## **Comments for the Editor**



## Participants Edit

Aris Mumpuni (arismumpuni)

# Messages Note From Dear Biodiversitas Journal Editor arismumpuni 2020-11-30 I hereby sent a maunscript entitled "DNA Barcoding of coprophilous 01:25 PM microfungi from Banyumas, Central Java, Indonesia" which is based on my recent research in Banyumas Regency, Central Java Province, Indonesia. This is a little piece of my continuous research in the same field : coprophiloud fungi. I hope this can be proceed accordingly. Thank you very much Sincerely yours Aris Mumpuni

## Add Message

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

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7 Abstract. Coprophilous fungi are a group of fungi that are ecologically interesting in relation to herbivores. These fungi are 8 cosmopolitan in distribution and spread cosmopolitely found wherever herbivorous animals are present, and They play a 9 predominant role in the decomposition of organic matter<sub>a</sub>- They are broken down by a in which organic matter passes through 10 series of events involving physical processes such as leaching and mechanical degradation; as well as through biological processes 11 such as degradation by microbes which involve by secreting several exoenzymes. The role of coprophilous fungi is very important 12 as the main decomposers of the lignocellulosic material of herbivorous animal waste which is widespread in nature. Previous 13 research on the inventory and identification of coprophilous fungi in the Banyumas region was still limited to the macroscopic 14 genera, so the results have not been able to provide a comprehensive picture of the presence of coprophilous fungi, especially in the 15 region. Identifying the types of microscopic coprophilous fungi that live in herbivorous animal waste such as lignocellulosic 16 material is necessary to reveal its taxonomy. This study aimed to invent and identify microscopic coprophilous fungi obtained in 17 the Banyumas Regency area. Based on the purposive random sampling method, the obtained fungi were analyzed using molecular 18 methods molecularly through the stages of DNA isolation, barcoding analysis, DNA sequencing and phylogeny analysis of fungal 19 cultures. The results of this study obtained species: Emmia lacerate, Ceriporia lacerate, Trichosporon insectorium and Lentinus 20 squarosulus; and genera: Fusarium sp., Aspergillus sp., and Trichosporon sp. 21 22 Keywords: coprophilous fungi, inventory, molecular identification 23 Running title: DNA barcoding and bioprospecting of coprophilous microfungi

#### INTRODUCTION

25 Coprophilous fungi are saprophytic fungi that live in animal dung. These fungi utilize the feces of various 26 animals, especially herbivores as their substrates (Melo et al., 2012). Masunga et al. (2006) stated that 27 coprophilous fungi are a group of Zygomycota, Ascomycota and Basidiomycota. According to Krug et al. (2004) 28 most of the coprophilous fungi are known to inhabit in the dung of herbivorous livestock such as sheep and cows. 29 According to Sinsabaugh et al. (1981), these fungi spread cosmopolitely wherever herbivorous animals are present 30 and play a predominant role in the decomposition of organic matter. The y organic matter are is broken down by a 31 series of events involving physical processes such as leaching and mechanical degradation; as well as through 32 biological processes such as degradation by microbes involving several exoenzymes.

33 Several researches on coprophilous fungi in Indonesia have been carried out, among others, by Mumpuni and 34 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, 35 36 Mumpuni et al. (2020) also reported that in the former Banyumas Residency (District: Banjarnegara, Purbalingga, 37 Banyumas, and Cilacap) there were 12 genera of macroscopic coprophilous fungi, namely Panaeolus, 38 Coprinopsis, Stropharia, Tricholoma, Lycoperdon, Ascobolus, Rhodocybe, Conocybe, Bolbitius, Leucocoprinus, 39 Mycena, and Hypholoma. The coprophilous fungi obtained from these studies were limited to the macroscopic 40 fungi that were found at the time of sampling. To obtain more comprehensive results, a broader research is needed 41 through the isolation of microscopic coprophilous fungi from the substrate of herbivorous animal waste.

42 Zuber et al. (2011) stated that the standard method for identifying fungal species is morphological analysis, 43 which consists of macroscopic and microscopic observations. Macroscopic analysis consists of the determination **Commented [UR1]:** This sentence is as it is repetition of abstract, please rephrase

**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 ..... 44 of the color, size and structure structure characteristics of the fruiting body. Further analysis of microscopic 45 characteristics was mainly carried out on comparisons of spore appearance. An alternative to morphological 46 analysis is the identification of fungal species based on their genetic studies. The molecular analysis method that 47 has been used is the DNA forensic method (Herbert et al., 2004), using polymorphism against 2 (two) nonencoding polymorphic Internal Transcriber Spacers (ITS), namely ITS1 and ITS2. According to Nilson et al. 48 49 (2008), DNA sequence analysis of these fragments has been successfully used for taxonomic studies on fungi. Lee 50 et al. (2000) revealed that ITS1 and ITS2 regions are common markers used in the identification of fungal species. 51 ITS fragments are extremely useful in species identification because of their long, sequential polymorphisms. 52 Studies that have been carried out have proven that the ITS region provides excellent results in molecular 53 systematics down to the species level as well as in the determination of geographical variations between species. 54 These fragments are present in multiple copies, so they can be amplified even on damaged marking material, 55 which still gives significant results in studies carried out for forensic purposes. The effectiveness of the ITS region 56 polymorphism analysis for forensic purposes has been tested, among others, on the differentiation of the 57 psychotropic fungi of the genera Panaeolus and Psilocybe based on the length of the polymorphisms in the 58 amplification products of this region.

59 Molecular tools complementing with morphological ones-characteristics are very-is a promising approach in 60 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 61 fungal species since the 1990s (White et al., 1991; Burns et al., 1991).; howeverHowever, the strategy based on the 62 63 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 64 65 latter involves the use of a standard DNA region that is specific for a taxonomic group. Badotti et al., (2017) stated 66 that one advantage of using the ITS region as a standard marker is that most fungal species have been identified 67 based on this genomic region. 68

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



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

#### 98 Procedures

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#### 99 Sampling, isolation and purification of the obtained coprophilous fungi-

100 This Present study was held carried out by for elaborating the survey methodon coprophilous fungi from Baturraden, Kedungbanteng, and Cilongok districts. Samples in the form of cow dung were taken from these 3 101 (three) three Districts in of Banyumas Regency: Baturraden District; Kedungbanteng District; and Cilongok 102 103 District. Cow dung samples were taken from a predetermined location using a pry tool from a maximum depth of 104 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<sup>-3</sup> - 10<sup>-5</sup> dilution series.<sub>3</sub> grew a <u>A</u> drop of the dilution diluted extract was 105 106 grown in Soil Extract Agar (SEA) media with the addition of Chloramphenicol. Incubation was carried out at room 107 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 108 109 inoculated into Malt Extract Broth (MEB) media. The cultures were incubated at room temperature for 15 days 110 until the mycelia filled the Erlenmeyer flask. Mycelia are harvested using filtration and washed twice with distilled 111 water. Wet mycelia are then directly used for DNA isolation, or freeze-dry and stored at -20°C for DNA isolation. 112

#### 113 Coprophilous fungal mycelium culture

#### 115 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:

- 118a. Pure cultures of coprophilous fungi (up to 2 x 108) from agar plates were inserted into 1.5 mL119microsentrifugation tubes and centrifuged 5,000 x g, 10 minutes. Then, tThe pellets were further processed and120the supernatant is removed.
- b. <u>Nearly</u> 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).
  interval 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 and 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 was put back in the 2 ml collection tube was discarded and the GD column in the collection tube was discarded and the GD column in the collection tube was discarded and the GD column was put back in the 2 ml 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 min utes 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).

#### 151 Barcoding analysis of the coprophilous fungi

- 152 Amplification of the ITS locus was carried out using ITS-1 primers with the base sequence DNA: 5'-
- 153 TCCGTAGGTGAACCTGCGG-3' and ITS-4 with the base sequence DNA: 5'-TCCTCCGCTTATTGATGC-3'.
- 154 The volume of the PCR mixture used was 25 util consisting of: 1 util genomic DNA template, 12.5 util 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 95°C for 3 minutes, denaturation stage at 95°C for 10 seconds, the primary attachment stage (annealing) at 52°C for 30 seconds, and the extension stage at 72°C for 45 seconds. DNA amplicon visualization using 1-2% agarose gel electrophoresis. PCR products were purified using ZymocleanTM Gel DNA Recovery Kit (Zymo Research).

#### 162 DNA sequencing

163 The purified PCR products are were then sequenced using the bi-directional method using ABI Prism Big Dye 164 Terminator Cycle Sequencing Ready Reaction Kit, v. 3.0 (Applied Biosystems) and the product reactions were 165 separated and <u>analyzed analyzed</u> using the ABI Prism 310 and/or the ABI Prism 3100 Genetic 166 AnalyzersAnalysers. Sequencing is carried out by a third party (PT Genetika Science Indonesia). Data was 167 submitted to GenBank (http://www.ncbi.nlm.nih.gov/) for data analysis.

#### 169 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

#### 177 Result

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By elaborating purposive random sampling technique in-of the coprophilous fungal isolates from 3 (three)-three Districts ( $\underline{viz}$ , Kedungbanteng, Baturraden, and Cilongok) of Banyumas Regency<sub>2</sub>, 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.)



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Figure 1. Choprophilous fungal isolates from Regency of Banyumas, 5 days old culture.

Table 1 Engel	DNA.	and the set of
Table 1. Fungal	genomic DNA	quantification

No.	Nama Sample	Conc. (ng/µl)	A260/280	A260/230	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. shows 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 aAn 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, <u>namely µ/z</u>, KN1-1, KN1-2, KN2-1, KN3-1, KN3-2, KN4-1, KB1-1, BJ1-1, 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  $\lambda 260 / 280$  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.

Table 2. Sequence assembly result - PCR products

No	Sample	Sequences
	Name	Sequence Assembly 636bp
		1 TGAACCTGCG GAAGGATCAT TATCGAGTTT TGAACGGGTT GTAGCTGGCC TTTAACGAG
		61 TATGTGCACG CCTGGCTCAT CCACTCTCAA CCTCTGTGCA CTTTATGTAA GAAACGGTO
		121 AAGCCAGCTA TTTAATAGTC GGTAATAAGC CTTTCTTATG TTTACTACAA ACGCTTCAC
		181 TATAGAATGT TTACTGTGTA TAACACAATT ATATACAACT TTCAGCAACG GATCTCTTC
		241 CTCTCGCATC GATGAAGAAC GCAGCGAAAT GCGATAAGTA ATGTGAATTG CAGAATTC
1.	KN1-1	301 TGAATCATCG AATCTTTGAA CCCATCC ACTCCTTCGT ATTCCGACGA GTATCCCT
		361 TEGRETERS TECHNETERS ACCOUNTS ATTENDED A CONTRACT
		421 TGGAGGTTGT GTCGGCTTCT AGTCGACTCC TCTGAAATGT ATTAGCGTGA ATCTTACGC
		481 TEGECTTCAG TETGATATT ATCTGCGCTC TEGTETTGAA GTATTATTA GTTCATECT
		541 ATACTOSTCT CTTACCAGE CARTTATOS CARTCAGE TOSATCAGE TAGGACTA
		601 CGCTGAACTT AAGCATATCA ATAAGCCGGA GGAAGG
		Sequence Assembly 533bp
		1 TAGGTGAACC TGCGGAAGGA TCATTAGTGA TTGCCTTTAT AGGCTTATAA CTATATCCA
		61 TTACACCTGT GAACTGTTCT ACTACTTGAC GCAAGTCGAG TATTTTTACA AACAATGTC
		121 AATGAACGTC GTTTTATTAT AACAAAATAA AACTTTCAAC AACGGATCTC TTGGCTCTC
2	KN1-2	181 CATCGATGAA GAACGCAGCG AATTGCGATA AGTAATGTGA ATTGCAGAAT TCAGTGAAT
<b>~</b>	10111-2	241 ATCGAATCTT TGAACGCAGC TTGCGCTCTC TGGTATTCCG GAGAGCATGC CTGTTTCA
		301 GTCATGAAAT CTCAACCACT AGGGTTTCCT AATGGATTGG ATTTGGGCGT CTGCGATT
		361 TGATCGCTCG CCTTAAAAGA GTTAGCAAGT TTGACATTAA TGTCTGGTGT AATAAGTT
		421 ACTGGGTCCA TTGTGTTGAA GCGTGCTTCT AATCGTCCGC AAGGACAATT ACTTTGAC
		481 TGGCCTGAAA TCAGGTAGGA CTACCCGCTG AACTTAAGCA TATCAATAAG CGG
-	KAID 4	Sequence Assembly 647bp
э.	KN3-1	1 AGGATCATCA CEGAGITIG FARCEGETTE INCEGAGETTE AGGAGETTE
- 1		121 AAGGGGCCTT CACGGGCTTT TTTCTTGCCT AGTTGTTACT GGGCCTACGT TTCACTAC
		181 ACACTTATAA AGTATCAGAA TGTGTATTGC GATGTAACGC ATCTATATAC AACTTTCA
		241 AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTC
		301 ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC TTGCCTCCT TGGTATTCC
		361 ACCCCTCC CTCTTCACT CTCATCAAAT TCTCAACCTA ACCCCTTCTT AACCCCCC
		421 COTTALCOT TOCACTTOCA COTTOCTTC ACCOUNTS ACCOUNTS
		421 GETTIAGET IGAACTIGGA GETETIGIE GEETIGETAAGT COGETECT
		401 RAAFIGCATT AGCITIGGTIC CIGCIGCAGA COGCICACGO IGTARIAATI GICIACGO
		501 ECCLORED ACCTCALT ACCTACACT ACCCCCCCAL CTIACAC
-		Sequence Assembly 584bn
		1 AGGTGAACCT GCGGAAGGAT CATTACCGAG TGCGGGTCCG CGTGGCCCAA CCTCCCAC
		61 GTGCCTATTG TACCCTGTTG CTTCGGCGGG CCCGCCAGCC TTCGGGCTGG CCGCCGGG
		121 GCGTCTCGCC CCCGGGCCCG TGCCCGCCGG AGACCCCCAAC ATGAACCCTG TTCTGAAA
		181 TTGGTGTCTG AGTGTGATTG TTTGCAATCA GTTAAAACTT TCAACAATGG ATCTCTTG
4.	KN3-2	241 TCCGGCATCG ATGAAGAACG CAGCGAAATG CGATAACTAA TGTGAATTGC AGAATTCAG
		301 GAATCATCGA GTCTTTGAAC GCACATTGCG CCCCCTGGTA TTCCGGGGGGG CATGCCTG
		361 CGAGCGTCAT TGCTGCCCTC AAGCCCGGCT TGTGTGTTGG GCCCTCGTCC CCCGGCTC
		421 GGGGGACGGG CCCGAAAGGC AGCGGCGGCA CCGCGTCCGG TCCTCGAGCG TATGGGGC
		481 TGTCTTCCGC TCTGCAGGCC CGGCCGGCGC CCGCCGACGC ATAACAACTT TTTTTCCAG
		541 TTGACCTCGG ATCAGGTAGG GATACCCGCT GAACTTAAGC ATAT
		Sequence Assembly 670bp
		ARCENEGGE RECEDENCE CTERNERE RECEGETTE ACCASE
		121 BACCACCO RECOCCET CICCACCO DIGCALIAC DIGGILIAC AGAGENA
		121 ARAGGAGAA AAGGGGCCTT CACGGGCTT TITCTIGCCT AGTIGTTACT GGGCCTAC
		181 FFCACTACAA ACACITATAA AGIATCAGAA TGTGIATTGC GATGIAACGC ATCIATAT
		241 AACTTTCAGC AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGA
5.	KN3-3	301 AGTAATGTGA ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC TTGCGCTCC
		361 TEGTATICUS AGGAGCATGC CTGTTTGAGT GTCATGAAAT TCTCAACCTA ACGGGTTC
		421 AACGUGACTT GCTTTAGGCT TGGACTTGGA GGTTCTTGTC GGCTTGCTTC AATGTCAAC
		481 CGGUTUUTUT TAAATGCATT AGCTTGGTTC CTGTGCGGAT CGGCTCACGG TGTGATAA
		541 GTCTACGCCG CGACCGTTGA AGCGTTTTTA TAGGCCAGCT TCTAGTCGTC TCTTTACG
		601 ACAATAATCA TCGAACTCTG ACCTCAAATC AGGTAGGACT ACCCGCTGAA CTTAAGCA
_		661 TCAATAAGGC
- 1		Sequence Assembly 522bp
		A AGGATUATT AUGAGTITA CARLICUCAA ACCULTGIGA ACATACUAAT TGTTGCCT
		b1 GUGGATUAGE CEGETECEGG TAAAACGGGA EGGECEGECA GAGGACECET AAACTETG
		121 TCTATATGTA ACTTCTGAGT AAAACCATAA ATAAATCAAA ACTTTCAACA ACGGATCT
6	KN4-1	181 TGGTTCTGGC ATCGATGAAG AACGCAGCAA AATGCGATAA GTAATGTGAA TTGCAGAA
		241 CAGTGAATCA TCGAATCTTT GAACGCACAT TGCGCCCGCC AGTATTCTGG CGGGCATG
		301 TGTTCGAGCG TCATTTCAAC CCTCAAGCCC CCGGGTTTGG TGTTGGGGAT CGGCGAGC
		361 TTGCGGCAAG CCGGCCCCGA AATCTAGTGG CGGTCTCGCT GCAGCTTCCA TTGCGTAG
		421 GTAAAACCCT CGCAACTGGT ACGCGGCGCG GCCAAGCCGT TAAACCCCCCA ACTTCTGA
		481 GTTGACCTCG GATCAGGTAG GAATACCCGC TGAACTTAAG CA
7.	KB1-1	Repeat Sequencing Process
		Sequence Assembly 516bp
8.	BJ3-1	61 TCCTTGCGA CGATTAGAGC GACGCTTCAL CACABITCAL CACATGAGAC MARGINA
		121 AGACATTAT CONTRACTOR CHOCKED TALCCORC CARDINARU TATTACA
		101 CANATCCART COLUMN CONTROL CANTER AND CONTROL CANTER AND COLUMN
- 1		241 CTCTCCCGLI TECCEGGGA CCCLIGCTCC CTTCLIGATE TCCLICATE ACCCLIC
		A A A A A A A A A A A A A A A A A A A
		301 GCAATTCACA TTACTTATCC CAATTCCCTC CCTTCTTCAT CCATCCCACA CCCAACAC
		301 GCAATTCACA TTACTTATCG CAATTCGCTG CGTTCTTCAT CGATGCGAGA GCCAAGAG
		301 GCAATTCACA TTACTTATCG CAATTCGCTG CGTTCTTCAT CGATGCGAGA GCCAAGAG 361 CCGTTCTTGA AAGTTTTATT TTGTTATAAT AAAACGACGT TCATTACACA TTGTTTGT 300 DAGAGCGACTGCCCAA CAAGACA CTGTTCATCACACA TTGTTTGTT 300 DAGAGCACTGCCCAA CAAGACA CAAGACACACACACACACACACACA
		301 GCAATTCACA TTACTATCG CAATTCGCTG CGTTCTTCAT CGATGCGAGA GCCAAAGG 361 CCGTTGTTGA AAGTTTATTT TTGTTATATA AAAACGACT TCATTACACA TTGTTTG 421 AAATACTCGA CTGGCGTGAA GTAGTAGAAC AGTTCACAGG TGTAAGTGGA TATAGTTA 481 ACCGTARDAA CGCAARGCAT ABACGACGTT CCCCACAGG TGTAAGTGGA TATAGTTA 481 ACCGTARDAA CCCAARGCAT ABACGACGTT CCCCACAGG TGTAAGTGGA TATAGTTA 481 ACCGTARDAA CCCAARGCAT ABACGACGTT CCCCACAGG TGTAAGTGGA TATAGTTA
		301 GCAATIGCA TRACTARG CAATIGGTG CETTGTTCAT CGATGGGGG GCCAAGA 301 CCGTGTTG AAGTTART TGTTATAAT AAACGACGT TGATGGGGA TATGTTG 421 AAATACTGG CTTGCGTCAA CFAGTAGAAC AGTTGACAGG GTAAGTGGGA TATAGTTA 481 AGCCTATAAA GGGAATGGCT AATGGATCGT CGCGAG
		<ul> <li>301 GCAATIGACA TFACTIATGG CAATIGGCGG CGTGCTTCAT CGATGGGAGA GCCAAGAG</li> <li>301 CCGTGTGTG AAGTTIATAT TGTTATATA AAAGAGCGT TCATTACCACA TTGTTGCT</li> <li>421 AAATACTGGA CTTGCGTCAA GTAGAGAAC AGTTGACAGG TGTAAGTGGA TATAGTTA</li> <li>481 AGCCTATAAA GGCAATCAT ATGATCTT CCGCAG</li> <li>Sequence Assembly S39bp</li> <li>1 TCCCTAGATG AACGTGCGGA AGGATCATTA GTGATTGCCT TATAGCTT ATAACTAT</li> </ul>
		<ul> <li>301 GCAATCACA TRACTATGE CAATCGCTG CGTTCTTCAT CGATGCGAGA GCCAAGAG</li> <li>301 CCGTTGTGG AAGTTTATT TGTTATATA HAAAGGACT TCATTACCACA TTOTTGT</li> <li>421 AAATACTCGA CTTGGCTGCAA GTAGTAGAAC AGTTGACAGG TGTAAGTGGA TATAGTTA</li> <li>481 AGCCTATARA GGCAATCACT AATGATCCTT CGCGGG</li> <li>Sequence Assembly 33bp</li> <li>1 TCCGTAGGTG AACCTGCGGA AGGATCATTA GTGATTGCCT TTATAGGCTT ATAACTAT</li> <li>61 CCGATTACC TGGGAATCG TTCTACTACT TGAGGCAAGT CGGTATTTT TACAAACA</li> </ul>

