stones from time to time.

To conclude, present investigation uncovered the existence of coprophilous microscopic fungi occurring in Banyumas Regency are as follows: (1) at the species level, the fungi identified were : *Emmia lacerata*, *Ceriporia lacerata*, *Trichosporon insectorium* and *Lentinus squarosulus*; (2) at the genus level, they wer identified as: *Fusarium* sp., *Aspergillus* sp., and *Trichosporon* sp. Further investigations are needs to to study the potential of these fungi for various human interests in various fields.

ACKNOWLEDGEMENTS

The author thanks to Rector and Head of LPPM of Universitas Jenderal Soedirman for providing permission and funding to run this research through the research scheme of Improvement of Competence 2020.

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Notifications

[biodiv] Editor Decision

2020-12-28 12:28 AM

Aris Mumpuni, Adi, Daniel:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "- DNA Barcoding of coprophilous microfungi from Banyumas, Central Java, Indonesia".

Our decision is: Revisions Required
Note: Kindly send your revised paper to professional English proofreader prior to resubmission.

Smujo Editors
editors@smujo.id

Reviewer A:

Dear authors and editor.

I've read the article carefully, and I do not find this work provies taxonomic and/or ecological data regarding coprophilous fungi to this region that deserves to be published. Firstly, the methodology for fungal isolation from dung is highly questionable, as a moist chambers where not mounted, and this resulted in a very low number of isolates. The authors also do not make it clear in the methodology how many samples were used for each one of the animals. Second, the sequencing the ITS region alone is not sufficient to delimit (resolve) all isolated species, and other loci must be considered. Yet, authors did not use morphological data, essential for the delimitation of species. Last but not least, the phylogenetic analyzes shown in this work are quite week, since the authors did not build the trees using bayesian and/or maximum parsimony analyzes including other genera and related species in the trees.

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Specific comments

Abstract: Major part of the abstract is describing background of the research theme while result obtained from the study are mentioned in the 2-3 lines. Conclusion should be added in brief

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Some of the species are unidentified because of mix result of DNA analysis, morpho-taxonomic features may be used for the further confirmation of the identity of the species, not all but at least some of the species will definitely identified. This is just suggestion, if authors wish they can because this will increase significance of the study.

Please correct the spelling of 'coprophilous', at several place 'h' needs to be deleted

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Recommendation: Revisions Required

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2. Perhaps the title of this manuscript can be changed "Molecular identification of coprophilous microfungi from Banyumas, Central Java, Indonesia".
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Molecular identification of coprophilous microfungi from Banyumas District, Central Java, Indonesia

Abstract. Coprophilous microfungi are a group of fungi that are ecologically interesting in relation to herbivores. These fungi play a predominant role in the decomposition of organic matter, in which the organic matter passes through a series of events involving mechanical degradation, as well as physical and biological processes. The role of coprophilous fungi as the main decomposers of the lignocellulosic material of herbivorous animal waste, which is widespread in nature, is very important. Previous research on the inventory and identification of coprophilous fungi in the Banyumas ~~District~~ district has been limited to macroscopic genera, so the results have not been able to provide a comprehensive picture of the presence of coprophilous fungi in the region. Identification of the types of microscopic coprophilous fungi that live in herbivorous animal waste, such as lignocellulosic material, is necessary to understand the taxonomy of these fungi. This study aimed to investigate and identify microscopic coprophilous fungi obtained in the Banyumas ~~District~~ district of Central Java, Indonesia. Based on the purposive random sampling method, the obtained fungi were analysed using the molecular methods of DNA isolation, barcoding analysis, DNA sequencing and phylogenetic analysis of fungal cultures. The following species and genera were identified: *Ceriporia lacerata*, *Trichosporon insectorium*, *Lentinus squarrosulus*, *Fusarium* sp., *Aspergillus* sp., and *Trichosporon* sp.

Keywords: molecular identification, coprophilous fungi, inventory

Running title: Molecular identification of coprophilous microfungi

INTRODUCTION

Coprophilous fungi are saprophytic fungi that live ~~in~~ on animal dung. These fungi utilize the faeces of various animals, especially herbivores, as their substrates (Melo et al., 2012). ~~Masunga et al. (2006) reported that coprophilous~~These fungi belong to the phyla Zygomycota, Ascomycota and Basidiomycota (Masunga et al. 2006). According to Krug et al. (2004), most coprophilous fungi inhabit the dung of herbivorous livestock, such as sheep and cattle. According to Sinsabaugh et al. (1981), these fungi spread cosmopolitely [widely?] wherever herbivorous animals are present and play a predominant role in the decomposition of organic matter. The organic matter is broken down by a series of events involving physical processes, such as leaching and mechanical degradation, as well as through biological processes, such as degradation by microbes involving several exoenzymes.

~~According to Mumpuni and Wahyono (2016), four~~Four genera of macroscopic coprophilous fungi, *Coprinopsis*, *Panaeolus*, *Mycena*, and *Stropharia*, were found in the coastal tourism area of Parangtritis, Yogyakarta, Indonesia (Mumpuni and Wahyono 2016). Furthermore, Mumpuni et al. (2020) reported 12 genera of macroscopic coprophilous fungi, *Panaeolus*, *Coprinopsis*, *Stropharia*, *Tricholoma*, *Lycoperdon*, *Ascobolus*, *Rhodocybe*, *Conocybe*, *Bolbitius*, *Leucocoprinus*, *Mycena*, and *Hypholoma*, in the former Banyumas ~~District~~ district (~~District:~~ Banjarnegara, Purbalingga, Banyumas~~,~~ and Cilacap). The studies on coprophilous fungi ~~obtained from those~~from the previous studies were limited to the macroscopic fungi found at the time of sampling. To obtain more comprehensive results, broader research involving the isolation of microscopic coprophilous fungi from herbivorous animal waste is needed.

Zuber et al. (2011) reported that the standard method for identifying fungal species is morphological analysis, which consists of macroscopic and microscopic observations. Macroscopic analysis consists of the determination of the colour, size and structural characteristics of the fruiting body. Further analysis of microscopic characteristics is performed mainly by comparison of spore appearance. An alternative to morphological analysis is the identification of fungal species based on phylogenetic studies. Among such studies, the DNA forensic method (Herbert et al., 2004) has been applied to evaluate polymorphisms in two noncoding polymorphic internal transcriber spacers (ITS1 and ITS2). The ITS regions are extremely useful for species identification because of their long, sequential polymorphisms. DNA sequence analysis of ITS1 and ITS2 has been successfully used for taxonomic studies of fungi (Nilson et al., 2008), and these regions are common markers used for the identification of fungal species (Lee et al., 2000). Studies have proven that the ITS region

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provides excellent results in molecular systematics down to the species level, as well as in the determination of geographical variations among species. ITS1 and ITS2 are present in multiple copies in the genome, so they can be amplified even in damaged marking material [not understood], which still gives significant results in forensic studies. Studies have evaluated the effectiveness of ITS polymorphism analysis for forensic purposes in the differentiation of psychotropic fungi of the genera *Panaeolus* and *Psilocybe*, based on the lengths of polymorphisms identified in ITS1/2 amplification products.

Use of molecular tools to complement morphological characteristics is a promising approach for rapid identification of species for reliable evaluation of biological diversity. These markers have been effectively and successfully used for the identification of fungal species since the 1990s (White et al., 1991; Burns et al., 1991). However, strategies based on sequencing of standardized genomic fragments (DNA barcoding) was recognized much later (Hollingsworth, 2007). The primary difference between molecular identification tools and the "DNA barcode" approach is that the latter involves the use of a standard DNA region that is specific for a taxonomic group. Badotti et al. (2017) suggested that one advantage of using the ITS region as a standard marker is that most fungal species have been identified based on this genomic region.

To reveal the taxonomic identity and bioprospection of coprophilous fungi, we investigated and identified microscopic coprophilous fungi obtained in the Banyumas ~~District~~ district in Central Java, Indonesia.

MATERIALS AND METHODS

Study area

~~We surveyed~~The survey of study area for the collection of the coprophilous fungi from ~~samples of~~ cow dung ~~collected from the~~was carried out in Baturraden, Kedungbanteng, and Cilongok districts (ranged between 7º03' – 7º38' South Latitude and 109º10' – 109º25' East Longitude) in the Banyumas District in Central Java, Indonesia .

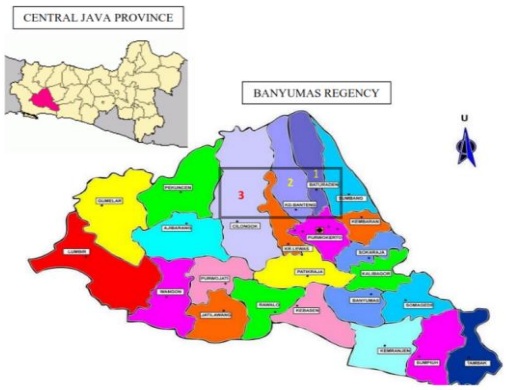

Figure 1. Sampling locations in Banyumas ~~District~~ district at districts Baturraden (1), Kedungbanteng (2), and Cilongok (3).

Sampling, isolation and purification of coprophilous fungi

~~Using a pry tool, the~~The dung samples were obtained from a maximum depth of 10 cm below the surface of a 1-month-old dung pile in a landfill with the help of a pry tool. The coprophilous fungi were isolated via a 10^{-3} to 10^{-5} ~~dilutio n~~dilution series. A drop of the diluted extract was placed on soil extract agar (glucose 1g; dipotassium phosphate 0.5g; soil extract 17.75g; agar 15g with final pH at 25°C 6.8±0.2) containing chloramphenicol and then incubated at room temperature for 3–7 days. The fungi grown on this medium were then purified by serial culture on potato dextrose agar until pure cultures were obtained. Subsequently, the purified fungi were inoculated into malt extract broth and incubated at room temperature for 15 days until the mycelia filled the Erlenmeyer flask. Mycelia were harvested via filtration and washed twice with distilled water. The wet mycelia were then either used immediately for DNA isolation or freeze-dried and stored at −20°C for later DNA isolation.

Molecular identification of coprophilous fungi

Isolation of DNA from the purified coprophilous fungal isolates was performed using the Presto™ Mini gDNA kit for yeast (Geneaid) until 100 µl of the DNA solution was obtained. DNA solutions were used immediately for PCR analysis or stored at −80°C for later analysis. The ITS locus was amplified using the primer sequences

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5'-TCCGTAGGTGAACCTGCGG-3' and 5'-TCCTCCGCTTATTGATGC-3'. The PCR mixture (25 µl total volume) consisted of 1 µl genomic DNA template, 12.5 µl 2× MyTaq Red Mix (Bioline), 1 µl each primer (20 µM/µl), and 9.5 µl double-distilled H_2O. Amplification was carried out for 35 cycles on the Applied Biosystems 96-Well GeneAmp 9700 thermal cycler using the following conditions: pre-denaturation at 95°C for 3 min, denaturation at 95°C for 10 s, annealing at 52°C for 30 s, and extension at 72°C for 45 s. The DNA amplicon was visualised using 1–2% agarose gel electrophoresis. The PCR products were purified using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research). The purified PCR products were then outsourced to PT Genetika Science Indonesia for DNA sequencing. The sequence data were submitted to GenBank (http://www.ncbi.nlm.nih.gov/) for data analysis.

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Data analysis

Electropherograms were edited manually, contigs were merged, and multiple alignments were made for all data sequences using Genetool software (Biotools Inc). The neighbour-joining distance algorithm with the Kimura2 parameter model using PAUP (v.4.0b10) (Swofford, 2000) was used for phylogenetic analysis. Heuristic analysis using parsimony was also performed.

RESULTS AND DISCUSSION

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Results

~~In this investigation,~~ Total16 samples of coprophilous fungal isolates exhibiting different somatic phase characteristics were obtained (Fig. 1). The fungal isolates were purified and subjected to DNA extraction.