**Commented [UR6]:** Please check this sentence that the meaning is not changing for the message researchers wants to convay.

LP1-3

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. 298

299	Table 3. Resul	lts of blast nucleotide analysis from the NCBI database

N	Sampla	Result	Links				
IN O	Sample	Description	Max	Total	Query	E	Dar Idant
0.	5	Description	Score	score	cover	value	Fel Idelit
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,K	J780757.	1,KF1518	51.1,KT844	687.1,KF	850375.1,L
		C431580.1,MK775821.1,KM388611.1,HQ891300.1,	LC312413	3.1			
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,N	IT136544	.1,MK267	768.1,MG2	41533.1,1	Y105711.
2	1010 1	1,K1900123.1,K1900118.1,K1282395.1,KM982986	.1	11.00	1000/	0.0	1000/
3	KN3-1	Lentinus squarrosulus isolate TAM1004	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucner wARRIPt	1108	1108	100%	0.0	100%
		Lentinus squarrosulus voucher WARRI34	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucner UNIP13	1108	1108	100%	0.0	100%
		Lentinus squarrosulus voucner Odi26	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucner IBD43	1168	1168	100%	0.0	100%
		Leninus sp BAB5000	1168	1168	100%	0.0	100%
		Lentinus squarrosulus voucher BORH0009	1162	1162	99%	0.0	100%

	1	T	1150	1150	1000/	0.0	00.054
		Lentinus squarrosulus small subunit	1159	1159	100%	0.0	99.85%
		ribosomai	1155	1155	100%	0.0	99.69%
		Lentinus squarrosulus strain wCR1201	(T)72200	1 1/17072/	270 1 1/2707	2270 1 127	272264-1
		KR155105.1,MH053154.1,KT956127.1	12/3380	.1, <b>K1</b> 2/3.	5/9.1, <b>K</b> 12/	5570.1,KI	273364.1,
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,M	/H292842	2.1,LC152	416.1,HQ60	)7958.1,M	K450628.1
		,MH292844.1,KM613137.1,MH865978.1,KX443215	5.1,KX443	211.1	1	I	
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%
		KT273379.1,KT273364.1,JN253598.1,MH053154.1,	KP28348	1, <b>K</b> 12733 4.1	70.1, K1950	0127.1, <b>K</b> 1	275575.1,
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,N 1 MT477704 1 MT466521 1 MT447544 1 MT43400	1T549849 5 1 mt37	.1,MN817 8328 1	705.1,MN8	17704.1,N	AT477707.
7	BJ3-1	Trichosporon asahii isolate SY4-1 clone SY4-	931	931	100%	0.0	100%
·	200 1	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	927	927	100%	0.0	99.81%
		Trichosporon asahii strain CU12015 21					
		http://www.ncbi.nlm.nih.gov/nuccore/KY963115.1,K	Y105746	1,KY105	745.1,KY10	5736.1,HI	<u>M802133.1,</u>
8	I P1-3	$\frac{1}{1}$	072	072	100%	0.0	100%
0	LI 1-5	Trichosporon faecale culture CRS 4826	973	973	100%	0.0	100%
		Trichosporon insectorium strain ATCC 20506	973	973	100%	0.0	100%
		Trichosporon insectorium ATCC MVA-4361	973	973	100%	0.0	100%
L	I	Thenosporon insectorium Tree MIR-4501	715	115	100/0	0.0	100/0

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,HM802	133.1,NR		
Trichosporon faecale CBS 4828         073         073         100%         0.0         1           Trichosporon insectorium culture CBS 10422         971         971         99%         0.0         1           Trichosporon asahii strain CU12015 6         968         968         100%         0.0         99.           Trichosporon asahii isolate M15         968         968         100%         0.0         99.           Trichosporon sp isolate EE(19)-CHc         968         968         100%         0.0         99.           Trichosporon asahii isolate M15         968         968         100%         0.0         99.           Trichosporon sp isolate E2(292         968         968         100%         0.0         99.           Trichosporon asahii isolate E22922         968         968         100%         0.0         99.           http://www.ncbi.nlm.nih.gov/nuccore/KY963115.1.KY105736.1.HM802133.1.NR         11353.1.NR073242.1.KY105746.1.MT482659.1.MT136544.1.MK605936.1.MK267768.1         148267768.1					

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 choprophilous fungal isolate















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

Figure 9. Phylogenetic tree of LP1-3 choprophilous fungal isolate

#### 409 Discussion

396

Based on the results of blast nucleotide analysis from the NCBI database (Table 3) and the resulting 410 411 phylogenetic trees (Fig, 2 to 9.), several samples that can be determined to the species level are as follows: (1) 412 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 413 414 samples that have not yet been determined to the species level are as follows: (1) the KN4-1 sample is thought to 415 be the genus Fusarium. KN4-1 cannot be determined at the species level because it has similarities with several 416 species. If necessary, the KN4-1 sample needs to be subjected to further blast nucleotide analysis using a more 417 specific database to determine the genus Fusarium, such as Fusarium ID; (2) The KN3-2 sample is thought to be 418 the genus Aspergillus, this sample cannot be determined at the species level because it has similarities with several 419 species; (3) Samples BJ3-1 and LP3-1 are thought to be of the genus Trichosporon, but they cannot be determined 420 at the species level because they are also similar to several species. Thus, if necessary, morphological analysis can 421 also be carried out, so that this morphological data can be used to complement the obtained molecular data. The 422 use of 16S rRNA markers in blast nucleotides from the NCBI database on samples of microorganisms such as 423 fungi is identical (similar) at the species level, namely if the percentage identity value is above 97.5%, while at the 424 genus level, namely if the percentage identity value is above 95% (Stackebrandt and Goebel, 1994).

425 The presence of these coprophilous fungi on the substrate of cow dung, both at the species and genus levels, 426 shows its adaptability to the environment with complex lignocellulosic materials. Cow dung provides a habitat for 427 various types of organisms, including coprophilous fungi, which break down the nutrient content in it for 428 recycling. Nutrients contained in cow dung, among others, were mentioned by Melsasail et al. (2019) namely that 429 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)/P2O5 value 0.22% - 0.34%; and potassium (K-total)/K2O value 0.36% -0.56%. 430

431 Several genera of the fungi that can be isolated and identified molecularly in this study have never been 432 reported as coprophilic fungi in previous studies, except for Trichosporon spp which was found in chicken manure 433 (Obire et al., 2008); buffalo dung (Lorliam et al., 2013); and rhino dung (Makhuvele et al., 2017). Meanwhile, 434 other Other fungi (Fusarium fujikuroi) were reported to be obtained from the soil as plant pathogens (Al-Ansari, 435 2018; Cen et al., 2020). On wood (Ceriporia lacerata) that was reported by Wulandari et al. (2018), who-found 436 that two resupinate fungal isolates in East Kalimantan which were classified as the Ceriporia species. C. inflata 437 and C. lacerata were identified based on morphological characteristics and analysis of the internal transcribed 438 spacer (ITS) and nuclear ribosomal large subunit (nL SU) data sequences; Lentinus squarrosulus, which is an 439 edible fungus commonly found growing in the wild on decaying tree trunks during the rainy season. Similar to 440 other macro fungal species, this fungus can grow in a wide variety of substrates and habitats. Many Lentinus 441 species have been reported to grow in nature on special substrates and can grow on pasteurized substrates (Morais 442 et al., 2000, Philippousis et al., 2001). Hu et al. (2013) reported the discovery of A. allahabadii on the rock face of 443 Angkor Thom Cambodia temples. Microbial biofilm on the surface of the temple stone destroys the integrity of the 444 substrate material and is a biodeteriogent that is responsible for the destruction of the temple stones from time to 445 time

446 To conclude, present investigation uncovered the existence of coprophilous microscopic fungi occurring in 447 Banyumas Regency are as follows: (1) at the species level, the fungi identified were : Emmia lacerata, Ceriporia 448 lacerata, Trichosporon insectorium and Lentinus squarosulus; (2) at the genus level, they wer identified as: 449 Fusarium sp., Aspergillus sp., and Trichosporon sp. Further investigations are needs to to study the potential of

450 these fungi for various human interests in various fields. 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.

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454

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# [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.

In my opinion, it is not acceptable for this article to be published with some unidentified

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

Smujo Editors editors@smujo.id


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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.

In my opinion, it is not acceptable for this article to be published with some unidentified

microfungi ......... Indonesia'. I have gone through the manuscript and found that the manuscript deserves publication in your esteemed journal but not in the present form. Manuscript is written satisfactorily but there is great scope for linguistic improvement. I have tried to improve the manuscript by editing some of the sentences. Particularly, in materials methods, 'Isolation of DNA from the culture' is written as lab protocol and I feel it should be written as simple paragraph and not like the way it is presented (off course it's my opinion).

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

Manuscript must be corrected linguistically with the help of native English speaker.

Some of the species are unidentified because of mix result of DNA analysis, morphotaxonomic 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

Manuscript is acceptable after including the suggestions and corrections done on the annotated copy while final decision is left to the editorial board of the journal.

Recommendation: Revisions Required

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Reviewer N:

- 1. Coprophilous fungi are an ecologically interesting group. This manuscript is entitled "DNA barcoding of ...", but is not about DNA barcoding indeed. DNA barcoding is a technology of identifying species by using the sequence variation between inter- and intra-species. DNA barcoding paper should investigate the intra- and inter-species sequence difference and evaluate the power of species identification. However, this manuscript does not contain the above study.
- 2. Perhaps the title of this manuscript can be changed "Molecular identification of coprophilous microfungi from Banyumas, Central Java, Indonesia".

3. In that case, it is necessary to delete the Figs 2 to 9.

4. "barcoding analysis" should be carried out after DNA sequencing in the line 17, https://smujo.id/biodiv/authorDashboard/submission/7293

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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 7 8 9 10 11 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 12 13 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 14 15 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 16 analysed using the molecular methods of DNA isolation, barcoding analysis, DNA sequencing and phylogenetic analysis of fungal 17 cultures. The following species and genera were identified: Ceriporia lacerata, Trichosporon insectorium, Lentinus squarrosulus, 18 Fusarium sp., Aspergillus sp., and Trichosporon sp.

19 Keywords: molecular identification, coprophilous fungi, inventory

20 Running title: Molecular identification of coprophilous microfungi

#### INTRODUCTION

23 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 24 25 belong to the phyla Zygomycota, Ascomycota and Basidiomycota (Masunga et al. 2006). According to Krug et al. (2004), 26 most coprophilous fungi inhabit the dung of herbivorous livestock, such as sheep and cattle. According to Sinsabaugh et 27 al. (1981), these fungi spread cosmopolitely [widely?] wherever herbivorous animals are present and play a predominant 28 role in the decomposition of organic matter. The organic matter is broken down by a series of events involving physical 29 processes, such as leaching and mechanical degradation, as well as through biological processes, such as degradation by 30 microbes involving several exoenzymes.

According to Mumpuni and Wahyono (2016), fourFour 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, Purbalingg, Banyumas, and Cilacap). The <u>studies on</u> coprophilous fungi obtained from thosefrom 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.

38 39 Zuber et al. (2011) reported that the standard method for identifying fungal species is morphological analysis, which 40 consists of macroscopic and microscopic observations. Macroscopic analysis consists of the determination of the colour, 41 size and structural characteristics of the fruiting body. Further analysis of microscopic characteristics is performed mainly 42 by comparison of spore appearance. An alternative to morphological analysis is the identification of fungal species based 43 on phylogenetic studies. Among such studies, the DNA forensic method (Herbert et al., 2004) has been applied to evaluate 44 polymorphisms in two noncoding polymorphic internal transcriber spacers (ITS1 and ITS2). The ITS regions are 45 extremely useful for species identification because of their long, sequential polymorphisms. DNA sequence analysis of 46 ITS1 and ITS2 has been successfully used for taxonomic studies of fungi (Nilson et al., 2008), and these regions are 47 common markers used for the identification of fungal species (Lee et al., 2000). Studies have proven that the ITS region Commented [AKG1]: Check spelling? Commented [AKG2]: ???

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48 provides excellent results in molecular systematics down to the species level, as well as in the determination of 49 geographical variations among species. ITS1 and ITS2 are present in multiple copies in the genome, so they can be 50 amplified even in damaged marking material [not understood], which still gives significant results in forensic studies. 51 Studies have evaluated the effectiveness of ITS polymorphism analysis for forensic purposes in the differentiation of 52 psychotropic fungi of the genera Panaeolus and Psilocybe, based on the lengths of polymorphisms identified in ITS1/2 53 amplification products.

54 55 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 56 identification of fungal species since the 1990s (White et al., 1991; Burns et al., 1991). However, strategies based on 57 sequencing of standardized genomic fragments (DNA barcoding) was recognized much later (Hollingsworth, 2007). The 58 primary difference between molecular identification tools and the "DNA barcode" approach is that the latter involves the 59 use of a standard DNA region that is specific for a taxonomic group. Badotti et al. (2017) suggested that one advantage of 60 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 61 62 coprophilous fungi obtained in the Banyumas District district in Central Java, Indonesia.

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#### MATERIALS AND METHODS

#### 64 Study area 65

We surveyed The survey of study area for the collection of the coprophilous fungi from samples of cow dung collected from thewas carried out in Baturraden, Kedungbanteng, and Cilongok districts (ranged between 7°03' – 7°38' South Latitude and 109°10' – 109°25' East Longitude) in the Banyumas District in Central Java, Indonesia. 66

67 68



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-monthold dung pile in a landfill with the help of a pry tool. The coprophilous fungi were isolated via a 10<sup>-3</sup> to 10<sup>-5</sup> dilutio <u>adilution</u> 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  $25^{\circ}$ 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.

#### 84 Molecular identification of coprophilous fungi

Isolation of DNA from the purified coprophilous fungal isolates was performed using the Presto<sup>TM</sup> Mini gDNA kit for 85 86 yeast (Geneaid) until 100 µl of the DNA solution was obtained. DNA solutions were used immediately for PCR analysis 87 or stored at -80°C for later analysis. The ITS locus was amplified using the primer sequences Commented [AKG3]: Confusing statement. Check and rewrite.

Commented [AKG4]: What does it means district of districts??

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<sub>2</sub>O. 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<sup>TM</sup> 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.

# 9697 Data analysis

98 Electropherograms were edited manually, contigs were merged, and multiple alignments were made for all data 99 sequences using Genetool software (Biotools Inc). The neighbour-joining distance algorithm with the Kimura2 parameter 100 model using PAUP (v.4.0b10) (Swofford, 2000) was used for phylogenetic analysis. Heuristic analysis using parsimony 101 was also performed.

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#### RESULTS AND DISCUSSION

103 Results

104 In this investigation, Total 16 samples of coprophilous fungal isolates exhibiting different somatic phase characteristics

105 106



9 Figure 1. Five-day-old cultures of coprophilous fungal isolates from Banyumas Districtdistrict, 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 **Commented [AKG5]:** Provide coding of promers.

**Commented [AKG6]:** 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.

Commented [AKG7]: ??

**Commented [AKG8]:** 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. 115 LP1-5) indicates indicated possible RNA contamination; while, and a ratio less than 1.8 (viz., KN3-3) indicates-indicated

116 possible protein contamination (Sambrook & Russel, 2001). Several isolates (viz., KN1-1, KN3-1, KN3-3, and LP1-2) had

117 concentrations substantially less than 20 ng/ $\mu$ l, which was not optimal for spectrophotometric analysis; however, in 118 general the DNA of these isolates exhibited reasonably good purity.

120 Table 1. Fungal genomic DNA quantification

No.	Nama Sample	Conc. (ng/µl)	A260/280	A260/230	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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No	Name				Sequence	15		
		Sequenc	e Assembly	636bp				
		1 1	GAACCTGCG	GAAGGATCAT	TATCGAGTTT	TGAACGGGTT	GTAGCTGGCC	TTTAACGAG
		01	TATGTGCACG	CCTGGCTCAT	CCACTCTCAA	CCTCTGTGCA	CTTTATGTAA	GAAACGGTG'
		121	AAGCCAGCTA	TTTAATAGTC	GGTAATAAGC	CTTTCTTATG	TTTACTACAA	ACGCTTCAG
		241	TATAGAATGT	CATCAACAAC	CCACCCAATT	ATATACAACT CCCATAACTA	ATCTCAGCAACG	CACAATTCA
1.	KN1-1	301	TCAATCATCC	ATCTTTCAA	CCCACCTTCC	ACTOCATOCAT	ATTCCCACCA	CTATCCCTC
		361	TTGACTCTCA	TCCAATTCTC	AACCCCTAAA	TTTTCTAATC	ALTCCGAGGA	GIAIGCOIG
		421	TGGAGGTTGT	GTCGGCTTCT	AGTEGACTEC	TCTGAAATGT	ATTAGCGTGA	ATCTTACGG
		481	TCGCCTTCAG	TGTGATAATT	ATCTGCGCTG	TGGTGTTGAA	GTATTTATTA	GTTCATGCT
		541	ATAGTCGTCT	CTTACCGAGA	CAATTTATGA	CAATCTGAGC	TCAAATCAGG	TAGGACTAC
		601 C	GCTGAACTT	AAGCATATCA	ATAAGCCGGA	GGAAGG		
		Sequenc	e Assembly	533bp				
		1 7	AGGTGAACC	TGCGGAAGGA	TCATTAGTGA	TTGCCTTTAT	AGGCTTATAA	CTATATCCA
		101 1	TACACCTGT	GAACTGTTUT	ACTACTIGAC	GCAAGTCGAG	TATTTTTACA	AACAATGTG
		121 4	ATGAACGTC	GITTTATTAT	AACAAAATAA	AACTITCAAC	AACGGATUTC	TIGGCTUTC
2.	KN1-2	181 0	ATCGATGAA	GAACGCAGCG	AATTGCGATA	AGTAATGTGA	ATTGCAGAAT	TCAGTGAAT
	0.0000000000000000000000000000000000000	301 6	TCATCAAAT	CTCAACGCAGC	ACCOUNTCOL	ANTOCATTOC	ATTTCCCCCCT	CTGCCATT
		361 7	GATCGCTCG	CCTTABABAGA	GTTAGCAACT	TTGACATTAA	TGTCTGGTGT	AATAACTTT
		421 2	CTEGETCCA	TTGTGTTGAA	GCGTGCTTCT	AATCGTCCGC	AACCACAATT	ACTTTCACT
		481 T	GGCCTGAAA	TCAGGTAGGA	CTACCCGCTG	AACTTAAGCA	TATCAATAAG	CGG
		Sequenc	e Assembly	647bp				
3.	KN3-1	1 A	GGATCATTA	TCGAGTTTTG	AAACGGGTTG	TAGCTGGCCT	TCCGAGGCAT	GTGCACGCC
		61 T	GCTCATCCA	CTCTACACCT	GTGCACTTAC	TGTGGGTTTC	AGGAGCTTCG	AAAGCGAGA
		121 /	AGGGGCCTT	CACGGGCTTT	TTTCTTGCCT	AGTTGTTACT	GGGCCTACGT	TTCACTACA
		181 4	CACTTATAA	AGTATCAGAA	TGTGTATTGC	GATGTAACGC	ATCTATATAC	AACTTTCAG
		291 8	ACGGATUTC	TIGGCICICG	ATCGATGAA	GAACGCAGCG	AAATGCGATA	AGTAATGTG
		361	CCACCATCC	CTOTTTCACT	CTCATCAPAT	TOTCALCOTA	ACCOUNT	AACCOCRCT
		421 P	CTTTACCOT	TCCACTTCCA	GICATGAAAT	GCCTTCCAACCTA	ACCOGGITTCTT	AACGGGACT
		491 7	AAATCCATT	ACCTTCCTTC	CTCTCCCCAT	CCCCTCACCC	TOTOATAATT	CTCTACCCC
		541	GACCGTTCA	AGCGTTTTTA	TAGGCCAGCT	TCTAGTCGTC	TCTTTACCAC	ACAATAATC
		601 T	CGAACTCTG	ACCTCAAATC	AGGTAGGACT	ACCCGCTGAA	CTTAAGC	
_		Sequenc	e Assembly	584bp				
		1 A	GGTGAACCT	GCGGAAGGAT	CATTACCGAG	TGCGGGTCCG	CGTGGCCCAA	CCTCCCACC
		61 0	TGCCTATTG	TACCCTGTTG	CTTCGGCGGG	CCCGCCAGCC	TTCGGGCTGG	CCGCCGGGG
		121 0	CGTCTCGCC	CCCGGGCCCG	TGCCCGCCGG	AGACCCCAAC	ATGAACCCTG	TTCTGAAAG
		181 7	TGGTGTCTG	AGTGTGATTG	TTTGCAATCA	GTTAAAACTT	TCAACAATGG	ATCTCTTGG
4.	KN3-2	241 7	CCGGCATCG	ATGAAGAACG	CAGCGAAATG	CGATAACTAA	TGTGAATTGC	AGAATTCAG
		301 0	AATCATCGA	GTCTTTGAAC	GCACATTGCG	CCCCCTGGTA	TTCCGGGGGGG	CATGCCTGT
		361 0	GAGCGTCAT	TGCTGCCCTC	AAGCCCGGCT	TGTGTGTTGG	GCCCTCGTCC	CCCGGCTCC
		421 0	GGGGGACGGG	CCCGAAAGGC	AGCGGCGGCA	CCGCGTCCGG	TCCTCGAGCG	TATGGGGCT
		481 7	GTCTTCCGC	TCTGCAGGCC	CEECCEECEC	CCGCCGACGC	ATAACAACTT	TTTTTCCAG
		541 T	TGACCTCGG	ATCAGGTAGG	GATACCCGCT	GAACTTAAGC	ATAT	
		Sequenc	e Assembly	670bp	TOCACTERTS	AAAccommo	TACCTCCCCT	TCCCACCCA
		61 0	TGCACGCCC	TGCTCATCCA	CTCTACACCT	GTGCACTTAC	TGTGGGTTTC	AGGAGCTTC
		121 7	AAGCGAGAA	AAGGGGCCTT	CACGGGGCTTT	TTTCTTGCCT	AGTTGTTACT	GGGCCTACG
		181 7	TCACTACAA	ACACTTATAA	AGTATCAGAA	TGTGTATTGC	GATGTAACGC	ATCTATATA
		241 #	ACTTTCAGC	AACGGATCTC	TTGGCTCTCG	CATCGATGAA	GAACGCAGCG	AAATGCGAT
5.	KN3-3	301 P	GTAATGTGA	ATTGCAGAAT	TCAGTGAATC	ATCGAATCTT	TGAACGCACC	TTGCGCTCC
		361 7	GGTATTCCG	AGGAGCATGC	CTGTTTGAGT	GTCATGAAAT	TCTCAACCTA	ACGGGTTCT
		421 7	ACGGGACTT	GCTTTAGGCT	TGGACTTGGA	GGTTCTTGTC	GGCTTGCTTC	AATGTCAAG
		481 0	GGCTCCTCT	TAAATGCATT	AGCTTGGTTC	CTGTGCGGAT	CGGCTCACGG	TGTGATAAT
		541 0	TCTACGCCG	CGACCGTTGA	AGCGTTTTTA	TAGGCCAGCT	TCTAGTCGTC	TCTTTACGA
		601 F	CAATAATCA	TCGAACTCTG	ACCTCAAATC	AGGTAGGACT	ACCCGCTGAA	CTTAAGCAT
-		001 T	CAATAAGGC					
		1 2	GGGATCATT	ACCGAGTTTA	CAACTCCCAA	ACCCCTGTGA	ACATACCAAT	TGTTGCCTC
		61 0	CGGATCACC	CCGCTCCCCC	TAAAACGGCA	CGGCCCGCCA	GAGGACCCCT	AAACTCTCT
		121 7	CTATATGTA	ACTTCTGAGT	AAAACCATAA	ATAAATCAAA	ACTTTCAACA	ACGGATCTC
-	1000000000	181 7	GGTTCTGGC	ATCGATGAAG	AACGCAGCAA	AATGCGATAA	GTAATGTGAA	TTGCAGAAT
6.	KN4-1	241 0	AGTGAATCA	TCGAATCTTT	GAACGCACAT	TGCGCCCGCC	AGTATTCTGG	CGGGCATGC
		301 7	GTTCGAGCG	TCATTTCAAC	CCTCAAGCCC	CCGGGTTTGG	TGTTGGGGAT	CGGCGAGCC
		361 7	TGCGGCAAG	CCGGCCCCGA	AATCTAGTGG	CGGTCTCGCT	GCAGCTTCCA	TTGCGTAGT
		421 0	TAAAACCCT	CGCAACTGGT	ACGCGGCGCG	GCCAAGCCGT	TAAACCCCCA	ACTTCTGAA
		481 G	TTGACCTCG	GATCAGGTAG	GAATACCCGC	TGAACTTAAG	CA	
7.	KB1-1			Rep	eat Sequencin	g Process		
- 8		Sequence	Assembly	516bn				
		1	TGATATGCTT	AAGTTCAGCG	GGTAGTCCTA	CCTGATTTCA	GGCCAGAGTC	AAAGTAATT
8.	BJ3-1	61	TCCTTGCGGA	CGATTAGAAG	CACGCTTCAA	CACAATGGAC	CCAGTGAAAC	TTATTACAC
		121	AGACATTAAT	GTCAAACTTG	CTAACTCTTT	TAAGGCGAGC	GATCAGAGAT	CGCAGACGC
		181	CAAATCCAAT	CCATTAGGAA	ACCCTAGTGG	TTGAGATTTC	ATGACACTGA	AACAGGCAT
		241	CTCTCCGGAA	TACCAGAGAG	CGCAAGCTGC	GTTCAAAGAT	TCGATGATTC	ACTGAATTC
		301	GCAATTCACA	TTACTTATCG	CAATTCGCTG	CGTTCTTCAT	CGATGCGAGA	GCCAAGAGA
		361	CCGTTGTTGA	AAGTTTTATT	TTGTTATAAT	AAAACGACGT	TCATTACACA	TTGTTTGTA
		421	AAATACTCGA	CTTGCGTCAA	GTAGTAGAAC	AGTTCACAGG	TGTAAGTGGA	TATAGTTAT
		481	AGCCTATAAA	GGCAATCAC	AATGATCCT	r ccgcag		
		Sequenc	e Assembly	539bp				
		1	CCGTAGGTG	AACCTGCGGA	AGGATCATTA	GTGATTGCCT	TTATAGGCTT	ATAACTATA
		61	CACTTACAC	CTGTGAACTG	TTCTACTACT	TGACGCAAGT	CGAGTATTTT	TACAAACAA
		121	GTGTAATGAA	CGTCGTTTTA	TTATAACAAA	ATAAAACTTT	CAACAACGGA	TCTCTTGGC
9.	LP1-3	181	CTCGCATCGA	TGAAGAACGC	AGCGAATTGC	GATAAGTAAT	GTGAATTGCA	GAATTCAGT
~		241	AATCATCGAA	TCTTTGAACG	CAGCTTGCGC	TCTCTGGTAT	TCCGGAGAGC	ATGCCTGTT
		301	CAGTGTCATG	CHCCCCCTTA	LACTAGGGTT	AACTTAATGGA	TIGGATTTGG	GCGTCTGCG
		421	TTTCACTO	TCCATTON	TGAAGCORGO	TTOTATO	CCCCARCERC	ANTTACTO
		491	ACTCTCCCCT	CABATCAGGT	ACCACTACCO	CCTCAACTT	ACCATATCA	TRACCOCA
			and the second sec	second second 1 1 - FR1 all all all	management of a state of the	and the second sec	and the second sec	a second bit is an in the second bit is a s

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

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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 145 146 147

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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. 149

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

N		Result Links					
IN	Samples	Description	Max	Total	Query	E	Den Island
0.		Description	Score	score	cover	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,k	J780757.	1,KF1518	51.1,KT844	4687.1,KF	850375.1,L
		C431580.1,MK775821.1,KM388611.1,HQ891300.1,	LC312413	3.1			
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,M	IT136544	.1,MK267	768.1,MG2	41533.1,1	Y105711.
		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,F	T273380	.1,KT273	379.1,KT27	3370.1,KT	[273364.1,
		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,M	AH292842	2.1,LC152	416.1,HQ6	07958.1,M	IK450628.1
		,MH292844.1,KM613137.1,MH865978.1,KX443215	5.1,KX443	3211.1			
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%
	1	Lantinus squamoqulus voucher WADD124	1120	1120	0.80/	0.0	1000/

**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,K	T273380.	1,KT2733	70.1,KT956	5127.1,KT	273373.1,
		KT273379.1,KT273364.1,JN253598.1,MH053154.1,	KP283484	4.1			
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,N	IT549849	.1,MN817	705.1,MN8	17704.1,N	AT477707.
		1,MT477704.1,MT466521.1,MT447544.1,MT434005	5.1,MT37	8328.1			
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,K	Y105746.	1,KY105	745.1,KY10	5736.1,H	M802133.1,
		<u>NR</u> 111353.1,EF153624.1,NR 073242.1,MT482659.1	1,MT4826	58.1			
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(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,K	Y105736.	1,HM802	133.1,NR		
		111353.1,NR073242.1,KY105746.1,MT482659.1,MT	Г136544.1	,MK6059	36.1,MK26	7768.1	





159 160 161

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

**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.

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





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









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



#### 176 177 178

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



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



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

#### 186 Discussion

Based on the nucleotide BLAST searches (Table 3) and the resulting phylogenetic trees (Figs. 2-9), several of the 187 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*. 188 189 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 190 191 192 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*). 193 194 Furthermore, morphological analyses can be performed to complement the obtained molecular data. The 16S rRNA 195 markers of microorganisms such as fungi tend to be very similar or identical at the species level when the identity exceeds 196 97.5%, whereas the identity threshold is 95% at the genus level (Stackebrandt and Goebel, 1994).

197 The presence of these coprophilous fungi in cow dung demonstrates their adaptability to complex lignocellulosic 198 materials. Cow dung provides a habitat for various types of organisms, including coprophilous fungi, which break down 199 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<sub>2</sub>O<sub>5</sub> (0.22–0.34%), and potassium as (total K)/K<sub>2</sub>O (0.36–0.56%) (Melsasail et al., 2019). Commented [AKG11]: Add as results.

201 The fungal genera isolated and identified in this study have never been reported as being coprophilic, except for 202 Trichosporon spp., which has been found in chicken manure (Obire et al., 2008), buffalo dung (Lorliam et al., 2013), and 203 rhino dung (Makhuvele et al., 2017). Fusarium comprises soil-borne plant pathogenic species (e.g., F. fujikuroi) (Al-204 Ansari, 2018; Cen et al., 2020). Ceriporia lacerate grows on wood; Wulandari et al. (2018), found two resupinate fungal 205 specimens in East Kalimantan classified as Ceriporia species, C. inflata and C. lacerata, which were identified based on 206 morphological characteristics and the ITS and nuclear ribosomal large subunit sequences. L. squarrosulus is an edible 207 fungus commonly found growing in the wild on decaying tree trunks during the rainy season. Similar to other macrofungal 208 species, this fungus can grow in a wide variety of substrates and habitats. Many Lentinus species have been reported to 209 grow in nature on special substrates as well as on pasteurized substrates (Morais et al., 2000, Philippousis et al., 2001). Hu 210 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 211 responsible for the destruction of the temple stones over time. 212

213 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 214 the genus level, we identified Fusarium sp., Aspergillus sp., and Trichosporon sp. Further investigations are needed to 215 identify the fungi morphologically and to evaluate the utility of these fungi for various human interests. 216

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## Molecular identification of coprophilous microfungi from Banyumas DistrictRegency, Central Java, Indonesia

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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 7 8 9 10 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 regencydistrict has been limited to macroscopic genera, sp the results have not been able to provide a comprehensive picture of the presence of coprophilous fungi in the region. Identification of 12 13 the types of microscopic coprophilous fungi that live in herbivorous animal waste, such as lignocellulosic material, is necessary to 14 15 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 16 analysed using the molecular methods of DNA isolation, gene amplification bareoding analysis, DNA sequencing and phylogenetic 17 analysis of fungal cultures. The following species and genera were identified: Ceriporia lacerata, Trichosporon insectorium, Lentinus 18 squarrosulus, Fusarium sp., Aspergillus sp., and Trichosporon sp.