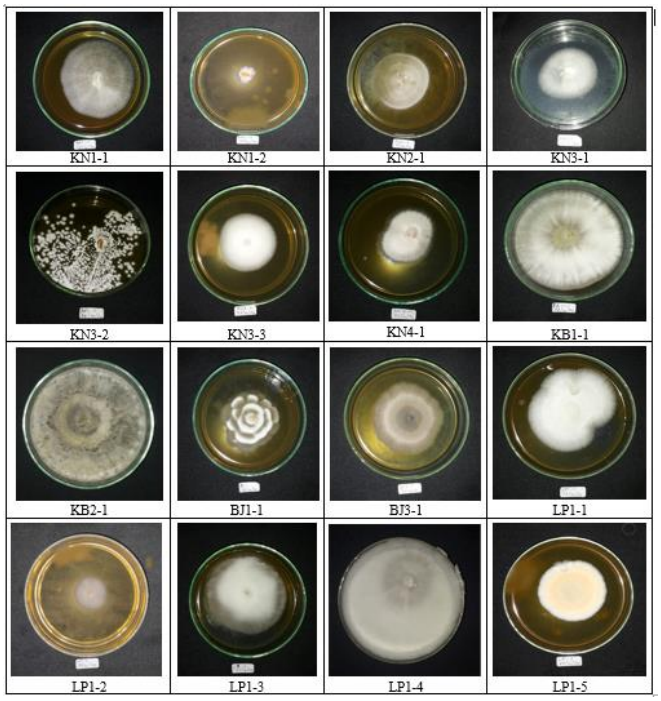

Figure 1. Five-day-old cultures of coprophilous fungal isolates from Banyumas ~~District~~district, Central Java, Indonesia.

Table 1 shows the of genomic DNA quantification results for DNA extracts from the coprophilous fungal isolates. The purity of each DNA extract was determined according to the 260/280 nm absorbance ratio. A ratio of 1.8 (viz. [not understood], samples KB2-1, LP1-1, and LP4-1) indicated a pure sample free of RNA and protein contamination; a ratio greater than 1.8 (viz., KN1-1, KN1-2, KN2-1, KN3-1, KN3-2, KN4-1, KB1-1, BJ1-1, BJ3-1, LP1-2, LP1- 3, and

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LP1-5) ~~indicates~~ indicated possible RNA contamination; while, ~~and~~ a ratio less than 1.8 (viz., KN3-3) ~~indicates~~ indicated possible protein contamination (Sambrook & Russel, 2001). Several isolates (viz., KN1-1, KN3-1, KN3-3, and LP1-2) had concentrations substantially less than 20 ng/µl, which was not optimal for spectrophotometric analysis; however, in general the DNA of these isolates exhibited reasonably good purity.

Table 1. Fungal genomic DNA quantification

No.	Nama Sample	Conc. (ng/µl)	$A_{260/280}$	$A_{260/230}$	Volume (µl)
1	KN1-1	14.2	1.98	0.30	40
2	KN1-2	31.6	1.98	0.14	40
3	KN2-1	29.0	1.93	0.41	40
4	KN3-1	9.3	2.02	0.14	40
5	KN3-2	22.3	1.90	0.17	40
6	KN3-3	9.6	1.65	0.39	40
7	KN4-1	22.3	1.90	0.17	40
8	KB1-1	18.0	2.01	0.19	40
9	KB2-1	96.9	1.89	0.82	40
10	BJ1-1	18.0	1.94	0.12	40
11	BJ3-1	26.7	1.94	0.11	40
12	LP1-1	23.1	1.89	0.04	40
13	LP1-2	11.7	1.98	0.11	40
14	LP1-3	24.5	1.92	0.28	40
15	LP1-4	21.1	1.86	0.27	40
16	LP1-5	55.5	1.93	0.58	40

We also measured the 260/230 absorbance ratio. According to Boyer (2005), a ratio ranging from 2.0 to 2.2 indicates a lack of polysaccharide contamination. The relatively low 260/230 ratios observed in our samples suggested possible contamination with carbohydrates, organic matter, or other chemicals.

Figure 2 shows DNA amplification of the ITS gene locus from coprophilous fungal samples. Of the 16 samples of coprophilic fungi isolated from cow dung, only 9 (KN1-1, KN1-2, KN3-1, KN3-2, KN3-3, KN4-1, KB1-1, BJ3-1, and LP1-3) showed optimal DNA amplification, as evidenced by a specific, single, thick DNA band, which indicates optimal quantity and purity of the extracted genomic DNA (Sambrook & Russel, 2001). According to Agrawal (2008), the purity of the DNA sample can affect the PCR results. Consequently, DNA sequencing was performed in these nine samples (Table 2).

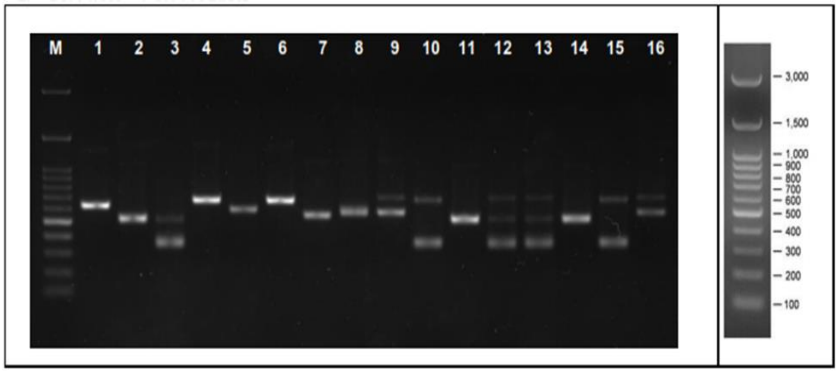

Figure 2. Amplified ITS gene loci from coprophilous fungal samples. Well "M", DNA ladder 100 bp; wells 1–16, coprophilous fungal DNA samples

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Table 2. DNA sequence assemblies of PCR-amplified noncoding polymorphic internal transcriber spacers from coprophilous fungal samples.

No	Sample Name	Sequences
1.	KN1-1	**Sequence Assembly 636bp** 1 TGAACCTGCG GAAGGATCAT TATCGAGTTT TGAACGGGTT GTAGCTGGCC TTTAACGAGG 61 TATGTGCACG CCTGGCTCAT CCACTCTCAA CCTCTGTGCA CTTTATGTAA GAAACGGTGT 121 AAGCCAGCTA TTTAATAGTC GGTAATAAGC CTTTCTTATG TTTACTACAA ACGCTTCAGT 181 TATAGAATGT TTACTGTGTA TAACACAATT ATATACAACT TTCAGCAACG GATCTCTTGG 241 CTCTCGCATC GATGAAGAAC GCAGCGAAAT GCGATAAGTA ATGTGAATTG CAGAATTCAG 301 TGAATCATCG AATCTTTGAA CGCACCTTGC ACTCCTTGGT ATTCCGAGGA GTATGCCTGT 361 TTGAGTCTCA TGGAATTCTC AACCCCTAAA TTTTGTAATG AAGTTTAGTG GGCTTGGACT 421 TGGAGGTTGT GTCGGCTTCT AGTCGACTCC TCTGAAATGT ATTAGCGTGA ATCTTACGGA 481 TCGCCTTCAG TGTGATAATT ATCTGCGCTG TGGTGTTGAA GTATTTATTA GTTCATGCTT 541 ATAGTCGTCT CTTACCGAGA CAATTTATGA CAATCTGAGC TCAAATCAGG TAGGACTACC 601 CGCTGAACTT AAGCATATCA ATAAGCGGGA GGAAGG
2.	KN1-2	**Sequence Assembly 533bp** 1 TAGGTGAACC TGCGGAAGGA TCATTAGTGA TTGCCTTTAT AGGCTTATAA CTATATCCAC 61 TTACACCTGT GAACTGTTCT ACTACTTGAC GCAAGTCGAG TATTTTTACA AACAATGTGT 121 AATGAACGTC GTTTTATTAT AACAAAATAA AACTTTCAAC AACGGATCTC TTGGCTCTCG 181 CATCGATGAA GAACGCAGCG AATTGCGATA AGTAATGTGA ATTGCAGAAT TCAGTGAATC 241 ATCGAATCTT TGAACGCAGC TTGCGCTCTC TGGTATTCCG GAGAGCATGC CTGTTTCAGT 301 GTCATGAAAT CTCAACCACT AGGGTTTCCT AATGGATTGG ATTTGGGCGT CTGCGATTTC 361 TGATCGCTCG CCTTAAAAGA GTTAGCAAGT TTGACATTAA TGTCTGGTGT AATAAGTTTC 421 ACTGGGTCCA TTGTGTTGAA GCGTGCTTCT AATCGTCCGC AAGGACAATT ACTTTGACTC 481 TGGCCTGAAA TCAGGTAGGA CTACCCGCTG AACTTAAGCA TATCAATAAG CGG
3.	KN3-1	**Sequence Assembly 647bp** 1 AGGATCATTA TCGAGTTTTG AAACGGGTTG TAGCTGGCCT TCCGAGGCAT GTGCACGCCC 61 TGCTCATCCA CTCTACACCT GTGCACTTAC TGTGGGTTTC AGGAGCTTCG AAAGCGAGAA 121 AAGGGGCCTT CACGGGCTTT TTTCTTGCCT AGTTGTTACT GGGCCTACGT TTCACTACAA 181 ACACTTATAA AGTATCAGAA TGTGTATTGC GATGTAACGC ATCTATATAC AACTTTCAGC 241 AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA 301 ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC TTGCGCTCCT TGGTATTCCG 361 AGGAGCATGC CTGTTTGAGT GTCATGAAAT TCTCAACCTA ACGGGTTCTT AACGGGACTT 421 GCTTTAGGCT TGGACTTGGA GGTTCTTGTC GGCTTGCTTC AATGTCAAGT CGGCTCCTCT 481 TAAATGCATT AGCTTGGTTC CTGTGCGGAT CGGCTCACGG TGTGATAATT GTCTACGCCG 541 CGACCGTTGA AGCGTTTTTA TAGGCCAGCT TCTAGTCGTC TCTTTACGAG ACAATAATCA 601 TCGAACTCTG ACCTCAAATC AGGTAGGACT ACCCGCTGAA CTTAAGC
4.	KN3-2	**Sequence Assembly 584bp** 1 AGGTGAACCT GCGGAAGGAT CATTACCGAG TGCGGGTCCG CGTGGCCCAA CCTCCCACCC 61 GTGCCTATTG TACCCTGTTG CTTCGGCGGG CCCGCCAGCC TTCGGGCTGG CCGCCGGGGG 121 GCGTCTCGCC CCCGGGCCCG TGCCCGCCGG AGACCCCAAC ATGAACCCTG TTCTGAAAGC 181 TTGGTGTCTG AGTGTGATTG TTTGCAATCA GTTAAAACTT TCAACAATGG ATCTCTTGGT 241 TCCGGCATCG ATGAAGAACG CAGCGAAATG CGATAACTAA TGTGAATTGC AGAATTCAGT 301 GAATCATCGA GTCTTTGAAC GCACATTGCG CCCCCTGGTA TTCCGGGGGG CATGCCTGTC 361 CGAGCGTCAT TGCTGCCCTC AAGCCCGGCT TGTGTGTTGG GCCCTCGTCC CCCGGCTCCC 421 GGGGGACGGG CCCGAAAGGC AGCGGCGGCA CCGCGTCCGG TCCTCGAGCG TATGGGGCTT 481 TGTCTTCCGC TCTGCAGGCC CGGCCGGCGC CCGCCGACGC ATAACAACTT TTTTTCCAGG 541 TTGACCTCGG ATCAGGTAGG GATACCCGCT GAACTTAAGC ATAT
5.	KN3-3	**Sequence Assembly 670bp** 1 AACCTGCGGA AGGATCATTA TCGAGTTTTG AAACGGGTTG TAGCTGGCCT TCCGAGGCAT 61 GTGCACGCCC TGCTCATCCA CTCTACACCT GTGCACTTAC TGTGGGTTTC AGGAGCTTCG 121 AAAGCGAGAA AAGGGGCCTT CACGGGCTTT TTTCTTGCCT AGTTGTTACT GGGCCTACGT 181 TTCACTACAA ACACTTATAA AGTATCAGAA TGTGTATTGC GATGTAACGC ATCTATATAC 241 AACTTTCAGC AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGATA 301 AGTAATGTGA ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC TTGCGCTCCT 361 TGGTATTCCG AGGAGCATGC CTGTTTGAGT GTCATGAAAT TCTCAACCTA ACGGGTTCTT 421 AACGGGACTT GCTTTAGGCT TGGACTTGGA GGTTCTTGTC GGCTTGCTTC AATGTCAAGT 481 CGGCTCCTCT TAAATGCATT AGCTTGGTTC CTGTGCGGAT CGGCTCACGG TGTGATAATT 541 GTCTACGCCG CGACCGTTGA AGCGTTTTTA TAGGCCAGCT TCTAGTCGTC TCTTTACGAG 601 ACAATAATCA TCGAACTCTG ACCTCAAATC AGGTAGGACT ACCCGCTGAA CTTAAGCATA 661 TCAATAAGGC
6.	KN4-1	**Sequence Assembly 522bp** 1 AGGGATCATT ACCGAGTTTA CAACTCCCAA ACCCCTGTGA ACATACCAAT TGTTGCCTCG 61 GCGGATCAGC CCGCTCCCGG TAAAACGGGA CGGCCCGCCA GAGGACCCCT AAACTCTGTT 121 TCTATATGTA ACTTCTGAGT AAAACCATAA ATAAATCAAA ACTTTCAACA ACGGATCTCT 181 TGGTTCTGGC ATCGATGAAG AACGCAGCAA AATGCGATAA GTAATGTGAA TTGCAGAATT 241 CAGTGAATCA TCGAATCTTT GAACGCACAT TGCGCCCGCC AGTATTCTGG CGGGCATGCC 301 TGTTCGAGCG TCATTTCAAC CCTCAAGCCC CCGGGTTTGG TGTTGGGGAT CGGCGAGCCC 361 TTGCGGCAAG CCGGCCCCGA AATCTAGTGG CGGTCTCGCT GCAGCTTCCA TTGCGTAGTA 421 GTAAAACCCT CGCAACTGGT ACGCGGCGCG GCCAAGCCGT TAAACCCCCA ACTTCTGAAT 481 GTTGACCTCG GATCAGGTAG GAATACCCGC TGAACTTAAG CA
7.	KB1-1	**Repeat Sequencing Process**
8.	BJ3-1	**Sequence Assembly 516bp** 1 TGATATGCTT AAGTTCAGCG GGTAGTCCTA CCTGATTTCA GGCCAGAGTC AAAGTAATTG 61 TCCTTGCGGA CGATTAGAAG CACGCTTCAA CACAATGGAC CCAGTGAAAC TTATTACACC 121 AGACATTAAT GTCAAACTTG CTAACTCTTT TAAGGCGAGC GATCAGAGAT CGCAGACGCC 181 CAAATCCAAT CCATTAGGAA ACCCTAGTGG TTGAGATTTC ATGACACTGA AACAGGCATG 241 CTCTCCGGAA TACCAGAGAG CGCAAGCTGC GTTCAAAGAT TCGATGATTC ACTGAATTCT 301 GCAATTCACA TTACTTATCG CAATTCGCTG CGTTCTTCAT CGATGCGAGA GCCAAGAGAT 361 CCGTTGTTGA AAGTTTTATT TTGTTATAAT AAAACGACGT TCATTACACA TTGTTTGTAA 421 AAATACTCGA CTTGCGTCAA GTAGTAGAAC AGTTCACAGG TGTAAGTGGA TATAGTTATA 481 AGCCTATAAA GGCAATCACT AATGATCCTT CCGCAG
9.	LP1-3	**Sequence Assembly 539bp** 1 TCCGTAGGTG AACCTGCGGA AGGATCATTA GTGATTGCCT TTATAGGCTT ATAACTATAT 61 CCACTTACAC CTGTGAACTG TTCTACTACT TGACGCAAGT CGAGTATTTT TACAAACAAT 121 GTGTAATGAA CGTCGTTTTA TTATAACAAA ATAAAACTTT CAACAACGGA TCTCTTGGCT 181 CTCGCATCGA TGAAGAACGC AGCGAATTGC GATAAGTAAT GTGAATTGCA GAATTCAGTG 241 AATCATCGAA TCTTTGAACG CAGCTTGCGC TCTCTGGTAT TCCGGAGAGC ATGCCTGTTT 301 CAGTGTCATG AAATCTCAAC CACTAGGGTT TCCTAATGGA TTGGATTTGG GCGTCTGCGA 361 TCTCTGATCG CTCGCCTTAA AAGAGTTAGC AAGTTTGACA TTAATGTCTG GTGTAATAAG 421 TTTCACTGGG TCCATTGTGT TGAAGCGTGC TTCTAATCGT CCGCAAGGAC AATTACTTTG 481 ACTCTGGCCT GAAATCAGGT AGGACTACCC GCTGAACTTA AGCATATCAA TAAGCGGAG