19 Keywords: molecular identification, coprophilous fungi, inventory

20 Running title: Molecular identification of coprophilous microfungi

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#### INTRODUCTION

Coprophilous fungi are saprophytic fungi that live <u>in-on</u> animal dung. These fungi utilize the faeces of various animals, especially herbivores, as their substrates (Melo et al., 2012). <u>Masunga et al. (2006) reported that coprophilousThese</u> fungi belong to the phyla Zygomycota, Ascomycota and Basidiomycota (<u>Masunga et al. (2006)</u>. 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 <u>cosmopolitely widely [widely?]</u> 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.

31 According to Mumpuni and Wahyono (2016), four Four genera of macroscopic coprophilous fungi, Coprinopsis 32 Panaeolus, Mycena, and Stropharia, were found in the coastal tourism area of Parangtritis, Yogyakarta, Indonesi 33 34 (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, 35 Leucocoprinus, Mycena, and Hypholoma, in the former Banyumas District-residencedistriet (District-regencies of 36 Banjarnegara, Purbalingga, Banyumas, and Cilacap). The studies on coprophilous fungi obtained from those from t 37 38 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 39 needed.

40 Zuber et al. (2011) reported that the standard method for identifying fungal species is morphological analysis, which 41 consists of macroscopic and microscopic observations. Macroscopic analysis consists of the determination of the colour, 42 size and structural characteristics of the fruiting body. Further analysis of microscopic characteristics is performed mainly 43 by comparison of spore appearance. An alternative to morphological analysis is the identification of fungal species based 44 on phylogenetic studies. Among such studies, the DNA forensic method (Herbert et al., 2004) has been applied to evaluate 45 polymorphisms in two noncoding polymorphic internal transcriber spacers (ITS1 and ITS2). The ITS regions are 46 extremely useful for species identification because of their long, sequential polymorphisms. DNA sequence analysis of 47 ITS1 and ITS2 has been successfully used for taxonomic studies of fungi (Nilson et al., 2008), and these regions are 48 common markers used for the identification of fungal species (Lee et al., 2000). Studies have proven that the ITS region

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49 provides excellent results in molecular systematics down to the species level, as well as in the determination of 50 geographical variations among species. ITS1 and ITS2 are present in multiple copies in the genome, so they can be 51 amplified even in damaged marking material [not understood], which still gives significant results in forensic studies. 52 Studies have evaluated the effectiveness of ITS polymorphism analysis for forensic purposes in the differentiation of 53 psychotropic fungi of the genera Panaeolus and Psilocybe, based on the lengths of polymorphisms identified in ITS1/2 54 amplification products.

55 56 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 57 identification of fungal species since the 1990s (White et al., 1991; Burns et al., 1991). However, strategies based on 58 sequencing of standardized genomic fragments (DNA barcoding) was recognized much later (Hollingsworth, 2007). The 59 primary difference between molecular identification tools and the "DNA barcode" approach is that the latter involves the 60 use of a standard DNA region that is specific for a taxonomic group. Badotti et al. (2017) suggested that one advantage of 61 using the ITS region as a standard marker is that most fungal species have been identified based on this genomic region.

62 To reveal the taxonomic identity and bioprospection of coprophilous fungi, we investigated and identified microscopic 63 coprophilous fungi obtained in the Banyumas District district regency in Central Java, Indonesia.

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#### MATERIALS AND METHODS

#### 65 Study area 66

We surveyed The survey of study area for the collection of the coprophilous fungi from samples of cow dung collected from thewas carried out in Baturraden, Kedungbanteng, and Cilongok districts (ranged between 7°03' – 7°38' South Latitude and 109°10' – 109°25' East Longitude) in the Banyumas District in Central Java, Indonesia. 67

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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-monthold dung pile in a landfill with the help of a pry tool. The coprophilous fungi were isolated via a 10<sup>-3</sup> to 10<sup>-5</sup> dilutio <u>adilution</u> 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  $6.8\pm0.2$ ) 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.

#### 85 Molecular identification of coprophilous fungi

Isolation of DNA from the purified coprophilous fungal isolates was performed using the Presto<sup>TM</sup> Mini gDNA kit for 86 87 yeast (Geneaid) until 100 µl of the DNA solution was obtained. DNA solutions were used immediately for PCR analysis 88 or stored at -80°C for later analysis. The ITS locus was amplified using the primer sequences of ITS1 Commented [AKG3]: Confusing statement. Check and rewrite.

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89 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATGC-3'). The PCR mixture (25 µl total 90 volume) consisted of 1 µl genomic DNA template, 12.5 µl 2× MyTaq Red Mix (Bioline), 1 µl each primer (20 µM/µl), 91 and 9.5 µl double-distilled H2O. Amplification was carried out for 35 cycles on the Applied Biosystems 96-Well 92 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<sup>TM</sup> Gel DNA Recovery Kit (Zymo 93 94 95 Research). The purified PCR products were then outsourced to PT Genetika Science Indonesia for DNA sequencing. The 96 sequence data were submitted to GenBank (http://www.ncbi.nlm.nih.gov/) for data analysis.

#### 97 98 Data analysis

99 Electropherograms were edited manually, contigs were merged, and multiple alignments were made for all data 100 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 101 102 was also performed.

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#### RESULTS AND DISCUSSION

Results In this investigation, Total 16 samples of coprophilous fungal isolates exhibiting different somatic phase characteristics 105 106 were obtained (<u>(Fig. 1). Fig. 1).</u> The fungal isolates were purified and subjected to DNA extraction.

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Figure\_1. Five-day-old cultures of coprophilous fungal isolates from Banyumas Districtdistrictregency, Central Java, Indonesia. 112

LP1-4

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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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\_) indicates indicated area pure sample free of RNA and protein 119 contamination as they showed absorbance ratio of 1.8; samples a ratio greater than 1.8 (viz., KN1-1, KN1-2, KN2-1, KN3-120 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 121 indicated possible RNA contamination; while, and a ratio less than 1.8 (viz., KN3-3) indicates indicated possible protein 122 contamination (Sambrook & Russel, 2001). Several isolates (viz., KN1-1, KN3-1, KN3-3, and LP1-2) had concentrations 123 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.

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> 126 Table 1. Fungal genomic DNA quantification

No.	Nama Sample	Conc. (ng/µl)	A260/280	A260/230	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

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We also measured the 260/2330 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).

Table 1. shows the of genomic DNA quantification results for DNA extracts from the coprophilous fungal isolates. The



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.

1000	Name				and the second s			
		Sequer	nce 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
1.00	1000000000000	241	CTCTCGCATC	GATGAAGAAC	GCAGCGAAAT	GCGATAAGTA	ATGTGAATTG	CAGAATTCAG
1.	KN1-1	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		
-		Seque	nce Assembly	533bn				
		Judger	TAGGTGAACC	TGCGGBBGGB	TCATTACTCA	TTCCCTTTAT	ACCOUTATAA	CTATATCCAC
		61	TTACACCTCT	GAACTGTTCT	ACTACTTGAC	GCABGTCGAG	TATTTTACA	AACAATGTGT
		121	AATGAACGTC	GTTTTATTAT	AACAAAATAA	AACTTTCAAC	AACGGATCTC	TTGGCTCTCG
		1.01	CATCCATCAA	CANCECACEC	AATTCCCATA	ACTAATCTCA	ATTCCACAAT	TCACTCAATC
2.	KN1-2	241	ATCGAATCTT	TGAACGCAGC	TTGCGCTCTC	TGGTATTCCG	GAGAGCATCC	CTGTTTCAGT
		301	GTCATGAAAT	CTCAACCACT	AGGGTTTCCT	AATGGATTGG	ATTTGGGCGT	CTGCGATTTC
		361	TGATCGCTCG	CCTTABABAGA	GTTAGCAAGT	TTGACATTAA	TGTCTGGTGT	AATAAGTTTC
		421	ACTEGETCCA	TTGTGTTGAA	GCGTGCTTCT	AATCGTCCGC	AAGGACAATT	ACTTTGACTC
		4.91	TCCCCTCAAA	TCACCTACCA	CTACCCCCTC	AACTTAACCA	TATCAATAAC	CCC
-		Conver	1000010AAA	CAThe	CINCCOULD	ANGLINAGUA	INTONNIANG	000
2	KNI2 4	Sequer	ACCARCATOR	Deco commence	AAAccounte	TACCTOCCT	TOCOLOGIA	CRECTCOCCC
э.	KING-1	61	TCCTCATCA	CTCTACACCT	CTCCACTTAC	TRUCIGOCCI	ACCACCOTTCC	ARACCCACER
1		121	AACCCCCTT	CACGGGGCTTT	TTTCTTCCCT	AGTTGTTACT	GGGCCTACGT	TTCACTACAA
		181	ACACTTATAA	AGTATCAGAA	TGTGTATTGC	GATGTAACGC	ATCTATATAC	AACTTTCAGC
		241	AACCGATCTC	TTGGCTCTCG	CATCGATGAA	GAACGCAGCG	AAATGCGATA	AGTAATGTCA
		301	ATTCCACAAT	TCAGTGAATC	ATCGAATCTT	TGAACGCACC	TTGCGCTCCT	TEGTATTCCC
		361	ACCACCATCC	CTCTTTCACT	CTCATCAAAT	TCTCAACCTA	ACCOUNT	AACCCCACTT
		421	COTTACCOT	TECACTTECA	COTTOTACTO	GCCTTCCTTC	ANTOTCANCT	CCCCTCCTCT
		491	TAAATCCATT	ACCTTCCTTC	CTCTCCCCAT	CCCCTCACCC	TCTCATAATT	CTCTACCCCC
		541	CCACCCTTCA	ACCOTTTTTA	TACCCCACCT	TCTACTCCTC	TOTORIGAL	ACAATAATCA
		601	TCCAACTCTC	ACCTCARATC	ACCTACCACT	ACCCCCTCAA	CTTAACC	ACABIABICA
_		Contract	Assembly	EQ.4ha	noornor	Heeseren	CI ITUIGO	
		Sequer	ACCTCAACCT	CCCCAACCAT	CATTRACCAC	Teccomece	CORCECCAN	COTOCOLOGO
		63	AGGI GRACCI	TACCORCETC	CHITACCORG	CCCCCCCCCCC	TRECCCCTCC	COLCECTOCC
		121	GIGCUTATIG	CCCCCCCCCCC	Teccoccocc	ACACCCCAAC	ATCAACCCTC	TTCTCARACC
		101	TTCCTCTCTC	ACTICATION	TUTCCASTCA	CTTADACCCARC	TCALCANTCC	ATCTCTCTTCCT
	KNI2 0	241	Treccenter	AGIGIGATIG	CACCCARATCA	CCATAACTAA	TOTACANTOG	ACCONTRACT
	KN3-2	301	CAATCATCCA	CTCTTTCAAC	CCACATTECE	CCCCCCTCCTA	TTCCCCCCCCC	CATCCCTCTC
		361	CGAGCGTCAT	TECTECCTC	AAGCCCGGCT	TGTGTGTTTGG	GCCCTCGTCC	CCCGGCTCCC
		421	GGGGGACGGG	CCCGAAAGGC	AGCGGCGGCA	CCGCGTCCGG	TCCTCGAGCG	TATGGGGGCTT
		481	TGTCTTCCGC	TCTGCAGGCC	CGGCCGGCGC	CCGCCGACGC	ATAACAACTT	TTTTTCCAGG
		541	TTGACCTCGG	ATCAGGTAGG	GATACCCGCT	GAACTTAAGC	ATAT	
1		Sequer	nce Assembly	670bp				
		1	AACCTGCGGA	AGGATCATTA	TCGAGTTTTG	AAACGGGTTG	TAGCTGGCCT	TCCGAGGCAT
				macman maca	CTCTACACCT	CTCCACTTAC	TGTGGGTTTC	ACCACCTTCC
		61	GIGCACGCCC	IGUIUMIUUM	O T P T LEP LEP P T	GIGCUCIINC	TO TO DO D T T TO	110011001100
		121	AAAGCGAGAAA	AAGGGGCCTT	CACGGGGCTTT	TTTCTTGCCT	AGTTGTTACT	GGGCCTACGT
		61 121 181	AAAGCGAGAAA TTCACTACAA	AAGGGGGCCTT	CACGGGCTTT	TTTCTTGCCT TGTGTATTGC	AGTTGTTACT	GGGCCTACGT
		61 121 181 241	AAAGCGAGAA TTCACTACAA AACTTTCAGC	AAGGGGGCCTT ACACTTATAA AACGGATCTC	CACGGGCTTT AGTATCAGAA TTGGCTCTCG	TTTCTTGCCT TGTGTATTGC CATCGATGAA	AGTTGTTACT GATGTAACGC GAACGCAGCG	GGGCCTACGT ATCTATATAC AAATGCGATA
5.	KN3-3	61 121 181 241 301	AAAGCGAGAA TTCACTACAA AACTTTCAGC AGTAATGTGA	AAGGGGCCTT ACACTTATAA AACGGATCTC ATTGCAGAAT	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC	TTTCTTGCCT TGTGTATTGC CATCGATGAA ATCGAATCTT	AGTTGTTACT GATGTAACGC GAACGCAGCG TGAACGCACC	GGGCCTACGT ATCTATATAC AAATGCGATA TTGCGCTCCT
5.	KN3-3	61 121 181 241 301 361	AAAGCGAGAA TTCACTACAA AACTTTCAGC AGTAATGTGA TGGTATTCCG	AGGGGGCCTT ACACTTATAA AACGGATCTC ATTGCAGAAT AGGAGCATGC	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTTGAGT	TTTCTTGCCT TGTGTATTGC CATCGATGAA ATCGAATCTT GTCATGAAAT	AGTTGTTACT GATGTAACGC GAACGCAGCG TGAACGCACC TCTCAACCTA	GGGCCTACGT ATCTATATAC AAATGCGATA TTGCGCTCCT ACGGGTTCTT
5.	KN3-3	61 121 181 241 301 361 421	GIGCACGCCC AAAGCGAGAA TTCACTACAA AACTTTCAGC AGTAATGTGA TGGTATTCCG AACGGGACTT	AGGGGCCTT ACACTTATAA AACGGATCTC ATTGCAGAAT AGGAGCATGC GCTTTAGGCT	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTTGAGT TGGACTTGGA	TTTCTTGCCT TGTGTATTGC CATCGATGAA ATCGAATCTT GTCATGAAAT GGTTCTTGTC	AGTTGTTACT GATGTAACGC GAACGCAGCG TGAACGCACC TCTCAACCTA GGCTTGCTTC	AGGCCTACGT ATCTATATAC AAATGCGATA TTGCGCTCCT ACGGGTTCTT AATGTCAAGT
5.	KN3-3	61 121 181 241 301 361 421 481	ATAGCGAGAA TTCACTACAA AACTTTCAGC AGTAATGTGA TGGTATTCCG AACGGGACTT CGGCTCCTCT	AAGGGGCCTT ACACTTATAA AACGGATCTC ATTGCAGAAT AGGAGCATGC GCTTTAGGCT TAAATGCATT	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTTGAGT TGGACTTGGA AGCTTGGTTC	TTTCTTGCCT TGTGTATTGC CATCGATGAA ATCGAATCTT GTCATGAAAT GGTTCTTGTC CTGTGCGGAT	AGTIGITACT GATGTAACGC GAACGCAGCG TGAACGCACC TCTCAACCTA GGCTTGCTTC CGGCTCACGG	GGGCCTACGT ATCTATATAC AAATGCGATA TTGCGCTCCT ACGGGTTCTT AATGTCAAGT TGTGATAATT
5.	KN3-3	61 121 181 241 301 361 421 481 541	ATAGCGAGAA TTCACTACAA AACTTTCAGC AGTAATGTGA TGGTATTCCG AACGGGACTT CGGCTCCTCT GTCTACGCCG	AAGGGGCCTT ACACTTATAA AACGGATCTC ATTGCAGAAT AGGAGCATGC GCTTTAGGCT TAAATGCATT CGACCGTTGA	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTTGAGT TGGACTTGGTC AGCGTTTTTA	TTTCTTGCCT TGTGTATTGC CATCGATGAA ATCGAATCTT GCTCATGAAAT GGTTCTTGCC CTGTGCGGAT TAGGCCAGCT	AGTIGITACI GATGTAACGC GAACGCAGCG TGAACGCACC TCTCAACCTA GGCTTGCTTC CGGCTCACGG TCTAGTCGTC	GGGCCTACGT ATCTATATAC AAATGCGATA TTGCGCTCCT ACGGGTTCTT AATGTCAAGT TGTGATAATT TCTTTACGAG
5.	KN3-3	61 121 181 241 301 361 421 481 541 601	ATAGCGAGAA TTCACTACAA AACTTTCAGC AGTAATGTGA TGGTATTCCG AACGGGACTT CGGCTCCTCT GTCTACGCCG ACAATAATCA	AAGGGGCCTT ACACTTATAA AACGGATCTC ATTGCAGAAT AGGAGCATGC GCTTTAGGCT TAAATGCATT CGACCGTTGA TCGAACTCTG	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTTGAGT TGGACTTGGAC AGCTTGGTTC AGCGTTTTTA ACCTCAAATC	TTTCTTGCCT TGTGTATTGC CATCGATGAA ATCGAATCTT GCCATGAAAT GGTTCTTGTC CTGTGCGGAT TAGGCCAGCT AGGTAGGACT	AGTIGITACI GATGTAACGC GAACGCAGCG TGAACGCACC TCTCAACCTA GGCTTGCTTC CGGCTCACGG TCTAGTCGTC ACCCGCTGAA	GGGCCTACGT ATCTATATAC AAATGCGATA TTGCGCTCCT ACGGGTTCTT AATGTCAAGT TGTGATAATT TCTTTACGAG CTTAACGATA
5.	KN3-3	61 121 181 241 361 421 481 541 601 661	GTGCACGCCC AAAGCGAGAA TTCACTACAA AACTTTCAGC AGTAATGTGA TGGTATTCCG AACGGGACTT CGGCTCCTCT GTCTACGCCG ACAATAATCA TCAATAAGGC	AGGGGCCTT ACACTTATAA AAGGGATCTC ATTGCAGAAT AGGAGCATGC GCTTTAGGCT TAAATGCATT CGACCGTTGA TCGAACTCTG	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTTGAGT TGGACTTGGAT AGCCTTGGTTC AGCGTTTTTA ACCTCAAATC	TTTCTTGCCT TGTGTATTGC CATCGATGAA ATCGAATCTT GTCATGAAAT GGTTCTTGTC CTGTGCGGAT TAGGCCAGCT AGGTAGGACT	GAGTGTAACGC GAACGCAGCG TGAACGCAGCG TCTCAACCTA GGCTTGCTTC CGGCTCACGG TCTAGTCGTC ACCCGCTGAA	GGGCCTACGT ATCTATATAC AAATGCGATA TTGCGCTCCT ACGGGTTCTT AATGTCAAGT TGTGATAATT TCTTTACGAG CTTAAGCATA
5.	KN3-3	61 121 181 241 361 421 481 541 601 661 <b>Sequen</b>	AAAGCGAGAA TTCACTACAA AACTATCAGA AGTAATGTGA TGGTATTCCG AACGGGACTT CGGCTCCCTCT GTCTACGCCG ACAATAATCA TCAATAATCA TCAATAAGGC	AAGGGGCCTT ACACTTATAA AACGGATCTC ATTGCAGAAT AGGAGCATGC GCTTTAGGCT TAAATGCATT CGACCGTTGA TCGAACTCTG	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTTGAGT TGGACTTGGA AGCTTGGTC AGCGTTTTTA ACCTCAAATC	TTTCTTGCCT TGTGTATTGC CATCGATGAA ATCGAATCTT GTCATGAAAT GGTTCTTGTC CTGTGCGGAT TAGGCCAGCT AGGTAGGACT	AGTTGTTACT GATGTAACGC GAACGCAGCG TGAACGCACC TCTCAACCTA GGCTTGCTTC CGGCTCACGG TCTAGTCGTC ACCCGCTGAA	GGGCCTACGT ATCTATATAC AAATGCGATA TTGCGCTCCT ACGGGTTCTT ACGGGTTCTT TGTGATAATT TCTTTACGAG CTTAAGCATA
5.	KN3-3	61 121 181 241 301 361 421 481 541 661 <b>Sequen</b> 1	GTGACGCCC AAAGCGAGAA TTCACTACAA AACTATCAGA AGTAATGTGA TGGTATTCCG AACGGACTT CGGCTCCTCT GTCTACGCCG ACAATAATCA TCAATAAGGC ACGGATCATT	AAGGGGCCTT ACACTTATAA AACGGATCTC ATTGCAGGAT AGGAGCATGC GCTTTAGGCT TAAATGCATT CGACCGTTGA TCGAACTCTG 522bp ACCGAGTTTA	CACGGGCTTT AGTATCAGAA TTGGCTCAGG CCGTTTGAATC CTGTTTGAGT TGGACTTGGA AGCTTTTGA ACCTCAAATC CAACTCCCAA	TTTCTTGCCT TGTGTATTGC CATCGATGAA ATCGAATCTT GTCATGAAAT GGTTCTTGTC CTGTGCGGAT TAGGCCAGCT AGGTAGGACT ACCCCTGTGA	AGTTGTTACT GATGTAACGC GAACGCAGCG TGGAACGCAGCG TGGACGCACCT GGCTTGCTCC CGGCTCAACGG TCTAGTCGTC ACCCGCTGAA ACATACCAAT	GGGCCTACGT ATCTATATAC AAATGCGCTCCT ACGGGTTCTT ACGGGTTCTT ACGGGTTCAT TGTGATAATT TCTTTACGAG CTTAAGCATA TGTTGCCTCG
5.	KN3-3	61 121 181 241 301 361 421 481 541 601 661 <b>Sequen</b> 1 61	GTACACGECE AAAGCGAGAA TTCACTACAA AGTAATGTGA TGGTATTCGG AACGGGACTT CGGCTCCTCT GTCTACGCCG ACAATAATGA TCAATAAGGC TCAATAAGGC AGGGATCAAT GCGGATCAGC	AAGGGGCCTT ACACCTATAAA AACGGATCTC ATTGCAGAAT AGGAGCATGC GCTTTAGGCT TAAATGCATT CGACCGTTGA TCGAACTCTG 522bp ACCGAGTTTA	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGGAATC CTGTTTGAGT AGCTTGGAT AGCTTGGTTC AGCGTTTTTA ACCTCAAATC CAACTCCCAA	TTTCTTGCCT TGTGTATTGC CATCGATGAA ATCGAATCTT GTTCATGAAATCTT GGTTCTTGTC CTGTGCGGAT TAGGCCAGCT AGGTAGGACT ACCCCTGTGA	AGTTGTTACT GATGTAACGC GAACGCAGCG TGAACGCACC TCTCAACCTA GGCTTGCTC CGGCTCACGG TCTAGTCGTC ACCCGCTGAA ACATACCAAT GAGGACCCCT	GGGCCTACGT ATCTATATAC AAATGCGCTCCT ACGGGTTCTT ACGGGTTCTT TGTGATAATT TCTTTACGAG CTTAAGCATA TGTTGCCTCG AAACTCTGTT
5.	KN3-3	61 121 181 241 301 361 421 481 541 601 661 1 21	GIGACGECE AAAGCGAGAA TTCACTACAA AACTTTCAGC AGTAATGTGA TGGTATTCGG AACGGGACTT CGGCTCCTCT GCTACGCCG ACAATAATCA TCAATAAGGC ACAATAATCA TCGATCAGC GCGATCAGC TCTATATGTA	AAGGGGCCTT ACACTTATAA AAGGGATCTC ATTGCAGAATCTC ATTGCAGAATCTC GCTTTAAATGCATT CGACCGTTGA TCGAACTCTG 522bp ACCGCGCCCGG ACTTCTGAGT	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTTGAAT AGCGTTGGAT AGCGTTTTTA ACCTCCAAATC CAACTCCCAA TAAAACGGA AAAACCATAA	TTTCTTGCCT TGTGTATTGC CATCGATGAA ATCGAATCTT GTCATGAAAT GGTTCTTGTC CTGTGCGGAT AGGTAGGACT AGGTAGGACT ACCCCTGTGA ACCCCTGTGA ATAAATCAAA	AGTTGTTACT GATGTAACGC CGACGCAGCG TCTAACGCACC GGCTTGCTTC CGGCTCACGG TCTAGTCGTC ACCCGCTGAA ACATACCAAT GAGGACCCCT ACTTCAACA	GGGCCTACGT ATCTATATAC AAATGCGATA TTGGGCTCCT ACGGGTTCTT AATGTCAAGT TGTTGATAATT TCTTTACCAG CTTAAGCATA TGTTGCCTCG AAACTCTGTT ACGGATCTCT
5.	KN3-3	61 121 181 241 301 361 421 481 541 601 661 121 181	GIGACGECE AAAGCGAGAA TTCACTACAA AACTTCAGC AGTAATCAG AGCAGGACTTC GGCTACGCCG ACAATAATCA CGGCTCCTCT GTCTACGCCG ACAATAATCA AGGATCATT GCGGATCATG GCGGATCAGC TCTATATGTA TGGTTCGGC	AGGGGCCTT ACACTTATAA AACGGATCTCA ATTGCAGAAT AGGAGCATGC GCTTTAAATGCATT CGACCGTTGA TCGAACTCTG 522bp ACCCAGTTTA CCGCTCCGGG ACTTCTGAGT ATCGATGAAG	CACGGGCTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTTTTGGACTTGA AGCTTGGTTC AGCGTTTTTA ACCTCAAATC CAACTCCCAA TAAAACGAGA AAAACCATAA AAACCATAA	ATTCTTECT TTTCTTECCT TGTETATEGC CATCGAATCAT GTCATGAAATCTT GTCATGAAACTT GGTCTTTCTC CTGTGCGGAT AAGGTAGGACT ACCCCTGTGA CGGCCCCCCA ATAAATCAAA AATCCGATAA	AGTTGTTACT GATCTAACGC GAACGCACCG TCTACAACCTA GGCTTGCTCC CGCCTCACGTA ACCCGCCCACGA ACCCGCCCACA GAGCACCCCTA ACATACCAAT GAGGACCCCT ACTTCAACA GTAATGTGAA	GGGCCTACGT ATCTATATAC AAATGCGATA TTGCGCTCCT AAGGTTCTT AATGTCAAGT TCTTACGAG CTTAAGCATA TGTTGCCTCG AAACTCTGTT ACGGATCTCT TTGCAGAATT
<b>5</b> . <b>6</b> .	KN3-3 KN4-1	61 121 181 241 301 361 421 481 541 661 661 121 181 241	AAACCGACGACA AAACCACACAA AACTTCACCACAA ACCTTCACC ACCAGACATCCCC ACCAGCACTCCCC CCCACCCCCCCCCC	IGETEATCER AAGGGGCCTT ACACGATCTC ATTCGAGAAT CGATCAGCATGC CGACCGTTGA TCGAACCTTG S22bp ACCGAGTTTA ACCGAGTTA ACCGAGTTA ACCGATGAG ACTCTCGAAC	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CACTTGGAC TGGACTTGGTC CACTCCCAA AGCGTTTTA ACCTCCAAATC CAACTCCCAA AAAACCATAA AACCGCAA AAACCACAA	ACCCCTGTGA ATCCCCTGGA GGTCTGAAAT GGTCTGCAAAT GGTCTGCGAAT TAGGCCAGCT AGGTAGGACT ACCCCTGTGA ACCCCCTGTGA AATCCCAAAA AATGCGATAA	AGTTGTTACT GATCGTACGC GAACCCACCG TGAACCCACG GCTTGCTACTC CGGCTGCTCC GGCTGCTCC ACCCGCTGAA ACATACCAAT ACATACCAAT GAGGACCCCT ACTTCAACA GTAATGTGAA AGTATTCTGG	GGGCCTACGT ATCTATATAC AAATGCGATA TTGGGCTCCT AAGGCTCCTT AAGTCAAGT TCTTAAGAG CTTAAGCATA TGTTGCCTCG AAACTCTCTT ACGGATCTCT TTGCAGAATTC