The DNA sequencing results of the nine selected samples are shown in Table 2. All but one (KB1-1) of the samples

exhibited good purity. According to Bruce et al. (2002), factors affecting DNA sequencing results include the denaturation, annealing and extension temperatures and the degree of DNA molecule separation during the purification and precipitation steps.

The results of nucleotide BLAST searches against the NCBI database are shown in Table 3. The samples KN1-1, KN1-2. KN3-1, KN3-2, KN3-3, BJ3-1, and LP1-3 exhibited consistent BLAST hits from one or two specific species; any differences were in the homotypic synonym, taxon synonym, or obligate synonym of the current name of the species.

Table 3. Results of nucleotide BLAST searches against the NCBI database.

No.	Samples	Result Links Description	Max Score	Total score	Query cover	E value	Per Ident
1	KN1-1	*Emmia lacerata* isolate A01	1136	1136	99%	0.0	99.84%
		Ceriporia lecerata isolate A1S5-D23	1135	1135	100%	0.0	99.69%
		Ceriporia lacerata isolate BPEF81	1123	1123	99%	0.0	99.52%
		Ceriporia lacerata isolate WS1JB14	1121	1121	97%	0.0	100.00%
		Ceriporia lacerata isolate X12	1118	1118	99%	0.0	99.21%
		Emmia lacerata MYA 12S07	1116	1116	99%	0.0	99.21%
		Emmia sp. strain Cef 13	1116	1116	99%	0.0	99.21%
		Ceriporia lacerata isolate CIFE 29	1116	1116	98%	0.0	99.52%
		Basidiomycota sp. SYBC-L17	1116	1116	99%	0.0	99.21%
		Ceriporia lacerata genes for 18S	1116	1116	99%	0.0	99.21%
		http://www.ncbi.nlm.nih.gov/nuccore/MH734799.1,KJ780757.1,KF151851.1,KT844687.1,KF850375.1,LC431580.1,MK775821.1,KM388611.1,HQ891300.1,LC312413.1					
2	KN1-2	*Trichosporon asahii* strain CU12015 6	962	962	100%	0.0	100%
		Trichosporon asahii isolate M15	962	962	100%	0.0	100%
		Trichosporon sp. isolate ~~EE~~(EE (19)-CHc	962	962	100%	0.0	100%
		Trichosporon asahii isolate E22922	962	962	100%	0.0	100%
		Trichosporon asahii strain DMic 165073	962	962	100%	0.0	100%
		Trichosporon asahii culture CBS 2497	962	962	100%	0.0	100%
		Trichosporon asahii strain V9	962	962	100%	0.0	100%
		Trichosporon asahii strain 18S	962	962	100%	0.0	100%
		Trichosporon asahii strain APMSU6	962	962	100%	0.0	100%
		Trichosporon asahii strain YCH116	962	962	100%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/MT482659.1,MT136544.1,MK267768.1,MG241533.1,KY105711.1,KT900123.1,KT900118.1,KT282395.1,KM982986.1					
3	KN3-1	*Lentinus squarrosulus* isolate TAM1004	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher WARRIPt	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher WARRI34	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher UNIP13	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher Odi26	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher IBD43	1168	1168	100%	0.0	100%
		Lentinus sp. BAB5060	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher BORH0009	1162	1162	99%	0.0	100%
		Lentinus squarrosulus small subunit ribosomal	1159	1159	100%	0.0	99.85%
		Lentinus squarrosulus strain WCR1201	1155	1155	100%	0.0	99.69%
		http://www.ncbi.nlm.nih.gov/nuccore/MH172168.1,KT273380.1,KT273379.1,KT273370.1,KT273364.1,KR155105.1,MH053154.1,KT956127.1					
4	KN3-2	*Aspergillus allahabadii* strain CGMC 3 03920	1054	1054	100%	0.0	100%
		Aspergillus allahabadii strain CGMC 3 02584	1054	1054	100%	0.0	100%
		Aspergillus allahabadii genes for 18S rRNA	1054	1054	100%	0.0	100%
		Aspergillus candidus isolate CY104	1054	1054	100%	0.0	100%
		Aspergillus allahabadii strain CMV004E2	1049	1049	100%	0.0	99.83%
		Aspergillus allahabadii strain CGMCC 3 01332	1049	1049	100%	0.0	99.83%
		Aspergillus niveus strain URM7046	1048	1048	99%	0.0	99.83%
		Aspergillus niveus strain CBS 132162	1045	1045	100%	0.0	99.66%
		Aspergillus allahabadii strain NN046949	1043	1043	98%	0.0	100%
		Aspergillus niveus strain NN043511	1043	1043	98%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/MH292843.1,MH292842.1,LC152416.1,HQ607958.1,MK450628.1,MH292844.1,KM613137.1,MH865978.1,KX443215.1,KX443211.1					
5	KN3-3	*Lentinus* sp. BAB-5060	1205	1205	99%	0.0	100%
		Lentinus squarrosulus voucher WARRIPt	1196	1196	98%	0.0	100%
		Lentinus squarrosulus voucher Odi26	1196	1196	98%	0.0	100%
		Lentinus squarrosulus strain WCR1201	1193	1193	99%	0.0	99.70%
		Lentinus squarrosulus voucher UNIP13	1191	1191	98%	0.0	100%
		Lentinus squarrosulus voucher WARRI34	1189	1189	98%	0.0	100%

Commented [AKG9]: If it denotes samples, then why KN1-1, KN1-2, etc. are presented singly.

		Lentinus squarrosulus IBD43	1189	1189	98%	0.0	100%
		Lentinus sp. S5	1188	1188	99%	0.0	99.55%
		Lentinus squarrosulus small subunit	1185	1185	98%	0.0	99.85%
		Lentinus squarrosulus voucher BORH0009	1180	1180	97%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/KR155105.1,KT273380.1,KT273370.1,KT956127.1,KT273373.1, KT273379.1,KT273364.1,JN253598.1,MH053154.1,KP283484.1					
6	KN4-1	*Fusarium proliferatum* strain CBB-4	942	942	100%	0.0	100%
		Fusarium fujikuroi strain S106	942	942	100%	0.0	100%
		Fusarium proliferatum strain 4156	942	942	100%	0.0	100%
		Fusarium proliferatum strain 4054	942	942	100%	0.0	100%
		Fusarium fujikuroi strainYT-4	942	942	100%	0.0	100%
		Fusarium diaminii strain YT-2	942	942	100%	0.0	100%
		Fusarium proliferatum strain BL4	942	942	100%	0.0	100%
		Fusarium proliferatum strain GFR39	942	942	100%	0.0	100%
		Fusarium annulatum strain F-6	942	942	100%	0.0	100%
		Fusarium proliferatum strain HYC1410080401	942	942	100%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/MT560212.1,MT549849.1,MN817705.1,MN817704.1,MT477707.1,MT477704.1,MT466521.1,MT447544.1,MT434005.1,MT378328.1					
7	BJ3-1	*Trichosporon asahii* isolate SY4-1 clone SY4-1B	931	931	100%	0.0	100%
		Trichosporon insectorium culture CBS 10422	931	931	100%	0.0	100%
		Trichosporon insectorium culture CBS 10421	931	931	100%	0.0	100%
		Trichosporon faecale culture CBS 4828	931	931	100%	0.0	100%
		Trichosporon insectorium strain ATCC 20506	931	931	100%	0.0	100%
		Trichosporon insectorium ATCCMYA-4361	931	931	100%	0.0	100%
		Trichosporon faecale strain DH545	931	931	100%	0.0	100%
		Trichosporon faecale CBS 4828	931	931	100%	0.0	100%
		Trichosporon asahii strain CU12015 6	927	927	100%	0.0	99.81%
		Trichosporon asahii strain CU12015 21	927	927	100%	0.0	99.81%
		http://www.ncbi.nlm.nih.gov/nuccore/KY963115.1,KY105746.1,KY105745.1,KY105736.1,HM802133.1, NR 111353.1,EF153624.1,NR 073242.1,MT482659.1,MT482658.1					
8	LP1-3	*Trichosporon asahii* isolate SY4-1 clone SY4	973	973	100%	0.0	100%
		Trichosporon faecale culture CBS 4826	973	973	100%	0.0	100%
		Trichosporon insectorium strain ATCC 20506	973	973	100%	0.0	100%
		Trichosporon insectorium ATCC MYA-4361	973	973	100%	0.0	100%
		Trichosporon faecale CBS 4828	073	073	100%	0.0	100%
		Trichosporon insectorium culture CBS 10422	971	971	99%	0.0	100%
		Trichosporon asahii strain CU12015 6	968	968	100%	0.0	99.81%
		Trichosporon asahii isolate M15	968	968	100%	0.0	99.81%
		Trichosporon sp. isolate ~~EF~~(EE (19)-CHc	968	968	100%	0.0	99.81%
		Trichosporon asahii isolate E22922	968	968	100%	0.0	99.81%
		http://www.ncbi.nlm.nih.gov/nuccore/KY963115.1,KY105736.1,HM802133.1,NR 111353.1,NR073242.1,KY105746.1,MT482659.1,MT136544.1,MK605936.1,MK267768.1					

The BLAST search results are displayed in phylogenetic trees (Figs. 2–9).

Commented [AKG10]: In my opinion, authors should go for some clustered phylogenies instead of providing separate for each sample. Moreover, no outgroup selected in phylogenies.

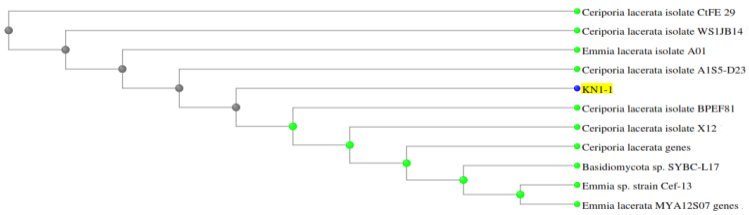

Figure 2. Phylogenetic tree for the KN1-1 coprophilous fungal isolate

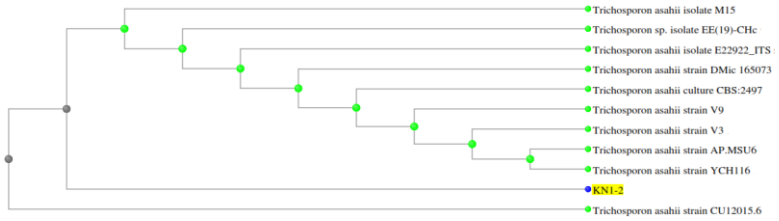

Figure 3. Phylogenetic tree for the KN1-2 coprophilous fungal isolate

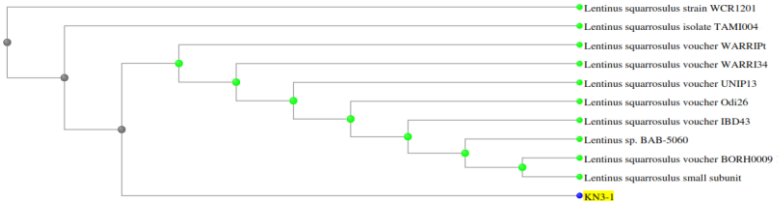

Figure 4. Phylogenetic tree for the KN3-1 coprophilous fungal isolate

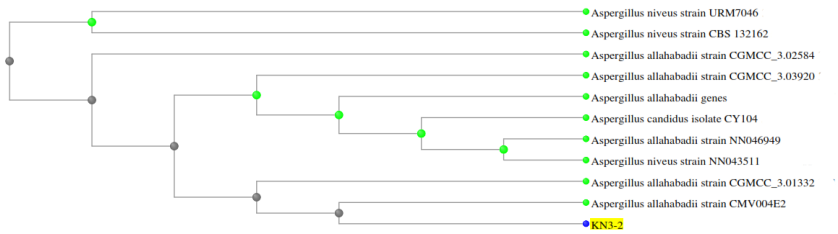

Figure 5. Phylogenetic tree for the KN3-2 coprophilous fungal isolate

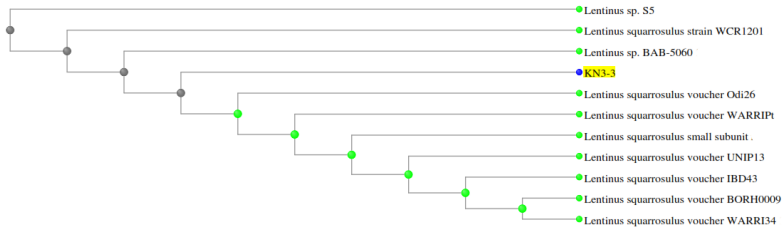

Figure 6. Phylogenetic tree for the KN3-3 coprophilous fungal isolate

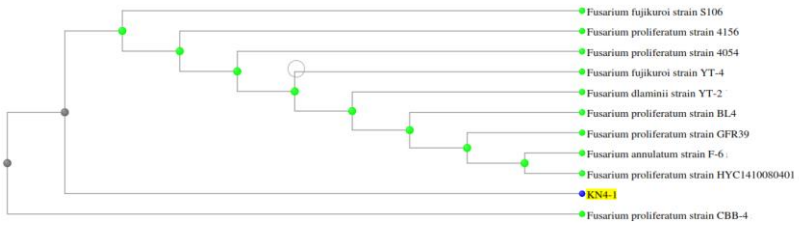

Figure 7. Phylogenetic tree for the KN4-1 coprophilous fungal isolate

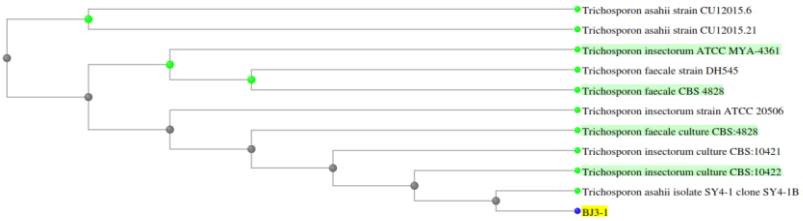

Figure 8. Phylogenetic tree for the BJ3-11coprophilous fungal isolate

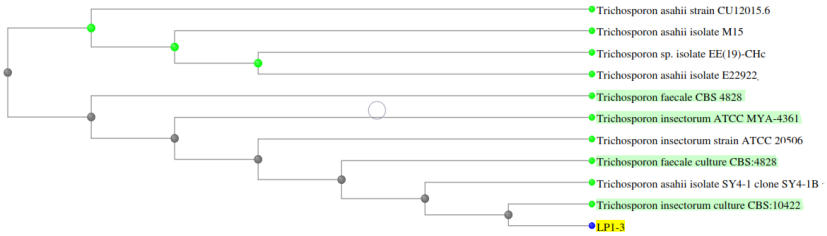

Figure 9. Phylogenetic tree for the LP1-3 coprophilous fungal isolate

Discussion

Based on the nucleotide BLAST searches (Table 3) and the resulting phylogenetic trees (Figs. 2–9), several of the coprophilous fungal samples could be identified at the species level. These samples were (1) KN1-1, identical to *Ceriporia lacerata*; (2) KN1-2, identical to *Trichosporon asahii*; and (3) KN3-1 and KN3-3, identical to *Lentinus squarrosulus*. Samples that could not be identified at the species level because they exhibit similarities with several species within a genus were (1) KN4-1, which probably belongs to the genus *Fusarium*; (2) KN3-2, which probably belongs to the genus *Aspergillus*; and (3) BJ3-1 and LP3-1, which probably belong to the genus *Trichosporon*. Further nucleotide BLAST searches against a more specific database, such as *Fusarium* ID, are needed for the KN4-1 sample (most likely *Fusarium*). Furthermore, morphological analyses can be performed to complement the obtained molecular data. The 16S rRNA markers of microorganisms such as fungi tend to be very similar or identical at the species level when the identity exceeds 97.5%, whereas the identity threshold is 95% at the genus level (Stackebrandt and Goebel, 1994).

The presence of these coprophilous fungi in cow dung demonstrates their adaptability to complex lignocellulosic materials. Cow dung provides a habitat for various types of organisms, including coprophilous fungi, which break down the nutrient content for recycling. The nutrients in cow dung include organic carbon (8.69–10.42%), total nitrogen (0.68–0.88%), phosphorus as (P)/P_2O_5 (0.22–0.34%), and potassium as (total K)/K_2O (0.36–0.56%) (Melsasail et al., 2019).

Commented [AKG11]: Add as results.

The fungal genera isolated and identified in this study have never been reported as being coprophilic, except for *Trichosporon* spp., which has been found in chicken manure (Obire et al., 2008), buffalo dung (Lorliam et al., 2013), and rhino dung (Makhuvele et al., 2017). *Fusarium* comprises soil-borne plant pathogenic species (e.g., *F. fujikuroi*) (Al-Ansari, 2018; Cen et al., 2020). *Ceriporia lacerate* grows on wood; Wulandari et al. (2018), found two resupinate fungal specimens in East Kalimantan classified as *Ceriporia* species, *C. inflata* and *C. lacerata*, which were identified based on morphological characteristics and the ITS and nuclear ribosomal large subunit sequences. *L. squarrosulus* is an edible fungus commonly found growing in the wild on decaying tree trunks during the rainy season. Similar to other macrofungal species, this fungus can grow in a wide variety of substrates and habitats. Many *Lentinus* species have been reported to grow in nature on special substrates as well as on pasteurized substrates (Morais et al., 2000, Philippousis et al., 2001). Hu et al. (2013) discovered *Aspergillis allahabadii* growing on the rock faces of Angkor Thom Cambodia temples. Microbial biofilms on the surface of the temple stone destroys the integrity of the substrate material and is a biodeteriogen responsible for the destruction of the temple stones over time.

To conclude, we have uncovered the existence of coprophilous microscopic fungi occurring in Banyumas District in Central Java, Indonesia. At the species level, we identified *C. lacerata*, *Trichosporon insectorium*, and *L. squarrosulus*. At the genus level, we identified *Fusarium* sp., *Aspergillus* sp., and *Trichosporon* sp. Further investigations are needed to identify the fungi morphologically and to evaluate the utility of these fungi for various human interests.

ACKNOWLEDGEMENTS

The author thanks Rector and Head of LPPM of Universitas Jenderal Soedirman, for providing the permission and funding to undertake this research through the research scheme of Improvement of Competence 2020.

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Molecular identification of coprophilous microfungi from Banyumas ~~District~~Regency, Central Java, Indonesia

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Manuscript received: xxx. Revision accepted: xxx December 2020.