5. 6.	KN3-3 KN4-1	61 121 181 241 301 361 421 481 601 661 121 181 241 301	ARACCACACO AAACCACACA AACTTCACCACAA ACTTCACCACAA ACTTCACCACACAC CONTRACTOR ACATAACCA CCACTACACAC ACAATAACCA CCACTAAGCC CCACAACAAC TCAATAAGC TCAATAACA AGGATCATC CCCAATCACC CCAGTCACCCA CAGTCACCAC CAGTCACCAC	AGGGCCTT ACACTATAA AAGGGCCTT ACCGATTCC ATTCCAGAAT TAAATGCATT CGACCGTTGA TCGAACTCTG S22bp ACCGAGTTTA CCGCTCCGGG ACTTCGACT ATCGATGAAG TCGATCTAC	CACGGGGTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTTGAGT TGGACTTGGA AGCTTGGTTC AGCTGGTTC AGCTGCACATCA AAAACCGCA AAAACCGCAG AAAACCGCAG AAAACCGCAG CACAGCACCATAA	ATTOCTOCCT TGTGATATGC CATCGATGAA ATCCAATCAT GGTCATGAAAT GGTCATGAAAT GGTCATGGAC AGGCCAGCA AGGCCAGCCA ATAAATCAAA AATGCGATAA TGCGCCGCCC CCGGGTTTGG	AGTTGTTACT GATCTAACGC GATCCAACGACC TCTAACCTA GCTTGCTTC CGCCTCACG TCTAGTCGTC ACCCCCCTGAA ACATACCAAT GAGGACCCCT ACTTCCAACA GTAATGTGAA AGTATTCTGG	GGGCCTACG ATCTATATAC AATGCGCTCT AATGCGCTCT AATGCAGATA TTGCGTCTACGAG CTTAACGAG CTTAACGAG CTTAACGAG ACCTCCTC TGCTCCCCCG AAACTCTCT TGCAGAATT CGGCGAGCCC
5. 6.	KN3-3 KN4-1	61 121 181 241 361 421 421 421 421 601 661 121 121 121 121 301 301 301 301 301 301 301 30	ARACCAGACAA AAACCAGACAA AACTTCAGC AGTAATGTGA TGGTATTCAG AGGGCACTC GGCTACCCCTC GCCTACGCCG ACAATAATCA CCAATAATCA CGCGATCATC GCCGAATCAATCA TGGTATCTGGC CAGTGAATCA TGGCGCAAG TTTCCGACCG	AGGGCCTT ACACTATAA AAGGGCCTT ACCGATTATGAGAT GGACCGTTAGGCT TAAATGCATT CGACCGTTGA TCGAACGTTG ACCGAGTTTA CCGCTCCCGG ACTTCTGAGT TCAATCTGAAC TCGAACCTT TCATTCAAC	CACCEGGCTT AGTATCAGA TTGCCTCTCG TCAGTAGATC CTCTTTCAGT TGGACTTGGA AGCTGGTTA AGCTGGTA ACCTCAAA ACCTCCAA AACCATAA AACCACATAA AACCACACA CCTCAAGCCC CCTCAAGCCC	TTTCTGCT GTGTATGCC CATCGATGAT ATCCAATCT GTCATGAAAT GGTCTTGTC CTCTGCGGAT AGGTAGGACT AGGTAGGACTA AATCGAA AATGGATAA AATGGATTAG CGGCCCCCC CCGGGTTCGCT	AGTTOTACT GATGTAAGGG GAACGCAGCG TGAAAGCAACG GGCTGAACGA CGGGTCAACGA CCGGCTCAACG ACCATACCAAT ACATACCAAT ACATACCAAT GAGGACCCCT ACTTTCAACA GTAATGTGAA GTAATGTGAGA	GGGGCTACGT ATCTATATAC AATCCCATA TTGGGCTCTT ACGGTTCTT ACGGTTCTACAGG CTTAAGAG CTTAAGAG TGTTGCCTCG AAACCTCTGTT ACGGATCTCT TTGCGAGATCC CGGGCAGCCC CGGGGAGCCC CGGGCAGCC
5.	KN3-3 KN4-1	61 121 181 241 301 361 421 601 661 121 181 241 301 61 121 181 241 301 361 421	GTGGACGCCC AAAGCGACAA AGCTACCAG AGCTACCAG AGCTACTCAG AGCACTACGG AGCGGCCCCC GCCTACGCCG ACATAATCA AGGGACAT CCAATAATCA AGGGACATCAG CGGACCACC CAGTAATCAG CGGACCCGCAG TGCTCCGGCCAG TGCTCCGGCCAG GCAAAACCCT	AGGGCCTTATCA ACCGATTATAA AGGGCCTTAGCT ATTCCGACAT TAGGACCATGC CCTTAGCCT CGACCGTTGA TCGAACTCTG S22bp ACCGAGTTA ACCGAGTTA ACCGAGTTA ACCGAGTTA ACCGAGTTA CGGACTCTG TCGTTTCAAC CCGCCCCGA	CACGGGGTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTTCAGT TGGACTTGGA AGCTTGGTTC AGCGTTTTA ACCTCAAATC CAACTCCCAA ATAAACGGGA AAAACCACATAA AAAACCACATAA AACGCACCAA ACCCCGCCGCG	ATTOCTGCCT TGTGTATGCC CATCGATGAA ATCCAATCAT GGTCCTGGAA GGTCCTGGAC CGGCCGGCA ACCCCTGTGA CGGCCCGCCA ATAAATCAAA AATGCGATAA AATGCGATGA CGGGTTGGC CGGGTTGGC CGCCACCCGT	AGTTGTTACT GATGCTACTG GATGCAGCG TGAACGCAGC GCACGCAGCG TCTCAACGACC CGGCTGACG CCGCCTGCA ACCATACCAAT GAGGACCCCT ACTTCCAACA GTAATCTGA AGTATTCTGG GCACCTTCCA	GGGCGACGT ATCTATATAC ANATGCGATA TGGGGTCCT ATGTCAAGT TGTGATAATT TCTTTACGAG TGTTACCAG AAACTCTCT ACGGATCTCT TGCGAGAATT CGGCGAGCAC TTGCGAGACT
6.	KN3-3 KN4-1	61 121 181 241 301 361 421 541 601 541 601 121 181 121 181 241 301 361 421 481	GTGACGCCC AAAGCGACAA TTCACTACAA AACTTTCAG AGTATTCAG AGTATTCAG ACATAATGTA ACATTAAGCG ACATTAAGCG ACATTAAGCG CACATTAAGCG CGGATCATC TCATATGTA TGGTTCGACCG CAGTGAATCA TTGCGCCAG GTTAAACCTC	AGGGCCTT ACACTATAA AAGGGCCTT AGGACATTAGACT TAAATGCATT CGACCGTTG TCGAACTCTG S22bp ACCGAGTTA ACCGGTCCCGG ACTTCTGAGT TCGAATCTTT TCATTCTAAC CCGCCCCCGG CCGCCCCCGA CCGCCCCCGA	CACEGGCTTT AGTATCAGAA TTGCCTCTCG TCACTTCAGT TGGACTTGGA AGCTGGTT AGCTGGTTTA ACCTCAAATCC CAACTCCCAA AAAACCATAA GAACGCACAT GAACGCACAT CCTCAAGCCC CCTCAAGCCC ACCCGCGCCGC GAATACCCCG	TTICTIGCT GTGTATIGC CATGAATGAA ATCCAATGT GTCATGAAAT GGTCTTGTG CGTGTGCGGAT AGGTAGGACA ACCCCTGTGA ACCCCTGTGA ACCCCCGCG CGGCCCGCC CGGGCCGCC CCGGGTTGGG CCCAGCCGT TGAACTTAAG	AGTTOTACT GATGTAAGGG GAAGGCAGCG TGAAAGGACCG CGGCTGAAGG CCGGCTGAAGG ACCGCCTGAA ACATACCAAT GAGGACCCCT ACTTCCACG GTAATGTGGAA AGTATTCTGG GATGTTGGGAAT CACCCCCA CA	GGGCCTACGT ATCTATATAC AATCCCATA TTCGCCTCT AATCCCAT TTGGCATAATT TCTTATACAAG CTTAAGAG CTTAAGCATA TGTTGCCTCG AAACTCTTGT ACGGATCTCT TGCGAGAATT CGGGCAAGCC CGGCGAGCCC CGGCGAGCCC
5.	KN3-3 KN4-1	61 121 181 241 361 421 481 541 601 661 121 181 241 301 361 421 481	GTEGACECEC AAAGCGACACA TTCACTACAA AACTITCAG AGTATTCAG AGTATTCAG CAGGTCCTCT GTCTACCCCG ACAATAATCA ACGGACTCAT CCAATAAGGC CAGTCAGCCACG CAGTCAATCA TCCACTCAGCC CAGTCAACCA GTTGACCTCG GTAAAACCT GTTGACCTCG	AGGGCCTTATAA AAGGGCCTT ACACTTATAA AGGAGCATGC GCTTTAGCCT TAAATGCATT CGACCGTTGA TCGACTCCGG S22bp ACCGAGGTTTA GCCCCCCGG ACTCCTGAG TCGATCTGAG TCGATCTGAG CCGCCCCGA GATCAGGTAG	CACEGGCTT AGTATCAGAA TTGGCTCTCG TCACTGAATC CTGTTTGAATC TGGACTTGGA AGCTTGGTTC ACCTCAAATC CAACTCCCAA TAAAACGGA AAAACCATAA AAGCCACAA GAACGCACAA GAACGCACAA CCTCAAGCC AATCACCCG GAATACCCCG	STITCTISCOT CATCETATOC CATCETATOC CATCETATO GETATOTOC GETATOTOC GETATOTOC GETACOGAT AGGTAGGACT ACCCCTGTA CGGCCCCCCCA ATAATCAAA AATCCAAA TGCCCCCCCC CCGGCTTCGC CGGCTCTCCC TGAACTTAAC	AGTIGUTACT GANCGAACGC GAACGAACGC GGATGCAACGC CCCACTAACCTA GGCTGCCTC CGGCTCAGCTGAA ACCACTACCAAT GAGGACCCCCT ACTITCAACA AGTATTCTGG GCAGCTTCCA CA	GGGCCTACGT ATCTATATAC ANTCTATATAC ANTCTCATATAC ACGGCTCCT ACGGCTCCT GTGATAATT TCTTTACGAG CTTAACGAT TCTTACGAG AAACTCTGTT ACGGCAACTC CGGCGAGCC TTGCCGAATT CGGCGAGCC TTGCGGAGTA ACTCTGAAT
5.	KN3-3 KN4-1 KB1-1	61 121 181 241 301 421 481 541 601 661 121 181 241 301 421 481	GTGCACCCCC ARACCGACACA TACCTTCACAC ACTAATCTCA ACTGATATTCCG CACTAATCACA CGCTACCCCC ACAATAATCA TCAATAAGC TCCAATCACAC CCAGTCAATCAT CCGGTCACCCC CAGTCAATCATCA TCTTTCGACCG CTTCCACCCCA GTTGACCCCCC	AGGGCCCTT ACACTATAA AAGGGCCTT AGGACCTTATAG AGGACATCC GCTTAGGCT CGACCGTTGA CCGCCCCGTGA ACCGCTCCGG ACTCTCGACT CGACCCCGG ACTCTCGAC CCGCCCCGA ACCGCCCCGA ACCGCCCCGA CACCGCCCGA CCGCACCTGGT GACCAGGTGG GACCACGGTGA CGCACCTGGT	CACEGGETTT AGTATCAGAA TTGGCTTCG TCAGTAGATC CTGTTTGAGT TGGACTTGGA AGCTGGTC AGCGTGTTTA ACCTCAAATC CAACTCCCAA AAACCATAA AACCCACA AAACCATAA GACCGCCCCA CTCAAGCCC AATTAGEGG GAATACCCCCC	TTCTTGCT GTGTATGCC ATCGATGATGA ATCGATGATGA GTCATGAAAT GGTCTTGTGC CTGTGCGGAT AGGTAGGACCT AGGTAGGACCT AGGCCCGCC ATAAATCAAA AATCGCAATAA GGGCCCGCC CCGGGTTCGGTT GGCAAGCCTTGA GGACTTAAG	AGTIGUTACI GATGTAAGGG GAAGGAAGG TGTAAGGACC GGATGAAGGACC GGGTAAGG TCTAGACGAC GAGGACCCCTAA ACATACCAAT ACATACCAAT AGAGACCCCTA ACTITCAACA GAGGACCCCCA GAGGACCCCCA CA	GGGCCTACGT ATCTATATAC ANTEGCATA TTGCGCTCCT ACGGCTCCT ATGCCATAAGT TGTAGATAATT TGTTACGAG CTTAAGCATA TGTTGCCTCG AAACTCTCT TGCGCACCC CGGCGACCCC CGGCGACCCC CGGCGACCC CGGCGACCA
<b>6</b> .	KN3-3 KN4-1 KB1-1	61 121 181 241 301 361 421 481 541 601 601 601 61 121 181 121 181 241 301 301 421 481 481 542 121 481 542 121 181 181 181 182 182 182 18	GIEGACCCCC AAAGCGACAAATGTGA AACTITCAGC AGTAATGTGA TGGTATTCCG AACGGGACTTCCC GTCTACCCCCC GTCTACCCCCC ACAATAATGTGA TCAATAATGA CCCCCCCCCC	IGCTCRACCUM AAGGGGCCTT ACACGTTATAA AAGGGATCTC ATTGCACATCT CACTTAGACTTT CGACCTGC S2200 ACCGCTCCGG S2200 ACCGCTCCGGC ACCCCTCGACGACTTT CACTCCACGACGACG ACCCCCCCCCC	CACEGGCTTT AGTATCAGAA TTGGCTCTCG TCACTGAATC CTGTTTGGAT TGGACTTGGTAC AGCTTTTGAA ACCTCCAA ACCTCCCAA AAAACCGACAA AAAACCGCACAA AAAACCGACAA AAACCGACCAA AACCACAACAA AACCACAACAA AACCACAACA	Internetion Generation Generation Generation Generation Generation Generation Accounting Accounting Accounting Accounting Accounting Accounting Accounting Accounting Accounting Content Accounting Content Co	AGTITOTACT GATCTAACGC GAACGAACGCACCG TCTCAACCTA GGCTGCTCC GGCTCACGTA ACCCCCCTGAA ACATACCAAT ACATACCAACA GTAATGTGAA AGTATTCTAG GTGTTGGGGAT CCACCCCCCCA CA	GGGCCTACGT ANCTATATAC ANTECATATAC ANTECCATA THOGGCTCT ACGGCTCCT ACGGCTCCT TGTTACGAG CTTAAGCATA TGTTACCAGA CTTAAGCATA ACGGATCCTCT TGCGGAGACCC CGGGGAGCCC TGCGTACTA
5. 6. 7.	KN3-3 KN4-1 KB1-1	61 121 181 241 301 301 421 481 541 661 121 181 241 301 361 421 481 541 641 121 181 241 8 6 8 8 9 9 9 9 9 9 9 9 9 9 9 9 9	ATAGCACCOC AAAACCACACAA TACTTTAAC AACCACATATTCCA ACTAATCTCA TCATATTCCCA CTCTACCCCC ACCAATAATCA TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC CAGTGAATCAT CCAGTCAATCA TCATTTCGACCCG CAGTGAATCAT TTCCCCCCCC CAGTGAACCCCC CTTCACCCCCC	Пактонатсов Асасттатла Акастаттала Алсасалата остатакасалат содассоятол тодаластото 522bp Ассологода Астособлода Астособлода Содасстоо Содасстоо Содасото Содасто Содасото Содасто Сода Содасто Сода Сода Сода Сода Сода Сода Сода Сод	CACEGGCTT AGTATCACAA AGTATCACAAA TTGGCTCTCG TCACTGAATC CTGTTTGGTC TGGCTTGGTC CTGCTTGGTC CACTCCCAA AGCTTTTA ACCTCAAATC CAACTCCCAA AAAACCACAA AAAACCACAA AAAACCACAA ACCACACCAA ACCACC	INTECTION TGTATTTGC CATCGATGA ATCGATTGA GATCATTGC GTATTGC CTATGCGATA AGCCCCTGTGA CGGCCCCCCA ATAATCAA AATCGATAA ATCGGATGA CGGCTCCCC CCGGTTGCC GCAAGCCGT IGAACTTAAG	AGTIGUTACT GATCTAACGC GATCTAACGC TGAACGCACC TCTCAACCTA GGCTTGCTTC CGGCTCACGTA ACATACCAAT GAGGACCCCT ACCTTCAACA GTAATGTGAA AGTATTCTAG GTGTTGGGGAT CA	GGGCCTACGT ATCTATATAC ANTEGCATA TTGCGCTCCT ACGGCTCCT ACGGCTCCT TGTAGATAATT TGTTGCCTCG ANACTCTGT TGCGCACGA CTTAAGCATA CGGCACCCC CGGCGACCCC CGGCGACCCC CGGCGACCCC
5. 6. 7.	кN3-3 КN4-1 КВ1-1 ВЈ3-1	61 121 181 241 301 361 541 661 661 121 181 301 361 121 181 301 361 481 <b>Sequei</b> 1 1 301 361 361 361 361 361 361 361 36	GIEGACCCCC AAAGCGACACA TCAACTACGA AACTITCAGC AGTAATCTGA TGGTATTCCT AAGGGACTTC GCTAGGCG ACAATAATCG CAATAAAGC TCAATAAAGC TCAATAAAGC TCAATAATCATCA CAGTCAATCA TCTTCGACGG GTAAAACCCT GTTGCACCGC AGTCAACCCTG TTGCACCCG	INCIDENCICAL ANGGGECCTT ACACGTATATA ANGGGATCTT ATAGGAACTCT ATAGGAACTCTG CGACCGTTAAGCT TGAACGTTG CGACCGTTG ACCCAGTGAG ACCCACTGAG ACCCACTGAG CGACCCCCGA CGGACCCCCGA CGGACCGCCCGA CGGACCGCCCGA CGGACCGCCGA CGGACCGCCGA CGGACCGCCGA CGGACCGCGA CGCCCCCCGA CGGCCCCCCA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCGA CGGCCCCCCCC	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCACTGAGTC CTGTTTGGACT TGGACTTGGTC AGCCTTTTA ACCTCAAATC CAACTCCCAA AAAACCGACAA AAAACCGACAA AAAACCGACAA AAACCGACCAA AATCAGCGCCGC GAATACCCGC CGTAACCCTCAA	Internet of the second	AGTITOTACT GATCTAACGC GAACCAACGCACCG TCTAACGCACC CGCACCAACCAA GCCTGCCTCC GGCCTCACGCAA ACATACCAAT GACGACCCCA ACATACCAACA GTAATCTGAACA GTAATCTGAACA CAATTCGACAC CAACTCCAACACCCCA CA	GGGCCTACGT ANCTATATAC ANTECTATAC ANTECCATA THOGOCTACT ACGGCTECT ACGGCTECT TGTACAAGT TGTTACCAAGT TGTTACCAAGT ANACTCACT TGCTACCAGAATA CGGCACCCC CGGCGACCCC CGGCGACCCC CGGCGACCCC CGGCGACCCC CGGCGACCCC CGGCGACCCC CGGCGACCCC CGGCACCCCCCCC