Abstract. Coprophilous microfungi are a group of fungi that are ecologically interesting in relation to herbivores. These fungi play a predominant role in the decomposition of organic matter, in which the organic matter passes through a series of events involving mechanical degradation, as well as physical and biological processes. The role of coprophilous fungi as the main decomposers of the lignocellulosic material of herbivorous animal waste, which is widespread in nature, is very important. Previous research on the inventory and identification of coprophilous fungi in the Banyumas ~~District~~ regency~~district~~ has been limited to macroscopic genera, so the results have not been able to provide a comprehensive picture of the presence of coprophilous fungi in the region. Identification of the types of microscopic coprophilous fungi that live in herbivorous animal waste, such as lignocellulosic material, is necessary to understand the taxonomy of these fungi. This study aimed to investigate and identify microscopic coprophilous fungi obtained in the Banyumas ~~District~~ regency~~district~~ of Central Java, Indonesia. Based on the purposive random sampling method, the obtained fungi were analysed using the molecular methods of DNA isolation, gene amplification~~barcoding analysis~~, DNA sequencing and phylogenetic analysis of fungal cultures. The following species and genera were identified: *Ceriporia lacerata*, *Trichosporon insectorium*, *Lentinus squarrosulus*, *Fusarium* sp., *Aspergillus* sp., and *Trichosporon* sp.

Keywords: molecular identification, coprophilous fungi, inventory

Running title: Molecular identification of coprophilous microfungi

INTRODUCTION

Coprophilous fungi are saprophytic fungi that live ~~in~~ on animal dung. These fungi utilize the faeces of various animals, especially herbivores, as their substrates (Melo et al., 2012). ~~Masunga et al. (2006) reported that coprophilous~~These fungi belong to the phyla Zygomycota, Ascomycota and Basidiomycota (Masunga et al. 2006). According to Krug et al. (2004), most coprophilous fungi inhabit the dung of herbivorous livestock, such as sheep and cattle. According to Sinsabaugh et al. (1981), these fungi spread ~~cosmopolitely~~ widely ~~[widely?]~~ wherever herbivorous animals are present and play a predominant role in the decomposition of organic matter. The organic matter is broken down by a series of events involving physical processes, such as leaching and mechanical degradation, as well as through biological processes, such as degradation by microbes involving several exoenzymes.

~~According to Mumpuni and Wahyono (2016), four~~Four genera of macroscopic coprophilous fungi, *Coprinopsis*, *Panaeolus*, *Mycena*, and *Stropharia*, were found in the coastal tourism area of Parangtritis, Yogyakarta, Indonesia (Mumpuni and Wahyono 2016). Furthermore, Mumpuni et al. (2020) reported 12 genera of macroscopic coprophilous fungi, *Panaeolus*, *Coprinopsis*, *Stropharia*, *Tricholoma*, *Lycoperdon*, *Ascobolus*, *Rhodocybe*, *Conocybe*, *Bolbitius*, *Leucocoprinus*, *Mycena*, and *Hypholoma*, in the former Banyumas ~~District~~ residence~~district~~ (~~District:~~ regencies of Banjarnegara, Purbalingga, Banyumas~~,~~ and Cilacap). The studies on coprophilous fungi ~~obtained from those~~from the previous studies were limited to the macroscopic fungi found at the time of sampling. To obtain more comprehensive results, broader research involving the isolation of microscopic coprophilous fungi from herbivorous animal waste is needed.

Zuber et al. (2011) reported that the standard method for identifying fungal species is morphological analysis, which consists of macroscopic and microscopic observations. Macroscopic analysis consists of the determination of the colour, size and structural characteristics of the fruiting body. Further analysis of microscopic characteristics is performed mainly by comparison of spore appearance. An alternative to morphological analysis is the identification of fungal species based on phylogenetic studies. Among such studies, the DNA forensic method (Herbert et al., 2004) has been applied to evaluate polymorphisms in two noncoding polymorphic internal transcriber spacers (ITS1 and ITS2). The ITS regions are extremely useful for species identification because of their long, sequential polymorphisms. DNA sequence analysis of ITS1 and ITS2 has been successfully used for taxonomic studies of fungi (Nilson et al., 2008), and these regions are common markers used for the identification of fungal species (Lee et al., 2000). Studies have proven that the ITS region

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provides excellent results in molecular systematics down to the species level, as well as in the determination of geographical variations among species. ~~ITS1 and ITS2 are present in multiple copies in the genome, so they can be amplified even in damaged marking material [not understood], which still gives significant results in forensic studies.~~ Studies have evaluated the effectiveness of ITS polymorphism analysis for forensic purposes in the differentiation of psychotropic fungi of the genera *Panaeolus* and *Psilocybe*, based on the lengths of polymorphisms identified in ITS1/2 amplification products.

Use of molecular tools to complement morphological characteristics is a promising approach for rapid identification of species for reliable evaluation of biological diversity. These markers have been effectively and successfully used for the identification of fungal species since the 1990s (White et al., 1991; Burns et al., 1991). However, strategies based on sequencing of standardized genomic fragments (DNA barcoding) was recognized much later (Hollingsworth, 2007). The primary difference between molecular identification tools and the "DNA barcode" approach is that the latter involves the use of a standard DNA region that is specific for a taxonomic group. Badotti et al. (2017) suggested that one advantage of using the ITS region as a standard marker is that most fungal species have been identified based on this genomic region.

To reveal the taxonomic identity and bioprospection of coprophilous fungi, we investigated and identified microscopic coprophilous fungi obtained in the Banyumas ~~District~~ ~~district~~regency in Central Java, Indonesia.

MATERIALS AND METHODS

Study area

~~We surveyed~~The survey of study area for the collection of the coprophilous fungi from ~~samples of~~ cow dung ~~collected from the~~was carried out in Baturraden, Kedungbanteng, and Cilongok districts (ranged between 7º03' – 7º38' South Latitude and 109º10' – 109º25' East Longitude) in the Banyumas District in Central Java, Indonesia .

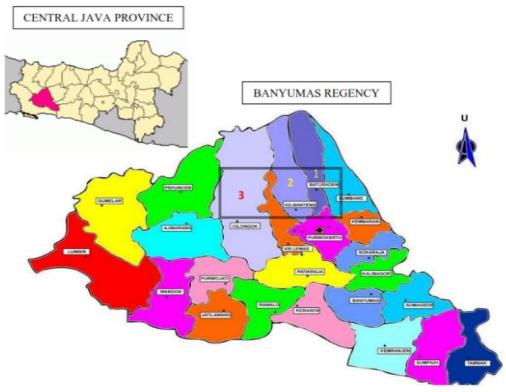

Figure 1. Sampling locations in Banyumas ~~District~~ regency~~district~~ at districts of Baturraden (1), Kedungbanteng (2), and Cilongok (3).

Sampling, isolation and purification of coprophilous fungi

~~Using a pry tool, the~~The dung samples were obtained from a maximum depth of 10 cm below the surface of a 1-month-old dung pile in a landfill with the help of a pry tool. The coprophilous fungi were isolated via a 10^{-3} to 10^{-5} ~~dilutio n~~dilution series. A drop of the diluted extract was placed on soil extract agar (glucose 1g; dipotassium phosphate 0.5g; soil extract 17.75g; agar 15g with final pH at 25°C 6.8±0.2) containing chloramphenicol and then incubated at room temperature for 3–7 days. The fungi grown on this medium were then purified by serial culture on potato dextrose agar until pure cultures were obtained. Subsequently, the purified fungi were inoculated into malt extract broth and incubated at room temperature for 15 days until the mycelia filled the Erlenmeyer flask. Mycelia were harvested via filtration and washed twice with distilled water. The wet mycelia were then either used immediately for DNA isolation or freeze-dried and stored at −20°C for later DNA isolation.

Molecular identification of coprophilous fungi

Isolation of DNA from the purified coprophilous fungal isolates was performed using the Presto™ Mini gDNA kit for yeast (Geneaid) until 100 µl of the DNA solution was obtained. DNA solutions were used immediately for PCR analysis or stored at −80°C for later analysis. The ITS locus was amplified using the primer sequences of ITS1

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(5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATGC-3'). The PCR mixture (25 µl total volume) consisted of 1 µl genomic DNA template, 12.5 µl 2× MyTaq Red Mix (Bioline), 1 µl each primer (20 µM/µl), and 9.5 µl double-distilled H_2O. Amplification was carried out for 35 cycles on the Applied Biosystems 96-Well GeneAmp 9700 thermal cycler using the following conditions: pre-denaturation at 95°C for 3 min, denaturation at 95°C for 10 s, annealing at 52°C for 30 s, and extension at 72°C for 45 s. The DNA amplicon was visualised using 1–2% agarose gel electrophoresis. The PCR products were purified using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research). The purified PCR products were then outsourced to PT Genetika Science Indonesia for DNA sequencing. The sequence data were submitted to GenBank (http://www.ncbi.nlm.nih.gov/) for data analysis.

Data analysis

Electropherograms were edited manually, contigs were merged, and multiple alignments were made for all data sequences using Genetool software (Biotools Inc). The neighbour-joining distance algorithm with the Kimura2 parameter model using PAUP (v.4.0b10) (Swofford, 2000) was used for phylogenetic analysis. Heuristic analysis using parsimony was also performed.

RESULTS AND DISCUSSION

Results

~~In this investigation,~~ Total 16 samples of coprophilous fungal isolates exhibiting different somatic phase characteristics were obtained (~~(~~Fig. 1). Fig. 1). The fungal isolates were purified and subjected to DNA extraction.

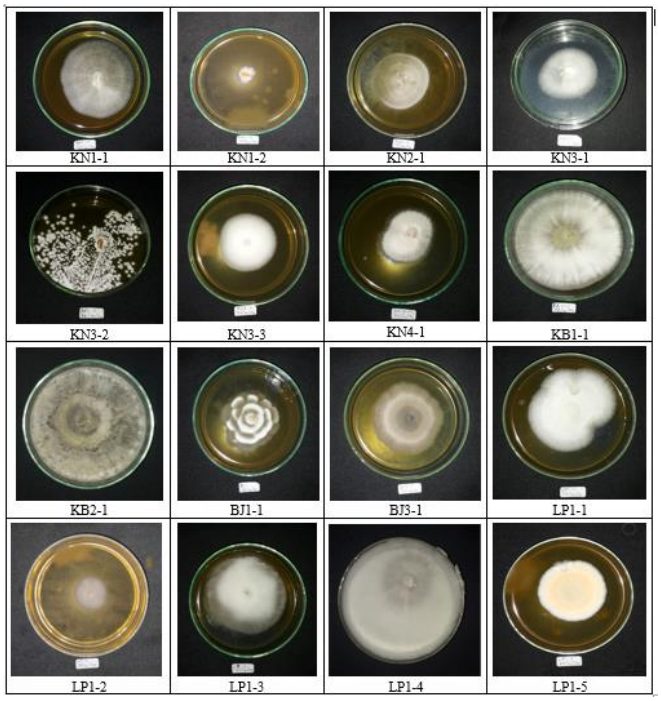

Figure 1. Five-day-old cultures of coprophilous fungal isolates from Banyumas ~~District~~~~district~~regency, Central Java, Indonesia.

Isolates KN1-1, KN1-2, KN2-1, KN3-1, KN3-2, KN3-3, and KN4-1 were obtained from Baturraden district; isolates KB1-1, KB2-1, BJ1-1, and BJ3-1 were obtained from Kedungbanteng district; isolates LP1-1, LP1-2, LP1-3, LP1-4, and LP1-5 were obtained from Cilongok district.

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Table 1. shows the of genomic DNA quantification results for DNA extracts from the coprophilous fungal isolates. The purity of each DNA extract was determined according to the 260/280 nm absorbance ratio. ~~A ratio of 1.8 (viz. [not understood],~~ ~~S~~samples KB2-1, LP1-1, and LP4-1 ~~) indicates indicated area pure sample~~ free of RNA and protein contamination as they showed absorbance ratio of 1.8; samples ~~a ratio greater than 1.8 (viz.,~~ KN1-1, KN1-2, KN2-1, KN3-1, KN3-2, KN4-1, KB1-1, BJ1-1, BJ3-1, LP1-2, LP1- 3, and LP1-5 with the absorbance ratio greater than 1.8~~)~~ ~~indicates~~ indicated possible RNA contamination; while, ~~and~~ a ratio less than 1.8 (viz., KN3-3) ~~indicates~~ indicated possible protein contamination (Sambrook & Russel, 2001). Several isolates (viz., KN1-1, KN3-1, KN3-3, and LP1-2) had concentrations substantially less than 20 ng/µl, which was not optimal for spectrophotometric analysis; however, in general the DNA of these isolates exhibited reasonably good purity.

Table 1. Fungal genomic DNA quantification

No.	Nama Sample	Conc. (ng/µl)	$A_{260/280}$	$A_{260/230}$	Volume (µl)
1	KN1-1	14.2	1.98	0.30	40
2	KN1-2	31.6	1.98	0.14	40
3	KN2-1	29.0	1.93	0.41	40
4	KN3-1	9.3	2.02	0.14	40
5	KN3-2	22.3	1.90	0.17	40
6	KN3-3	9.6	1.65	0.39	40
7	KN4-1	22.3	1.90	0.17	40
8	KB1-1	18.0	2.01	0.19	40
9	KB2-1	96.9	1.89	0.82	40
10	BJ1-1	18.0	1.94	0.12	40
11	BJ3-1	26.7	1.94	0.11	40
12	LP1-1	23.1	1.89	0.04	40
13	LP1-2	11.7	1.98	0.11	40
14	LP1-3	24.5	1.92	0.28	40
15	LP1-4	21.1	1.86	0.27	40
16	LP1-5	55.5	1.93	0.58	40

We also measured the 260/2~~3~~30 absorbance ratio. According to Boyer (2005), a ratio ranging from 2.0 to 2.2 indicates a lack of polysaccharide contamination. The relatively low 260/230 ratios observed in our samples suggested possible contamination with carbohydrates, organic matter, or other chemicals.

Figure 2 shows DNA amplification of the ITS gene locus from coprophilous fungal samples. Of the 16 samples of coprophilic fungi isolated from cow dung, only 9 (KN1-1, KN1-2, KN3-1, KN3-2, KN3-3, KN4-1, KB1-1, BJ3-1, and LP1-3) showed optimal DNA amplification, as evidenced by a specific, single, thick DNA band, which indicates optimal quantity and purity of the extracted genomic DNA (Sambrook & Russel, 2001). According to Agrawal (2008), the purity of the DNA sample can affect the PCR results. Consequently, DNA sequencing was performed in these nine samples (Table 2).

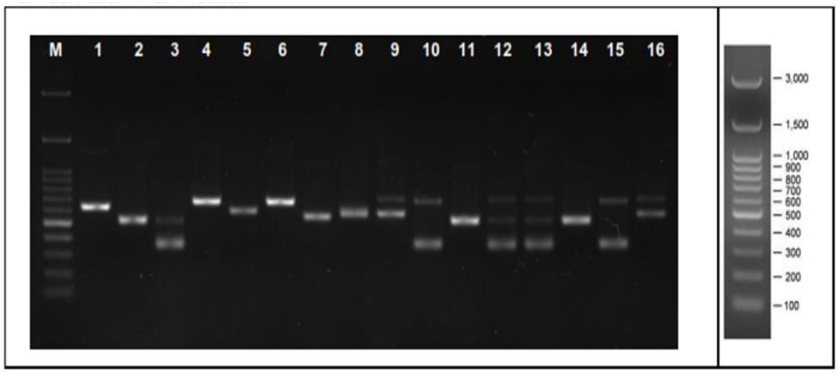

Figure 2. Amplified ITS gene loci from coprophilous fungal samples. Well "M", DNA ladder 100 bp; wells 1–16, coprophilous fungal DNA samples

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Table 2. DNA sequence assemblies of PCR-amplified noncoding polymorphic internal transcriber spacers from coprophilous fungal samples.

No	Sample Name	Sequences
1.	KN1-1	**Sequence Assembly 636bp** 1 TGAACCTGCG GAAGGATCAT TATCGAGTTT TGAACGGGTT GTAGCTGGCC TTTAACGAGG 61 TATGTGCACG CCTGGCTCAT CCACTCTCAA CCTCTGTGCA CTTTATGTAA GAAACGGTGT 121 AAGCCAGCTA TTTAATAGTC GGTAATAAGC CTTTCTTATG TTTACTACAA ACGCTTCAGT 181 TATAGAATGT TTACTGTGTA TAACACAATT ATATACAACT TTCAGCAACG GATCTCTTGG 241 CTCTCGCATC GATGAAGAAC GCAGCGAAAT GCGATAAGTA ATGTGAATTG CAGAATTCAG 301 TGAATCATCG AATCTTTGAA CGCACCTTGC ACTCCTTGGT ATTCCGAGGA GTATGCCTGT 361 TTGAGTCTCA TGGAATTCTC AACCCCTAAA TTTTGTAATG AAGTTTAGTG GGCTTGGACT 421 TGGAGGTTGT GTCGGCTTCT AGTCGACTCC TCTGAAATGT ATTAGCGTGA ATCTTACGGA 481 TCGCCTTCAG TGTGATAATT ATCTGCGCTG TGGTGTTGAA GTATTTATTA GTTCATGCTT 541 ATAGTCGTCT CTTACCGAGA CAATTTATGA CAATCTGAGC TCAAATCAGG TAGGACTACC 601 CGCTGAACTT AAGCATATCA ATAAGCGGGA GGAAGG
2.	KN1-2	**Sequence Assembly 533bp** 1 TAGGTGAACC TGCGGAAGGA TCATTAGTGA TTGCCTTTAT AGGCTTATAA CTATATCCAC 61 TTACACCTGT GAACTGTTCT ACTACTTGAC GCAAGTCGAG TATTTTTACA AACAATGTGT 121 AATGAACGTC GTTTTATTAT AACAAAATAA AACTTTCAAC AACGGATCTC TTGGCTCTCG 181 CATCGATGAA GAACGCAGCG AATTGCGATA AGTAATGTGA ATTGCAGAAT TCAGTGAATC 241 ATCGAATCTT TGAACGCAGC TTGCGCTCTC TGGTATTCCG GAGAGCATGC CTGTTTCAGT 301 GTCATGAAAT CTCAACCACT AGGGTTTCCT AATGGATTGG ATTTGGGCGT CTGCGATTTC 361 TGATCGCTCG CCTTAAAAGA GTTAGCAAGT TTGACATTAA TGTCTGGTGT AATAAGTTTC 421 ACTGGGTCCA TTGTGTTGAA GCGTGCTTCT AATCGTCCGC AAGGACAATT ACTTTGACTC 481 TGGCCTGAAA TCAGGTAGGA CTACCCGCTG AACTTAAGCA TATCAATAAG CGG
3.	KN3-1	**Sequence Assembly 647bp** 1 AGGATCATTA TCGAGTTTTG AAACGGGTTG TAGCTGGCCT TCCGAGGCAT GTGCACGCCC 61 TGCTCATCCA CTCTACACCT GTGCACTTAC TGTGGGTTTC AGGAGCTTCG AAAGCGAGAA 121 AAGGGGCCTT CACGGGCTTT TTTCTTGCCT AGTTGTTACT GGGCCTACGT TTCACTACAA 181 ACACTTATAA AGTATCAGAA TGTGTATTGC GATGTAACGC ATCTATATAC AACTTTCAGC 241 AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA 301 ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC TTGCGCTCCT TGGTATTCCG 361 AGGAGCATGC CTGTTTGAGT GTCATGAAAT TCTCAACCTA ACGGGTTCTT AACGGGACTT 421 GCTTTAGGCT TGGACTTGGA GGTTCTTGTC GGCTTGCTTC AATGTCAAGT CGGCTCCTCT 481 TAAATGCATT AGCTTGGTTC CTGTGCGGAT CGGCTCACGG TGTGATAATT GTCTACGCCG 541 CGACCGTTGA AGCGTTTTTA TAGGCCAGCT TCTAGTCGTC TCTTTACGAG ACAATAATCA 601 TCGAACTCTG ACCTCAAATC AGGTAGGACT ACCCGCTGAA CTTAAGC
4.	KN3-2	**Sequence Assembly 584bp** 1 AGGTGAACCT GCGGAAGGAT CATTACCGAG TGCGGGTCCG CGTGGCCCAA CCTCCCACCC 61 GTGCCTATTG TACCCTGTTG CTTCGGCGGG CCCGCCAGCC TTCGGGCTGG CCGCCGGGGG 121 GCGTCTCGCC CCCGGGCCCG TGCCCGCCGG AGACCCCAAC ATGAACCCTG TTCTGAAAGC 181 TTGGTGTCTG AGTGTGATTG TTTGCAATCA GTTAAAACTT TCAACAATGG ATCTCTTGGT 241 TCCGGCATCG ATGAAGAACG CAGCGAAATG CGATAACTAA TGTGAATTGC AGAATTCAGT 301 GAATCATCGA GTCTTTGAAC GCACATTGCG CCCCCTGGTA TTCCGGGGGG CATGCCTGTC 361 CGAGCGTCAT TGCTGCCCTC AAGCCCGGCT TGTGTGTTGG GCCCTCGTCC CCCGGCTCCC 421 GGGGGACGGG CCCGAAAGGC AGCGGCGGCA CCGCGTCCGG TCCTCGAGCG TATGGGGCTT 481 TGTCTTCCGC TCTGCAGGCC CGGCCGGCGC CCGCCGACGC ATAACAACTT TTTTTCCAGG 541 TTGACCTCGG ATCAGGTAGG GATACCCGCT GAACTTAAGC ATAT
5.	KN3-3	**Sequence Assembly 670bp** 1 AACCTGCGGA AGGATCATTA TCGAGTTTTG AAACGGGTTG TAGCTGGCCT TCCGAGGCAT 61 GTGCACGCCC TGCTCATCCA CTCTACACCT GTGCACTTAC TGTGGGTTTC AGGAGCTTCG 121 AAAGCGAGAA AAGGGGCCTT CACGGGCTTT TTTCTTGCCT AGTTGTTACT GGGCCTACGT 181 TTCACTACAA ACACTTATAA AGTATCAGAA TGTGTATTGC GATGTAACGC ATCTATATAC 241 AACTTTCAGC AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGATA 301 AGTAATGTGA ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC TTGCGCTCCT 361 TGGTATTCCG AGGAGCATGC CTGTTTGAGT GTCATGAAAT TCTCAACCTA ACGGGTTCTT 421 AACGGGACTT GCTTTAGGCT TGGACTTGGA GGTTCTTGTC GGCTTGCTTC AATGTCAAGT 481 CGGCTCCTCT TAAATGCATT AGCTTGGTTC CTGTGCGGAT CGGCTCACGG TGTGATAATT 541 GTCTACGCCG CGACCGTTGA AGCGTTTTTA TAGGCCAGCT TCTAGTCGTC TCTTTACGAG 601 ACAATAATCA TCGAACTCTG ACCTCAAATC AGGTAGGACT ACCCGCTGAA CTTAAGCATA 661 TCAATAAGGC
6.	KN4-1	**Sequence Assembly 522bp** 1 AGGGATCATT ACCGAGTTTA CAACTCCCAA ACCCCTGTGA ACATACCAAT TGTTGCCTCG 61 GCGGATCAGC CCGCTCCCGG TAAAACGGGA CGGCCCGCCA GAGGACCCCT AAACTCTGTT 121 TCTATATGTA ACTTCTGAGT AAAACCATAA ATAAATCAAA ACTTTCAACA ACGGATCTCT 181 TGGTTCTGGC ATCGATGAAG AACGCAGCAA AATGCGATAA GTAATGTGAA TTGCAGAATT 241 CAGTGAATCA TCGAATCTTT GAACGCACAT TGCGCCCGCC AGTATTCTGG CGGGCATGCC 301 TGTTCGAGCG TCATTTCAAC CCTCAAGCCC CCGGGTTTGG TGTTGGGGAT CGGCGAGCCC 361 TTGCGGCAAG CCGGCCCCGA AATCTAGTGG CGGTCTCGCT GCAGCTTCCA TTGCGTAGTA 421 GTAAAACCCT CGCAACTGGT ACGCGGCGCG GCCAAGCCGT TAAACCCCCA ACTTCTGAAT 481 GTTGACCTCG GATCAGGTAG GAATACCCGC TGAACTTAAG CA
7.	KB1-1	**Repeat Sequencing Process**
8.	BJ3-1	**Sequence Assembly 516bp** 1 TGATATGCTT AAGTTCAGCG GGTAGTCCTA CCTGATTTCA GGCCAGAGTC AAAGTAATTG 61 TCCTTGCGGA CGATTAGAAG CACGCTTCAA CACAATGGAC CCAGTGAAAC TTATTACACC 121 AGACATTAAT GTCAAACTTG CTAACTCTTT TAAGGCGAGC GATCAGAGAT CGCAGACGCC 181 CAAATCCAAT CCATTAGGAA ACCCTAGTGG TTGAGATTTC ATGACACTGA AACAGGCATG 241 CTCTCCGGAA TACCAGAGAG CGCAAGCTGC GTTCAAAGAT TCGATGATTC ACTGAATTCT 301 GCAATTCACA TTACTTATCG CAATTCGCTG CGTTCTTCAT CGATGCGAGA GCCAAGAGAT 361 CCGTTGTTGA AAGTTTTATT TTGTTATAAT AAAACGACGT TCATTACACA TTGTTTGTAA 421 AAATACTCGA CTTGCGTCAA GTAGTAGAAC AGTTCACAGG TGTAAGTGGA TATAGTTATA 481 AGCCTATAAA GGCAATCACT AATGATCCTT CCGCAG
9.	LP1-3	**Sequence Assembly 539bp** 1 TCCGTAGGTG AACCTGCGGA AGGATCATTA GTGATTGCCT TTATAGGCTT ATAACTATAT 61 CCACTTACAC CTGTGAACTG TTCTACTACT TGACGCAAGT CGAGTATTTT TACAAACAAT 121 GTGTAATGAA CGTCGTTTTA TTATAACAAA ATAAAACTTT CAACAACGGA TCTCTTGGCT 181 CTCGCATCGA TGAAGAACGC AGCGAATTGC GATAAGTAAT GTGAATTGCA GAATTCAGTG 241 AATCATCGAA TCTTTGAACG CAGCTTGCGC TCTCTGGTAT TCCGGAGAGC ATGCCTGTTT 301 CAGTGTCATG AAATCTCAAC CACTAGGGTT TCCTAATGGA TTGGATTTGG GCGTCTGCGA 361 TCTCTGATCG CTCGCCTTAA AAGAGTTAGC AAGTTTGACA TTAATGTCTG GTGTAATAAG 421 TTTCACTGGG TCCATTGTGT TGAAGCGTGC TTCTAATCGT CCGCAAGGAC AATTACTTTG 481 ACTCTGGCCT GAAATCAGGT AGGACTACCC GCTGAACTTA AGCATATCAA TAAGCGGAG