5. 6. 7. 8.	КN3-3 КN4-1 КВ1-1 ВЈ3-1	61 121 181 241 301 361 421 481 661 661 661 121 361 421 481 361 421 481 561 421 481 121 181 361 421 481 121 481 561 421 481 561 421 481 561 481 561 481 561 481 561 481 561 561 561 561 561 561 561 56	ARACCARCACO ARACCARCARA TACATTACAR ACTANTERIA ACTRANTERIA TGATANTERIA TGATANGCA TCAATAAGCA TCAATAAGCA TCAATAAGCA TCAATAAGCA TCAATAAGCA TCAATAAGCA TCAATAAGCA TCAATAAGCA TCAATAAGCA TCAATAAGCA TCAATAAGCA TCAATAAGCA TCAATAACCA TTGCGCCAAG ATTACCACA TCAATAGCAT TCATATACCACA TCAATAAGCACA TCAATAAGCACA TCAATAAGCACA TCAATAAGCACA TCAATAGCAT	Постоятсоя Асасттатал Алсасататал Алсасалата остатадасалас седасосятал тодалстого S22bp Ассологода Астособлода Астособлода Сосаласатал Сосасособа Сосаласала Сосасасала Сосасала Сосасала Сосасала Сосасала Сосасала Сосасала Сосасала Сосасала Сосасала Сосасала Сосасала Сосасала Сосасала Сосасасала Сосасасала Сосасасала Сосасасала Сосасасала Сосасасала Сосасасала Сосасасала Сосасасала Сосасасала Сосасасала Сосасасала Сосасасала Сосасасала Сосасаса Сосасаса Сосасаса Сосасаса Сосасаса Сосасасаса Сосасасаса Сосасасаса Сосасасаса Сосасаса Сосасасаса Сосасасаса Сосасасасаса Сосасасасаса Сосасасасаса Сосасасасаса Сосасасасаса Сосасасасаса Сосасасасаса Сосасасасаса Сосасасасасасасасаса Сосасасасасасасасасасасасасасасасасасаса	CACEGGETT AGTATCAGAA AGTATCAGAAA TTGGACTTGGAC CTGTATTGGAC TGGACTTGGAC AGCTTTTA ACCTCAAATC CAACTCCCAA AAAACCACAA AAAACCACAA AAAACCACAA CCTCAAAGCC CAACTCCCCA AATCTACTCG ACCCCGCCCCA ACTCACCCCC CAACTCCCAA	CCTCGATTCAC CATCCGATCGA GATCCGATCGA GATCCATATCAT GTATTGACGATCAT GGATCTTGTC CTGTGCGGAT AGGCCCCCCGCA ATAAATCAAA AATCCGATCAA AATCCGATCAG GCCAGCTCGC GCGATCTCAC GCCAATCCGAT CCCCGATTTCAC CCCGATTTCAC	AGTITATACT GATCTAACGC GATCTAACGC TGAACGCACC TCTCAACCTA GGCTTGCTTC CGGCTCACGTA ACATACCAAT GAGGACCCCT ACCTTCAACA GTAATGTGAA GTAATGTGAA GAGTTTCGGGCA CA GCCCACACTC CCACCCCCA	GGGCCTACGT ATCTATATAC ANTEGCATA ATCTAGGCTCT ACGGCTCCT ACGGCTCCT TGTAGCATAAGT TGTTGCCTCG ANACTCTGT TGCGCACGAGACCC CGGCGAGCCC CGGCGAGCCC CGGCGAGCCC AAACTCAATG TTATTACACC
5. 6. 7.	КN3-3 КN4-1 КВ1-1 ВЈ3-1	61 121 181 241 301 361 421 481 541 661 121 181 361 421 481 <b>Seque</b> 1 361 421 481 541 661 121 121 121 121 121 181 121 181 121 181 121 181 121 181 121 181 191 191 191 191 191 191 19	GIEGACCCCC AAAGCGACAAC TICACTACAA AACTITCAC AGTAATCTGA AACTITCAC AGTAATCTGA AACTITCAC AACTATCAC AACTATCAC GICTACGCC GICTACGCC CACTAATAATCA CAATAATCAC CAGTCAATCAC TICACGCCAA GITAAACCCT GITGACCCCG CAATAATCCCA TICCCCCCAACA CATCACCCCG CAATAACCCT GITGACCTCCAA CAATATCCCAA CACTCCCAAC		CACGGGCTTT AGTATCAGAA TTGGCTCTCG TGACTGAACC CTGTTTGGACT TGGACTTGGTC AGCCTTTTA ACCTCAAATC CAACTCCCAA AAAACCGGA AAAAACGGA AAAAACGGA AAAACCGACAA AAACCACAA AACCACACAA AACCACACAA AACCACACAA CCACGGCCCGC CAATCACCCCC CAACTCCCAA CCACGGCCCCCC CAATCACCCCCC	Internetion General Construction Construct	AGTITOTACT GATCTAACGC GAACCAACGCACCG TCTAACGCACC CGCATCCAACCTA GGCTGCCTC CGGCTCACGC ACCCCCCGCGAA ACATACCAACA GTAATCCAACA GTAATCCAACA GTAATCTCAG GGCCACGACTCCA CAACTTCCACACA CAACTCCACACAC CACCCCCCCC	GGGCCTACGT ANTCTATATAC ANTCTATATAC ANTCCCATA TGCGCTCCT ACGGCTCCT TGCGATAATT TGTTACCAAGT TGTTACCAAGT TGTTACCACG ANACTCTCTT TGCGGAGACCC CGGGCAACCC CGGGCAACCC CGGCAACCC TTCCCTAAT ACTTCTGAAT
5. 6. 7. 8.	КN3-3 КN4-1 КВ1-1 ВЈ3-1	61 121 181 241 301 361 421 421 421 421 601 61 121 121 181 421 421 421 421 421 421 421 42	ARACCARCACO ARACCARCARCA ARACCARCARCARCA ACTAATCACA ACTAATACTCA ACTAATACTCA CONTACTCCA CONTACTCCA CONTACTCCA CONTACTCA CASSEMUL AGGATCATT CCASTACATTCA CASSEMUL AGGATCATTCA CASTACATTCA CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCC CASTCARCCCCC CASTCARCCCCC CASTCARCCCCC CASTCARCCCCC CASTCARCCCCC CASTCARCCCCC CASTCARCCCCCCCC CASTCARCCCCCC CASTCARCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	Постоятсоя Асхасттатла Алсасаттатла Алсасалата Состатладаслатас содассоста тодаластого 322bp Алсосадатала Ассосадатала Ассосадатала Сосдастосода Сосдастосода Сосдастаса Катасаладаа Сосдастасатала Алсосадала Сосдастаса Алсосадала Сосдастаса Алсосадала Сосдастаса Алсосадала Сосдастаса Алсосадала Сосдастаса Алсосадала Сосдастаса Сосдаста Сос	CACEGGCTTT AGTATCACAA AGTATCACAAA TTGGCTTCGA TCACTGAATC CTGTTTGGTC TGGCTTGGTC CTGTTGGTC CACTCCCAA AGCTTGGTAC CAACTCCCAA AAAACCAGA AAAAACGGA AAAACCACAA AATCTACTGG ACCCGCCGCGCG SATGCCCCA CACCCTCAA CACCCTCAA CACCCTCAA CACCCTCAA CACCCTCAA CACCCTCCTAA CACCCTCCTAA CACCCTCCTAA CACCCTCCTAA	CCTGATTAAC CGTCCATCAA ATCCAATCAT GTCATATGAC GTTCTTGTC CTGTQCGATT AGGCCACCT AGGCACCACCT AGGTAGGACCT ACCCCTGTGA CGGCCCCCCC CCGGCTTGGC CCGGCTTGCG CCAAGCCGT GCCAAGCCGT GCCAAGCCGT CGCCAAGCCGT CCCGATTTCAA CCCCGATTTCA CCCGATTTCAA CCCCGATTTCA CCCGATTTCAA CCCCGATTTCAA CCCCGATTTCAA CCCCGATTCAA	AGTIGATACG GATCGAACGA GATCGAACGA GAACGACCACC CCCACCAACGA GCCTGCTCC GGCCTCACGA ACATACCAAT GAGACCCCCA ACATACCAACA GTAATGCGAA GAATGCGAA GAACTCCAACA CA GCCCACACCCCA CA CA CCCCCCCACACCCCA CA CA	GGGCCTACGT ATCTATATAC ANTEGCATA ATCTATACCATA TGCGCTCCT ACGGTCCT TGTGATAATT TGTTGCCTCG AAACTCATT TGTGCCCCG GGCATCCC CGGCATCCC CGGCATCCC CGGCATCCC CGGCATCCC CGGCATCCC CGGCATCCC CGACAGCATG
5. 6. 7.	КN3-3 КN4-1 КВ1-1 ВЈ3-1	61 121 181 301 361 421 481 541 661 181 241 181 241 481 501 361 121 181 241 601 181 241 301 361 421 481 541 481 541 661 181 241 301 301 421 181 181 181 181 181 181 181 1	GIEGACCCCC AAAGCGACAACAA AACTITCAGC AGTAATCIGA AACTITCAGC AGTAATCIGA AGCOGACTICAGC AACAATCAGC GICTACGGCG GICTACGGCG GICTACGGCG CAATAATCACC TCAATAAAGC TCAATAAAGC TCAATAAGC TCAATAAGC GITAAACCCCA GITGACCCCG GITAAACCCAAT CAATACCCAAT CAATACCCAAT CAATACCCAAT CAATACCCAAT		CACGGGCTTT AGTATCAGAA TTGGCTCTCG TGGACTTGGAC TGGACTGGTC TGGACTTGGAC AGCTTTTGAA AGCTTGGTTC AACCTCAAATC CAACTCCCAA AAAACGGA AAAACGGACAA AAACCGACAA AATCAGCACCAA AATCAGCACCAA AATCAGCACCAA AATCAGCACCAA AATCAGCACCAA AATCAGCACCAA CACGGCCCCCC CAATCACCTCCAA CGCACCTCCCAA	Internet of the second	AGTITOTACT GATCTAACGC GAACCAACGCACCG TCTAACGCACC CGCACCAACCAA CCCCCCCGACAA ACATACCAAT GACGACCCCA CACATACCAACA GTAATCTGA GACTTCCAACA GTAATCTGA GACTTCCAACA CAATTCGACAC CACTCCAACA CAATTCGACAC CACCCCCCACCCCA	GGGCCTACGT ATCTATATAC ANTCTATATAC ANTCTCATATAC ANTCTCATAC ANTCTCATAC ANTCTCATAG TGTGCCTCG ANACTCATAT TGTTACCAG CTTAAGCATAC ACGGATCCCC CGGCGAGCCC CGGCGAGCCC CGGCAACCCC CGCCGAGCCT ACCAGCAGCAC CCCCAGAGCCT
5. 6. 7.	КN3-3 КN4-1 КВ1-1 ВЈ3-1	61 121 181 301 421 421 421 601 601 601 61 121 181 421 481 541 121 181 121 121 121 121 121 12	ARACCARCACO ARACCARCARCA ARACCARCARCA ACTAATCACA ACTAATACTACA ACTAATACTACA CONTATTCCG CONTATTCCG CONTATTCCG CCARTARACCA TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC CAGTCAATCACA CAGTCAATCACA CAGTCACACCA CAGTCACACCAC CAGTCACACCAC CAGTCACACCAC CAGTCACACCAC CAGTCACACCAC CAGTCACACCAC CAGTCACACCAC CAGTCACACCAC CAGTCACACCAC CAGTCACACCAC CAGTCACACCACAC	Постояться Асасттатала Алсасататала Алсасалата алсасалата ассалата содассогтала тодаластого 522bp Алсосаяртала сосастосая Ассасатала сосастосая Алсосаяртала сосастосая Сосастоса Сосасто	CACEGGCTT AGTATCAGAA TTGGCTCTCG TCACTGAATC CTGTTTGGTC TGGCTTGGTC TGGCTTGGTC CACTCCAAATC CAACTCCCAA AACCTCCAAACCC CAACTCCCCA AAAACCAGAA AAACCACAA CCTCAAGCCC CAATCTCCTCA CACCCCCCC Bat Sequencin CGTAGTCCTCA CCCCCTCCAA CCCCCCCCCA	CCTGATTCAC CATCCATCAGA ATCCAATCAT GTCATTGAC GTTCTTGTC CTGTQCGATT AGGCCACCT AGGCACCACCT AGGCACCCCCCC CCGGCTCGCC CCGGCTCGCC CCGGCTTCGC CCCGATTCAA CCCCGATTCAA CCCCGATTCAA CCCCGATTCAA CCCGATTCAA CCCGATTCAA CCCGATTCAA CCCGATTCAA CCCGATTCAA CACAACCGCACCA CCCGATTCCAA CACAACCGCACCA CCCGATTCCAACA CCCGATTCCAACA CCCGATTCCAACA CCCGATTCCAACA CCCGATTCCAACA CCCGATTCCAACA CCCGACCACCA CCCGATTCCAACA CCCCGATTCCAACA CCCGACCACCACCACCACCACCACCACCACCACCACCACC	AGTIGTACT GATCTAACGC GATCTAACGC GATCTAACGC TCTACTCAACGTA GCTTGCTC CGGCTCACGG TCTAGTCGTC ACCCCCTGAA ACATACCACA GACATACCACA GACATACCACA GACATCCAGA GACATCCAGA CA CACCCCCCCAGACC CCACTGGAAC CA CACCCCCCCCAGACC CCACTGGAAC CACCCCCCCCCC	GGGCCTACGT ATCTATATAC ANTEGCATA ATCTATACCATA ATGCGCTCCT ACGGCTCCT TGTGATAATT TGTTGCCTCG AAACTCAGT TGTTGCCTCG AAACTCCTT TGCGGAGCCC CGGCGAGCCC CGGCGAGCCC TTGCGTAGTA ACTTATACACC CACAGCGC ACTCAATTCT CCCAAGAGTC
5. 6. 7. 8.	КN3-3 КN4-1 КВ1-1 ВЈ3-1	61 121 181 301 361 421 481 541 601 661 121 121 121 121 121 121 12	GIEGACCOUC AAAGCGACGACA TICACTACGA AACTITCAC AGTAATCIGA AGGATTCCA TGGTATTCCC AGGATCACC TCAATAATCA TCAATAATCA TCAATAATCA TCAATAATCA AGGATCATC TCAATAATCA TCAATAATCA TCAATAATCA TCAATAATCA TCAATAATCA TCAATAATCA TCAATAACCC TCAATAACCC TCAATAACCC TCAATAACCC TCAATAACCC TCAATAACCC TCAATACCCA TCAATACCCA TCAATACCCA CAATCACTCAA CAATCACCA CCATTCACA CCACTCACA CCATTCACA CCACTCACA	INTERPEDIA INTERPEDIA	CACGGGCTTT AGTATCAGAA TTGGCTTCGGT TGGACTTGGAAC TGGACTGGTC CTGTTGGAAC TGGACTTGGAAC AGCGTTGGTTC CAACTCCCAA AAAACGGAA AAAACGGACAA AAAACGGACAA AATCTACTGA GGAATACCGG GAATACCGG GAATACCCG CGAATCCGTG CACGGCTCAA CTAACTCTTA		AGTITATACT GATCTAACGC GAACCAACGCACC TCTCAACCTA GGCTGCTCC CGGCTCACGTA ACCCGCTGAAC ACATACCAAT GAGGACCCCT ACCTCCAACA GTAATCTGA GTATTCGGGT CTTGGGGT CATTCCAACA CAATTCGGAC CACTCCAACA CAATTCCAACA CAATTCGGAC CACTCCAACA CAATTCCAACA CAATTCCAACA CAATCCCCCA CACCGACCCCA CAATCCCCCACCACACA CAATCACACACACAC	СССССТАССТ АТСТАТАТАС АЛТСТАТАТАС АЛТСТСАТАТАС АЛТСТСАЛСТ ТГОССТССТ АЛТСССАЛСТ ТСТТАССАЛС СТТААССАТС АЛАСТСТСТ ТСССТСС АЛАСТСТСТ ТСССАДАЛТСТ ТСССАДАЛТСТ ТСССАДАЛТСТ АЛАСТАЛТТ АЛАСТАЛТТ АЛАСТАЛТТ АЛАСТАЛТТ АЛАСТАЛТТ СССАДАЛСТ АЛСССАДАТСТ ТТССТАЛАТСТ ТТАТТАСАСС СССАДАЛСТ ТТАТТАСАСС
5. 6. 7. 8.	КN3-3 КN4-1 КВ1-1 ВЈ3-1	61 121 121 241 301 421 421 421 601 601 61 121 121 121 421 421 421 421 42	ARACCARCACCE ARACCARCARCA ARACCARCARCARCA ACTAATCACA ACTAATCACA CONTACTOR ACCARCARCACCE CONTACTOR ACCARCARCARC CONTACTOR ACCARCARCARC CARTAAACCCE CARTAAACCCE CARTAATCCCA CARTAACCE CARTAC	Пактонатося Ассастатала Алсасататала Алсасалата Алсасалата ассалата седассертала тосаластоте 522bp Ассасатаса Ассасатаса Ассасатаса Сосассаса Сосастаса Сосасасала Сосасала Сосасасала Сосасасала Сосасаса Сосасаса Сосасаса Сосасасаса Сосасасаса Сосасасаса Сосасасаса Сосасасаса Сосасасаса Сосасасаса Сосасасаса Сосасасаса Сосасасасаса Сосасасаса Сосасасасасаса Сосасасасаса Сосасасасасасасасасаса Сосас	CACEGGCTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTGGTC TGGCTTGGTC CTGTTGGTC CACTCCAAATC CACTCCCAA TAAAACGGA AAAACCACAA AAACCACAA AACCACACA CCTCAAGCCC CAATCACCGC CAATCACCGC CAATCACCGC CAATCACCG CAATCCCTA CGCAAGCCCC CAATCCTCT ACCCTAGTCG CCAAGCTCCTA CCCCACTCGT ACCCCTAGTGG CCAAGCTCCTA CCCCACTCGTG CAATCCTTGTGAAAC CAACTCCTAGTGG CAATCCTTA	TTCCTTCCCT TGCTATTGC CATCGATGA ATCGATTGT GTCATTGC GTTCTTGC GTTCTTGC GTCTTGCGATT AGGCCAGCT AGGTAGGACT ACCCCTGTGA CGGCCCGCC CGGGTTGCG GCGATCGGC GCGATCGCC GCGATTGCG CGGTCTCCCT GCAATGGAC TGAACTTCA TAAGCGACA TGAGATTC CTTCAAGAC GTTCCAAGAC GTTCCAAGACCT AGACCACT	AGTIGTACT GATCTAACGC GATCTAACGC GGATCGAACGA GCTTGCTC CGGCTCACGTA TCTAGTCGTC ACCCCCTGAA ACATACCACA ACATACCACA GTAATGTGAA AGTATTCTGG GTTTGGGAT CACTTCCAACA GCACCGCCCA CA GCACGAGACCCC CA CA CACCACAGACTC CATCAGAGAT ACGACCAGACTC CGATCGGAGAT CGATCAGACTC CGATCGGAGAT CCGTAGACTCGACA TCATTACACA TCATACACAC	GGGCGTACGT ATCTATATAC ANTGCGTATATAC ANTGCGATAC ATGCGCTCCT ACGGGTCCT TGTGATAATT TGTTGCCTCG ANACTCATT TGTGCCCCG AAACTCTCT TGCGAGACCCC CGGCGAGCCCC CGCAGACCCC AACACTAATTG TTATTACACC AACGCATCTATAC
5. 6. 7. 8.	кN3-3 КN4-1 КВ1-1 ВЈЗ-1	61 121 121 121 121 121 121 241 301 541 541 541 541 601 601 601 121 121 241 301 361 121 121 241 1 61 121 121 241 1 61 121 241 301 361 241 241 241 361 361 241 241 361 361 361 361 361 361 361 361 361 36	GTGCACCCCC AAAGCGACAA AACTTCACTACAA AACTTCACC AGTAATGTGG GGCTACGCG AACAGGACTT CGGCTCACGCG ACAATAATCA TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAGCAT TCATACGAC TCAATACCT TCAATACCT TCCATCCAGA ACACCTCAAT CTTCCCGGAA GCAATCACTA CAATACCAT CAATCCCAG CAATACCCAT	AGGEGECCTT AAAGGEGECCTT AAAGGEGECCTT AAAGGEGECTT AGGAGCATGA CGCTTAAAGGATT CGACCGTTAA TCGAACTCTG S22bp ACCCAGTAGAA CCGCTCCGGG ACCCGATCAGTAA CCGGCCCCGA CCGACTCGTAG RCP S16bp AAGTCAGCGA CGATTAGAAG CCCATAGAAG CCCATAGAAG CCCATAGGAA TACCTAGGAA	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCACTGAATC CTGTTGGACT TGGACTTGGAC CTGTTGGAC TGGACTTGGAC AGCGTTGGTTC CACCTCCAA ACCCCCAAACCG CAACCACCAA CCACCCCCCC CACCAGCCCC CAACCACCAC CACCCCCCCA CACCCCCCCC	Internection Generation Arccastran Arccastran Generation Generation Arccastran Generation Arccastran Arccastran Arccastran Arcastran Arcastran Arcastran Arcastran Generation Generation Construct Cacastragen	AGTTOTACT GATCTAACGC GACCAACGCACC CCCACCAACGA GCCTGCCTCC CGCCTCACGA ACATACCAAT GACGACCCCT ACCCCCCGAGA ACATACCAAT GACGACCCCT GACTTCGGAT CATTCCAACA GAATACCAAC CACCACACACCCA CA CACTCCAACA CACCCCCCACACAC CACCACACACCCA CACCCCCC	GGGCCTACGT ATCTATATAC ANTECCATATAC ANTECCATAC ATGCGCTECT ACGGGTECT TGTAGATATT TGTTACGAG CTTAAGCATA AGGATCTCTT TGCGGAGCCC CGGCGAGCCC CGGCGAGCCC CGGCGAGCCC CGCGAGCCC CGCGAGCCC CGCGAGCCC CGCAGACCC CGCAGACCC CGCAGACCC CGCAGACCC CGCAGACCC CGCAGACCC CGCAGACCA ACTGTTATA