The DNA sequencing results of the nine selected samples are shown in Table 2. All but one (KB1-1) of the samples

exhibited good purity. According to Bruce et al. (2002), factors affecting DNA sequencing results include the denaturation, annealing and extension temperatures and the degree of DNA molecule separation during the purification and precipitation steps.

The results of nucleotide BLAST searches against the NCBI database are shown in Table 3. The samples KN1-1, KN1-2. KN3-1, KN3-2, KN3-3, BJ3-1, and LP1-3 exhibited consistent BLAST hits from one or two specific species; any differences were in the homotypic synonym, taxon synonym, or obligate synonym of the current name of the species.

Table 3. Results of nucleotide BLAST searches against the NCBI database.

No.	Samples	Result Links					
		Description	Max Score	Total score	Query cover	E value	Per Ident
1	KN1-1	*Emmia lacerata* isolate A01	1136	1136	99%	0.0	99.84%
		Ceriporia lecerata isolate A1S5-D23	1135	1135	100%	0.0	99.69%
		Ceriporia lacerata isolate BPEF81	1123	1123	99%	0.0	99.52%
		Ceriporia lacerata isolate WS1JB14	1121	1121	97%	0.0	100.00%
		Ceriporia lacerata isolate X12	1118	1118	99%	0.0	99.21%
		Emmia lacerata MYA 12S07	1116	1116	99%	0.0	99.21%
		Emmia sp. strain Cef 13	1116	1116	99%	0.0	99.21%
		Ceriporia lacerata isolate CIFE 29	1116	1116	98%	0.0	99.52%
		Basidiomycota sp. SYBC-L17	1116	1116	99%	0.0	99.21%
		Ceriporia lacerata genes for 18S	1116	1116	99%	0.0	99.21%
		http://www.ncbi.nlm.nih.gov/nuccore/MH734799.1,KJ780757.1,KF151851.1,KT844687.1,KF850375.1,LC431580.1,MK775821.1,KM388611.1,HQ891300.1,LC312413.1					
2	KN1-2	*Trichosporon asahii* strain CU12015 6	962	962	100%	0.0	100%
		Trichosporon asahii isolate M15	962	962	100%	0.0	100%
		Trichosporon sp. isolate ~~EE~~(EE (19)-CHc	962	962	100%	0.0	100%
		Trichosporon asahii isolate E22922	962	962	100%	0.0	100%
		Trichosporon asahii strain DMic 165073	962	962	100%	0.0	100%
		Trichosporon asahii culture CBS 2497	962	962	100%	0.0	100%
		Trichosporon asahii strain V9	962	962	100%	0.0	100%
		Trichosporon asahii strain 18S	962	962	100%	0.0	100%
		Trichosporon asahii strain APMSU6	962	962	100%	0.0	100%
		Trichosporon asahii strain YCH116	962	962	100%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/MT482659.1,MT136544.1,MK267768.1,MG241533.1,KY105711.1,KT900123.1,KT900118.1,KT282395.1,KM982986.1					
3	KN3-1	*Lentinus squarrosulus* isolate TAM1004	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher WARRIPt	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher WARRI34	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher UNIP13	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher Odi26	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher IBD43	1168	1168	100%	0.0	100%
		Lentinus sp. BAB5060	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher BORH0009	1162	1162	99%	0.0	100%
		Lentinus squarrosulus small subunit ribosomal	1159	1159	100%	0.0	99.85%
		Lentinus squarrosulus strain WCR1201	1155	1155	100%	0.0	99.69%
		http://www.ncbi.nlm.nih.gov/nuccore/MH172168.1,KT273380.1,KT273379.1,KT273370.1,KT273364.1,KR155105.1,MH053154.1,KT956127.1					
4	KN3-2	*Aspergillus allahabadii* strain CGMC 3 03920	1054	1054	100%	0.0	100%
		Aspergillus allahabadii strain CGMC 3 02584	1054	1054	100%	0.0	100%
		Aspergillus allahabadii genes for 18S rRNA	1054	1054	100%	0.0	100%
		Aspergillus candidus isolate CY104	1054	1054	100%	0.0	100%
		Aspergillus allahabadii strain CMV004E2	1049	1049	100%	0.0	99.83%
		Aspergillus allahabadii strain CGMCC 3 01332	1049	1049	100%	0.0	99.83%
		Aspergillus niveus strain URM7046	1048	1048	99%	0.0	99.83%
		Aspergillus niveus strain CBS 132162	1045	1045	100%	0.0	99.66%
		Aspergillus allahabadii strain NN046949	1043	1043	98%	0.0	100%
		Aspergillus niveus strain NN043511	1043	1043	98%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/MH292843.1,MH292842.1,LC152416.1,HQ607958.1,MK450628.1,MH292844.1,KM613137.1,MH865978.1,KX443215.1,KX443211.1					
5	KN3-3	*Lentinus* sp. BAB-5060	1205	1205	99%	0.0	100%
		Lentinus squarrosulus voucher WARRIPt	1196	1196	98%	0.0	100%
		Lentinus squarrosulus voucher Odi26	1196	1196	98%	0.0	100%
		Lentinus squarrosulus strain WCR1201	1193	1193	99%	0.0	99.70%
		Lentinus squarrosulus voucher UNIP13	1191	1191	98%	0.0	100%
		Lentinus squarrosulus voucher WARRI34	1189	1189	98%	0.0	100%

Commented [AKG9]: If it denotes samples, then why KN1-1, KN1-2, etc. are presented singly.

		Lentinus squarrosulus IBD43	1189	1189	98%	0.0	100%
		Lentinus sp. S5	1188	1188	99%	0.0	99.55%
		Lentinus squarrosulus small subunit	1185	1185	98%	0.0	99.85%
		Lentinus squarrosulus voucher BORH0009	1180	1180	97%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/KR155105.1,KT273380.1,KT273370.1,KT956127.1,KT273373.1, KT273379.1,KT273364.1,JN253598.1,MH053154.1,KP283484.1					
6	KN4-1	*Fusarium proliferatum* strain CBB-4	942	942	100%	0.0	100%
		Fusarium fujikuroi strain S106	942	942	100%	0.0	100%
		Fusarium proliferatum strain 4156	942	942	100%	0.0	100%
		Fusarium proliferatum strain 4054	942	942	100%	0.0	100%
		Fusarium fujikuroi strainYT-4	942	942	100%	0.0	100%
		Fusarium diaminii strain YT-2	942	942	100%	0.0	100%
		Fusarium proliferatum strain BL4	942	942	100%	0.0	100%
		Fusarium proliferatum strain GFR39	942	942	100%	0.0	100%
		Fusarium annulatum strain F-6	942	942	100%	0.0	100%
		Fusarium proliferatum strain HYC1410080401	942	942	100%	0.0	100%
		http://www.ncbi.nlm.nih.gov/nuccore/MT560212.1,MT549849.1,MN817705.1,MN817704.1,MT477707. 1,MT477704.1,MT466521.1,MT447544.1,MT434005.1,MT378328.1					
7	BJ3-1	*Trichosporon asahii* isolate SY4-1 clone SY4-1B	931	931	100%	0.0	100%
		Trichosporon insectorium culture CBS 10422	931	931	100%	0.0	100%
		Trichosporon insectorium culture CBS 10421	931	931	100%	0.0	100%
		Trichosporon faecale culture CBS 4828	931	931	100%	0.0	100%
		Trichosporon insectorium strain ATCC 20506	931	931	100%	0.0	100%
		Trichosporon insectorium ATCCMYA-4361	931	931	100%	0.0	100%
		Trichosporon faecale strain DH545	931	931	100%	0.0	100%
		Trichosporon faecale CBS 4828	931	931	100%	0.0	100%
		Trichosporon asahii strain CU12015 6	927	927	100%	0.0	99.81%
		Trichosporon asahii strain CU12015 21	927	927	100%	0.0	99.81%
		http://www.ncbi.nlm.nih.gov/nuccore/KY963115.1,KY105746.1,KY105745.1,KY105736.1,HM802133.1, NR 111353.1,EF153624.1,NR 073242.1,MT482659.1,MT482658.1					
8	LP1-3	*Trichosporon asahii* isolate SY4-1 clone SY4	973	973	100%	0.0	100%
		Trichosporon faecale culture CBS 4826	973	973	100%	0.0	100%
		Trichosporon insectorium strain ATCC 20506	973	973	100%	0.0	100%
		Trichosporon insectorium ATCC MYA-4361	973	973	100%	0.0	100%
		Trichosporon faecale CBS 4828	073	073	100%	0.0	100%
		Trichosporon insectorium culture CBS 10422	971	971	99%	0.0	100%
		Trichosporon asahii strain CU12015 6	968	968	100%	0.0	99.81%
		Trichosporon asahii isolate M15	968	968	100%	0.0	99.81%
		Trichosporon sp. isolate ~~EF~~(EE (19)-CHc	968	968	100%	0.0	99.81%
		Trichosporon asahii isolate E22922	968	968	100%	0.0	99.81%
		http://www.ncbi.nlm.nih.gov/nuccore/KY963115.1,KY105736.1,HM802133.1,NR 111353.1,NR073242.1,KY105746.1,MT482659.1,MT136544.1,MK605936.1,MK267768.1					

Based on the nucleotide BLAST searches (Table 3), several of the coprophilous fungal samples could be identified at the species level. These samples were (1) KN1-1, identical to *Ceriporia lacerata*; (2) KN1-2, identical to *Trichosporon asahii*; and (3) KN3-1 and KN3-3, identical to *Lentinus squarrosulus*. Samples that could not be identified at the species level because they exhibit similarities with several species within a genus were (1) KN4-1, which probably belongs to the genus *Fusarium*; (2) KN3-2, which probably belongs to the genus *Aspergillus*; and (3) BJ3-1 and LP3-1, which probably belong to the genus *Trichosporon*.

Nucleotide BLAST searches against a more specific database, such as *Fusarium* ID, are needed for the KN4-1 sample (most likely *Fusarium*).

~~The BLAST search results are displayed in phylogenetic trees (Figs. 2–9).~~

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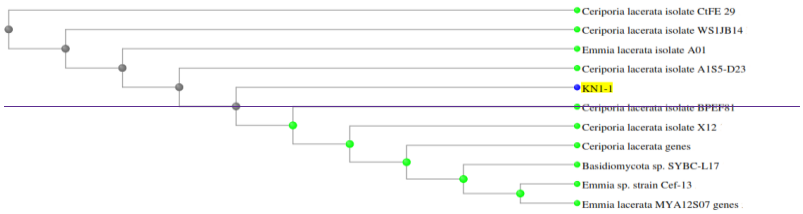
Commented [AKG11]: In my opinion, authors should go for some clustered phylogenies instead of providing separate for each sample. Moreover, no outgroup selected in phylogenies.



~~Based on the nucleotide BLAST searches (Table 3) and the resulting phylogenetic trees (Figs. 2–9), several of the coprophilous fungal samples could be identified at the species level. These samples were (1) KN1-1, identical to *Ceriporia lacerata*; (2) KN1-2, identical to *Trichosporon asahii*; and (3) KN3-1 and KN3-3, identical to *Lentinus squarrosulus*. Samples that could not be identified at the species level because they exhibit similarities with several species within a genus were (1) KN4-1, which probably belongs to the genus *Fusarium*; (2) KN3-2, which probably belongs to the genus *Aspergillus*; and (3) BJ3-1 and LP3-1, which probably belong to the genus *Trichosporon*. Further nucleotide BLAST searches against a more specific database, such as *Fusarium* ID, are needed for the KN4-1 sample (most likely *Fusarium*). Furthermore, morphological analyses can be performed to complement the obtained molecular data.~~ Molecular identification of coprophilous fungi obtained in Banyumas regency found *Ceriporia lacerata*, *Trichosporon insectorium*, and *Lentinus squarrosulus* at species level and *Fusarium* sp., *Aspergillus* sp., and *Trichosporon* sp. at genus level based on ITS1 and ITS4 in the 16S rRNA gene. According to Stackebrandt and Goebel (1994) the 16S rRNA markers of microorganisms such as fungi tend to be very similar or identical at the species level when the identity exceeds 97.5%, whereas the identity threshold is 95% at the genus level ~~(~~).

~~The 16S rRNA markers of microorganisms such as fungi tend to be very similar or identical at the species level when the identity exceeds 97.5%, whereas the identity threshold is 95% at the genus level (Stackebrandt and Goebel, 1994).~~

The fungal genera isolated and identified in this study have never been reported as being coprophilic, except for *Trichosporon* spp., which has been found in chicken manure (Obire et al., 2008), buffalo dung (Lorliam et al., 2013), and rhino dung (Makhuvele et al., 2017). *Fusarium* comprises soil-borne plant pathogenic species (e.g., *F. fujikuroi*) (Al-Ansari, 2018; Cen et al., 2020). *Ceriporia lacerate* grows on wood; Wulandari et al. (2018), found two resupinate fungal specimens in East Kalimantan classified as *Ceriporia* species, *C. inflata* and *C. lacerata*, which were identified based on morphological characteristics and the ITS and nuclear ribosomal large subunit sequences. *L. squarrosulus* is an edible fungus commonly found growing in the wild on decaying tree trunks during the rainy season. Similar to other macrofungal species, this fungus can grow in a wide variety of substrates and habitats. Many *Lentinus* species have been reported to grow in nature on special substrates as well as on pasteurized substrates (Morais et al., 2000, Philippousis et al., 2001). Hu et al. (2013) discovered *Aspergillis allahabadii* growing on the rock faces of Angkor Thom Cambodia temples. Microbial biofilms on the surface of the temple stone destroys the integrity of the substrate material and is a biodeteriogen responsible for the destruction of the temple stones over time.

To conclude, we have uncovered the existence of coprophilous microscopic fungi occurring in Banyumas ~~District~~ regency in Central Java, Indonesia~~. At the species level, we~~ identified as *Ceriporia*~~,~~ *lacerata*, *Trichosporon insectorium*, and *Lentinus*~~L.~~ *squarrosulus*~~,~~. ~~At the genus level, we identified~~ *Fusarium* sp., *Aspergillus* sp., and *Trichosporon* sp. Further investigations are needed to identify the fungi morphologically and to evaluate the utility of these fungi for various human interests.

ACKNOWLEDGEMENTS

The author thanks Rector and Head of LPPM of Universitas Jenderal Soedirman, for providing the permission and funding to undertake this research through the research scheme of Improvement of Competence 2020.

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Molecular identification of coprophilous microfungi from Banyumas Regency, Central Java, Indonesia

ARIS MUMPUNI♥, ADI AMURWANTO, DANIEL JOKO WAHYONO

TABLE OF RESPONSE 28 FEBRUARY 2021

Reviewer Ajay K Gautam (Name of file : A-7293-Article Text-37311-1-4-20210202)

Num-ber	Line Number	Reviewer's comment	Author's response
1	26-27	Check spelling? Reviewer's comment : Check spelling of "cosmopolitely" and word "widely" (as suggested by textcheck)	We choose "widely" to explain how do coprophlous fungi are cosmopolite organisms
2	48-49	Confusing statement. Check and rewrite Reviewer's comment : ITS1 and ITS2 are present in multiple copies in the genome, so they can be amplified even in damaged marking material [not understood], which still gives significant results in forensic studies.	We drop this sentence as we found that this has similar meaning with the prior and afterward sentence.
3	70	What does it means district of districts?? Reviewer's comment : Figure 1. Sampling locations in Banyumas district at districts Baturraden (1), Kedungbanteng (2), and Cilongok (3).	We changed it into : Figure 1. Sampling locations in Banyumas regency at districts of Baturraden (1), Kedungbanteng (2), and Cilongok (3).
4	87	Provide coding of promers. Reviewer's comment : 5' TCCGTAGGTGAACCTGCGG-3' and 5' TCCTCCGCTTATTGATGC-3'.	We changed it into : The ITS locus was amplified using the primer sequences of ITS1 (5' TCCGTAGGTGAACCTGCGG-3') and ITS4 (5' TCCTCCGCTTATTGATGC-3').
5	101	RESULTS AND DISCUSSION	We deleted Fig. 2 – 9

		Reviewer's comment : Although, the results of the present study are quite interesting, however, presented mostly either as table of figures. It is advised authors to present summarized results w.r.t. higher to lower, and other ways as text also to strengthen the obtained results.	We made correction according to the Reviewers suggestion in the rest of the manuscript.
6	111-112	(viz. [not understood], samples KB2-1, LP1-1, and LP4-1) indicated a pure sample free of RNA and protein contamination; a ratio greater than 1.8 (viz., KN1-1, KN1-2, KN2-1, KN3-1, KN3-2, KN4-1, KB1-1, BJ1-1, BJ3-1, LP1-2, LP1- 3, Reviewer's comment : What is the meaning of these codes? Authors does not mention anywhere in the manuscript. It is better to elaborate these codes somewhere in the text.	This the complete sentence after correction : Table 1 shows the of genomic DNA quantification results for DNA extracts from the coprophilous fungal isolates. The purity of each DNA extract was determined according to the 260/280 nm absorbance ratio. Samples KB2-1, LP1-1, and LP4-1 are free of RNA and protein contamination as they showed absorbance ratio of 1.8; samples KN1-1, KN1-2, KN2-1, KN3-1, KN3-2, KN4-1, KB1-1, BJ1-1, BJ3-1, LP1-2, LP1- 3, and LP1-5 with the absorbance ratio greater than 1.8 indicated possible RNA contamination; while, a ratio less than 1.8 (viz., KN3-3) indicated possible protein contamination (Sambrook & Russel, 2001).
7	152	Samples Reviewer's comment : If it denotes samples, then why KN1-1, KN1-2, etc. are presented singly.	We have corrected it into "Sample"
8	156	The BLAST search results are displayed in phylogenetic trees (Figs. 2–9). Reviewer's comment : In my opinion, authors should go for some clustered phylogenies instead of providing separate for each sample. Moreover, no outgroup selected in phylogenies.	We have deleted Figure 2 - 9

9	186 - 191	Based on the nucleotide BLAST searches (Table 3) and the resulting phylogenetic trees (Figs. 2–9), several of the coprophilous fungal samples could be identified at the species level. These samples were (1) KN1-1, identical to Ceriporia lacerata; (2) KN1-2, identical to Trichosporon asahii; and (3) KN3-1 and KN3-3, identical to Lentinus squarrosulus. Samples that could not be identified at the species level because they exhibit similarities with several species within a genus were (1) KN4-1, which probably belongs to the genus Fusarium; (2) KN3-2, which probably belongs to the genus Aspergillus; and (3) BJ3-1 and LP3-1, which probably belong to the genus Trichosporon. Reviewer's comment : Add as results.	We have moved this into "Results" and rewrite the first paragraph 1 in "Discussions" as follows : Molecular identification of coprophilous fungi obtained in Banyumas regency found Ceriporia lacerata, Trichosporon insectorium, and Lentinus squarrosulus at species level and Fusarium sp., Aspergillus sp., and Trichosporon sp. at genus level based on ITS1 and ITS4 in the 16S rRNA gene. According to Stackebrandt and Goebel (1994) the 16S rRNA markers of microorganisms such as fungi tend to be very similar or identical at the species level when the identity exceeds 97.5%, whereas the identity threshold is 95% at the genus level.

Note : We keep using REGENCY after BANYUMAS to explain administrative level of government below province (Central Java) instead of DISTRICT as a subordinate government administrative of regency.

Notifications

[biodiv] Editor Decision

2021-03-04 07:36 AM

ARIS MUMPUNI, ADI AMURWANTO, DANIEL JOKO WAHYONO:

The editing of your submission, "Molecular identification of coprophilous microfungi from Banyumas District, Central Java, Indonesia," is complete. We are now sending it to production.

Submission URL: https://smujo.id/biodiv/authorDashboard/submission/7293

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Biodiversitas Journal of Biological Diversity

Notifications

[biodiv] Editor Decision

2021-03-04 07:36 AM

ARIS MUMPUNI, ADI AMURWANTO, DANIEL JOKO WAHYONO:

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Notifications

[biodiv] Editor Decision

2021-03-04 07:36 AM

ARIS MUMPUNI, ADI AMURWANTO, DANIEL JOKO WAHYONO:

The editing of your submission, "Molecular identification of coprophilous microfungi from Banyumas District, Central Java, Indonesia," is complete. We are now sending it to production.

Submission URL: https://smujo.id/biodiv/authorDashboard/submission/7293

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Biodiversitas Journal of Biological Diversity