5. 6. 7. 8.	KN3-3 KN4-1 KB1-1 BJ3-1	01 121 121 121 121 121 121 121 121 121 1		Abstructure     According	CACEGGCTTT AGTATCAGAA AGTATCAGAAA TTGGCTTTGGAC TCAGTGAATC CTGGACTTGGTAC TGGACTTGGTAC CACTCCCAA AGCTTTTA ACCTCAAATC CAACTCCCAA AAAACCAGAA AAAACCAGAA AAAACCAGAA AAATCTAGTGG ACGCAGCACA CCTCAAGCCC CAACTCCTA CACGCTCCAA CGGAACCCTCA CTAACTCTT ACCTCAGTGCC CAATCGTTGAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGTCCAA CACGCTCCAA CACGTCCAA CACGTCCAA CACGCTCCAA CACGCTCCAA CACGTCCAA CCAACTCCTACTGC CAATCGCTG CAATCGCTG CAATCGCTG CAATCGCTG CAATCGCTG CAATCGCTG CAATCGCTG CAATCGCTG CAACTCCTAA CCCAACTCCAACCCC CAATCGCTG CAACTCCTACTCAACCCC CAATCGCTG CAACTCCTACTCAACCCC CAACTCCTACTCAACCCCCAACCCCCCAACCCCCCCC	TTCCTTCCCT GGTCATTGC ATCCAATCAT GTCCAATCAT GTCCATGAACAT GGTCCTTGC CTGTGCGACT AGGCCACCTGGA CGGCCCCCCC ACCCCGCCGCC CCGGTCTGCC CCGGTCTCCCT GCCAACCGCT GCCAACCGCT TGAACTTAAC CCCCGATTGA CCCCGATTGAC CCCGATTGCA CACAATGGAC GTCCAACGGCC ACTCCTCCAT GTCCAACGCC GTCCAACGCCC CGTCCCAACGCCC TAAGCCGACC TGACCTCTCCAT GTCCAACGCC ACTCCCCCC GTCCAACGCCC ACTCCCCCC CGTCCCCCCCCCC	AGTIGTACT GATCTAACGC GATCTAACGC GGATCGAACGA GCATGCACCA CTCTACTCAACGA ACATACCAAT GACGACCCCT ACCCGCTGAA ACATACCAAA GAAATGTGAA AGTATTCTGG GGTTGGGAAT CCACTACCACA GGCCAGACTCC CAGTCAGAGAT ATGACACTGA ATGACACTGA TCGATGGAATC CGATCAGAGAT TCGATAGACTG	GGGCGTACGT ATCTATATAC ANTGCGTATATAC ANTGCGCTCCT ACGGGTCCT TGTGATAATT TGTTGCCTCG ANACTCAGT TGTTGCCTCG ANACTCTCT TGCGGAGACCC CGGCGAGCCC CGGCGAGCCC AAAACTAATTG CGCAAGCCCT ACGGACTCATTC CGCAAGCCCC AACAGCATC ATGTTACTATA ATAACTATAT
5. 6. 8.	кN3-3 КN4-1 КВ1-1 ВЈЗ-1	61 121 121 121 121 121 121 121 1	ARACCARCACOL ARACCARCARA ARCTITCAGC ARCHICLAGC ACTATICAGC ACTATICAGC ACTATICAGC ACCARCARACA CONTINUE CONTINUE CONTINUE ACCARTARAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAGCAT CAATACCAT CAATACCAT CATCCCCAG ACCATTAAT CCTTCCCGGAA ACCACTTATACCAT CAATCCCAT CAATCCCAT CAATCCCAT	AGGEGECCTTA AAGGGGECCTTA AAGGGGECCTTA AAGGGACCTTA AGGAGCATGA CGCATGAGAT TGAACGATTA CGGACCGTTA ACCGAGCTTGA TCGAACTCTG GACCAGCAGAGA ACCGATGAGA ACCGAGTAGAG CGGATCAGGAG GCGATTAGAAG CGGATTAGAAG CGCATTAGGAA TACCCAGGAGA CGCATAGGAACTGC TACTTACGAACTGC CTGGGAACTGCCGAA	CACEGGCTT AGTATCAGAA AGTATCAGAA TTGGCTCTGG TCAGTGAATC CTGTTGGACT TGGACTTGGAA AGCTTGGTAC CACCTCCAA ACCTCCAA AACCCACAA AAAACCGGA AAAACCACAA AACCACACAA CTAACCGCC CAATCACCGC CAATCACCGC CGAATCACCGC GGTAGTCCTA ACCTAGTGG CGCAACCGC CAATCGAGCCC CAATCGAGCCC CAATCCAGTGG CGCAACCGCC ACCGCTCAA TTGTACATCT	Internection Grant and a second second arccantrate arccantrate arccantrate arccantrate arccantrate arccantrate arccantrate arccattrate arcan	AGTIGTACT GATCTAAGC GATCTAAGC CGACCCACCC CCCACCAACCTA GGCTGCTCC CGCCTCACGTA ACATACCAAT GAGGACCCCT ACCTCCAACA GTAATGTGAA AGTATTCTGG GTTTGGGAT CATTCCAACA CACTTCCAACA GCCAGGAGCCCCA CA CACTTCCAACA CACTTCGGACA CACTACCAACA CACTACCAACA CACTACCACACA CACTACCACACA CACTACCACA CACTACCACACACA	СССССТАССТ АТСТАТАТАС АЛТСТАТАТАС АЛТСТСАТАТАС АЛТСТСАЛСТАСТ ТССТТАССАСТ ТСТТАЛССАЛСТ ТСТТАЛССАЛС АЛАСТСТСТ ТСССТАСАДАТСТ ТССССАДАЛТТ ТССССАДАЛТТ ССССССССССС ССССССАДАТСТ ТСССТАЛАТАТ АЛАСТАЛАТТС ССССССССССС
5. 6. 7. 8.	КN3-3 КN4-1 ВЈ3-1	61 121 121 121 121 121 121 121 1	ARACCARCACO ARACCARCARA ARACCARCARA ARACCARCARA ARACCARCARA CONTACTOR ARACCARCARA CONTACTOR ARACCARCARA CONTACTOR ARACARA CASTANACC CASTANACCA CASTANACCA CASTANACCA CASTANACCA CASTANACCA CASTANACCA CASTANACCA CASTANACCA CASTANACCA CASTANACCA CASTANACCA CASTANACCA CASTANACCA CASTANACCA CONTACA	Пактонатося Пактонатов Ассасттатаа Алсасататаа Алсасататаа Алсасалата ассалатата седассоттаа ассасатаа ассасастаа Ассасастаа Ассасатааа Седассоссад Ассасатааа Седассастаа Седассастаа Седасастаа Седасастаа Седасастаа Седасастаа Седасатааа Седасастаа Седасастаа Седасатааа Седасастаа Седасатааа Седасастааа Седасатааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастаааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастааа Седасастасатаа	CACEGGCTTT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTGGTC TGGCTTTGGTC TGGCTTGGTA AGCTTTTA ACCTCAAATC CAACTCCCAA TAAAACGGA AAAACCACAA AAACCACAA AAACCACAA AAACCACAA AACCACACA CCTCAAGCCC CAACTCCTCA GAATACCGGC CAATCACCGC CAATCCTCA CGCAAGCCCCC CAATCCTCT ACCCTCATG CGCAAGCTCCTA CTGACTCCTA GGCATCCTTA TGTATAAT GTAGTACACA	TTCCTTCCCT GGTGATTGC GATCGATGA ATCGAATGA GGTCGATGA GGTCTTGTC CTGTGCGACT AGGCAGCT AGGCAGCCGCC CCGGTCTGC CCGGTCTGCC GCCAATGGAC GGTCCGCC TGAACTTAA GCCCCGCC CCGGTTTGC GCCAATGGAC TGAACTTCACAG GTCCAAGCGG TGAACTGTCACAG GTCCAAGCGC GTCCAAGCGC AGTTCCCCG GTCCAAGCGC AGTTCCCCG GTCCAAGCCC AGTTCCCCG AGTTCCCCG GTCCAAGCCC AGTTCCCCG GTCCAAGCCC AGTTCCCCG GTCCAAGCCC AGTTCCCCG GTCCAAGCCC AGTTCCCCG GTCCACGCAG TGACCCCAG TGACCCCAGC TGACCCCAG TGACCCAG TGACCCCCCCCCC	AGTIGTACT GATCTAACGC GATCTAACGC GGATCGAACGCA CTCTACTAACGC GGCTGCTC GGCTCACGTA ACATACCAAT GAGGACCCCT ACCTTCAACA GTAATGTGAA GTAATGTGAA GGACCACGA CACTACGACACCCA CA GGCCAGACTC CCATCAGAGAT ATGACACTGA TCGATCAGAGT TCGATCAGACT TCGATCAGCT CGATCAGCATCA TCGATAGTTC	GGGCGTACGT ATCTATATAC ANTGCGCTACA ATGTGACTATAC ANTGCGCTCCT ACGGGTCCT TGTGATAATT TGTTGCCTCG ANACTCATA TGTTGCCTCG ANACTCTT TGCGGACTCC CGGCGACCCC CGGCGACCCC CGCAGACCCC AACACTAATTG CTATACACAAT TGTTACTATAT ACTACTATAT
5. 6. 7. 8.	кN3-3 КN4-1 ВЈ3-1	81 121 121 121 121 121 121 121 1	GTGCACCCCC AAAGCGACAACAA AACTTCACTACAA AACTTCACC AGTAATGTGG CAACTACAACAA AACTACTCACC AGGACAACAA CAACTACAACAA CAACTACAACAACAACAACAACAACAACAACAACAACAAC	AGGEGECCTT AAAGGGECCTT AAAGGGECCTT AAAGGGECCTT AGGAGCATGC CGTTAGGAT TGAACGATT CGACCGATGA CGGCTCGGAT ACCGAGTTTA CGGCCCGGATGA CCGGCCCGGA CGGATCAGGAG CGATTAGAAG CGGATTAGAAG CGCATTAGAAG CGCATTAGAAG CGCATTAGAA CCCCATGGGA ACTGCGCAACTGC CTTGCCTCCGGA	CACGGGCTTT AGTATCAGAA TTGGCTCTCG TCACTGAATC CTGTTGGACT TGGACTTGGAC CTGTTGGAC TGGACTTGGAC AGCGTTGGTAC CACCTCCAA ACCCCCAA AAAACCGGA AAAACCGCA AAACCACAA AACCACAA CTAACCGCC CACCGCCCCC CATCCACCCC CAATCTACTCG GGAAGCCCC CAATCCAGCCC CAATCCAGCCC CAATCCAGCCC CAATCCAGCCC CAATCCAGCCC CAATCCAGCCC CAATCCAGCCC CAATCCACTCA TGTATAGAAC AACGAACTCTT TTATAGAAA AGCGAATTCC	Internection Grant and a second seco	AGTIGTACT GATCTAAGC GATCTAAGC CGACCCACCCAC CCCACCAACTA GGCTGCTCC CGGCTCACGC ACCCCCCGAA ACATACCAAT GAGGACCCCT ACTTCCAACA GTAATGTGAA AGTATTCTGG GGTCTGGGAT CGACCCCAC CAC GGCCAGAGTCC CCACTGAAAC GACAGGACCCA CATCAGAAGA CGACCAGACTCA TCAAGCGAC TCAAGCGACA TCAAGCGAC	GGGCCTACGT ATCTATATAC ANTECCATATAC ANTECCATATAC ANTECCATGCATA TTGCGCTECT ACGGTTCTT TCTTTACGAG CTTAAGCATA TGTTGCCTCG ANACTCTTT TGCGGACTCC CGGCGACCCC CGGCGACCCC CGGCGACCCC CGCGACCCC CGCGACCCC CGCGACCCC CGCAGACCC CGCAGACCC CGCAGACCC CGCAGACCCA ACTGTTATACACC
5. 6. 7. 8.	КN3-3 КN4-1 ВЈ3-1 LP1-3	61 121 121 121 121 121 121 121 1	ARACCARCACCO ARACCARCARCA ARACCARCARCARCA ACTAATCACA ACTAATCACA CONTACTOR ACCARCARCAC CONTACTOR ACCARCARCAC CONTACTOR ACCARCARCAC CONTACTOR ACCARCARCA CONTACTOR ACCARCARCA CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CONT	Indicationation According to a construction and a construction of a construction according to a construction accor	CACEGGETT AGTATCAGAA TTGGCTCTCG TCAGTGAATC CTGTTGGTC TGGCTTTGGTC TGGCTTGGTA CACTCCAAATC CACTCCCAA TAAAACGGA AAAACCACAA AAAACCACAA AAACCACAA AAACCACAA CCTCAAGCCC CAATCACCGC CAATCACCGC CAATCACCGC CAATCACCGC CAATCACCTCA CGCAAGCCCC CAATCACCTA CCCCAAGCCC CAATCACCTA CACGCTCCAA CCCCACTCGTG CAATCACCTA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CACGCTCCAA CCCAATCCCTA	TTCCTCACT GGTGATTGC ATCGATGA ATCGATGAT GGTCGATGA GGTCGTGC GGTCTTGC CTGGCGACT AGGCAGCC GGGTCGCG CCGGTCGCC GCGATCGCC GCGATCGCC GCGATCGCC TGAACTAA GCGCCGCC TGAACGATG TGAACTAA GTCCAATGGAC GTCCAATGGAC GTCCAAGCGC GTCCAAGCGC GTCCAAGCGC GTCCAAGCGC GTCCAAGCGC AGTCCCCGC GTCCAAGCCC GTCCAAGCCC GTCCAAGCCC GTCCAAGCCC GTCCAAGCCC AGTCCCCCG GTCCAAGCCC GTCCAGCC GTCCGC GTCCAGCC GTCCAGCC GTCCAGCC GTCCAGCC GTCCAGCC GTCCAGCC GTCCGC GTCCAGCC GTCCAGCC GTCCAGCC GTCCAGCC GTCCGC GTCCAGCC GTCCGC GTCCGC GTCCGC GTCCAGCC GTCCGC GTCGCC GTCCGCC GTCCGC GTCCGC GTCCGC GTCCGC GTCCGCC GTCCGCC GTCCGC GTCCGC GTCCGC GTCCGCC GTCCGC G	AGTIGTACT GATCTAACGC GATCTAACGC GATCTAACGC GGATCGAACGAC TCTACTCAACGA GCTTGCTC CGGCTCACGA ACATACCAAT GACGACCCCT ACTTCCAACA GTAATGTGAA GTATTGGGAT CATTACGACT CACTACGACAC CATCAGACATCA CATCAGACATCA CATCAGACATCA TCGATAGACTC CGATCAGACATCG CATCAGACATCG CATCAGACATCG TCATAACGCCT CGATAGGATTCCA TCATAACGACTA TCATAACGACTA	GGGCGTACGT ATCTATATAC ANTGCGCTACA ATGTGCGTCCT ACGGGTCCT TGTGATAATT TGTTGCGCCG AAAGTCAAGT TGTTAGGAG CTTAAGGATCTCT TGCGCACGGAGCCC CGGCGAGCCC CGGCAGCCC CGCAGACCCC AAAGCTAATTG TCGTTATACACCA ATTACTATAT ATAACTATAT ATAACTATAT
5. 6. 7. 8.	кN3-3 КN4-1 ВЈ3-1 LP1-3	81 121 121 121 121 121 121 121 1	GTEGACGCOC AAAGCGACAACAA AACTTCACGACAA AACTTCACGACA AGTATCAGC AGTATATCAG CAACAGGACT CGGCTCACGCG ACAATAATCA TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGC TCAATAGCAT CAGGAATCAGC TTCAGCCACG CAGTGAATCACT CAATACCATAA ACACCTCAAA AATACCATCAAA	AGGEGECCTT AAAGGGECCTT AAAGGGECCTT AAAGGGECCTT AGGAGGATGT GCTTAGGAT TGAAGGAT CGCATGAGAT CGCATGAGAT CGCACTCGG ACCCAGTAGA CCGACTCGGAT ACCCAGTAGAG CCGACTGCGAC Rep 516bp AAGTCAGCAGAC CGCATAGAAC CCCATAGAAG CGCATAGAAC CCCATAGAAC CCCATAGAAC CCCATAGGAA TACCTGCGCA AAGTCCCGCTTA	CACEGGCTT AGTATCAGAA AGTATCAGAA TTGGCTCTGG TCAGTGAATC CTGTTGGACT TGGACTTGGAC AGCTTGGAC CACTCCCAA ACCTCCAAATC CAACTCCCAA AAAACCGGA AAAAACGGA AAAACCACTAA AAACCACTAA AACCACAACAA CTAACTCCT CCTCAAGCCC CAATCAGTGG GGAAGCTCCA CGAACTCCTCAA CTAACTCAT AGGAACTCTT ATGAACCTT AGGATCCTA AGGAACTCT AGGATCATA AGGAACTCT TTATAACAAA AGCGAATTCC CACTGGCG	Internection Grant and a Contraction Arccantration Garactartac Garactartac Garactartac Garactartac Garactartac Accounter Accounter Accounter Accounter Accounter Accounter Accounter Contractartac Garactartac Cachangeos Tegacatartac Garactartac G	AGTIGTACT GATCTAAGC GATCTAAGC CGATCTAAGC CGACCCACCAC GGCTGCTCC CGGCTCACGG ACCTCCACCA ACCTCCACCA ACCTCCACA ACATACCAAT GAGACCCCC CACTGAAAC GACTACCACA CACTTCGGAT CACATCGACA CACTCCACACA CACATCGACA CACATCGACA CACATCGACAC CACTGAAAC GACCACGAC CACTGAAAC CACAGACGAC TCATAGGCTT CGACTATTTCCACGACA CGACACGACA	GGGCCTACGT ATCTATATAC ANTECCATATAC ANTECCATATAC ANTECCATCA ACGGATECT TGTGGCTCCT ACGGATCTCT TGTTACGAG CTTAAGCATA ACGGATCTCT TGCGAGATCCC CGGCGAGCCC CGGCGAGCCC CGCGAGCCC CGCGAGCCC CGCGAGCCC CGCAGAGCCT ACCAGCATCT TTATTACAAC CCCAGAGCAT ACCAGTATTG TTATTACAAC
5. 6. 7. 8.	KN3-3 KN4-1 BJ3-1 LP1-3	01 1121 121 121 121 121 121 121	ARACCARCACO ARACCARCARCA ARACCARCARCARCA ACTAATCACA ACTAATCACA CONTACTOR ACCARCARCAC CONTACTOR ACCARCARCAC CONTACTOR ACCARCARCAC CONTACTOR ACCARCARCA CONTACTOR ACCARCARCA CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CASSENDIA CONTACTOR CONTA	Пактосатося Пактосатора Сосаститата Алсосатитата Алсосансти одеосодитета содассто Содассотора Содассто Содасособ Асстособа Ассосастара Сосастособа Сособастособа Сособас	CACEGGCTTT AGTATCAGAA TTGGCTTCGG TCAGTGAATC CTGTTTGGTC TGGCTTGGTA GGCTTTGGTA CACTCCAAATC CACTCCCAA TAAAACGGA AAAACCACAA AAAACCACAA AAACCACAA AAACCACAA AAACCACAA AAACCACAA AACCACAA CCTCAAGCCC CAATCACCGC CAATCACCGC CAATCACCTCA GGATACCCTA CGCAAGCTCCA CTAACTCTTA ACCCTAGTGG CAATACCTTA CACGCTCAA GGATCATCT AGGATCCTA TGTATAATC GTAGTCCTA GGGATCATTA TCTAACAAA AGGAATTGCC CAATTACCAA	CCTGATTCAC GATCAATGAC ATCCAATGAA ATCCAATCT GTATTGCCATTGAC GGTCTTGTC CTGTGCGATT AGGCCAGCT AGGTAGGACT ACCCCTGTGA CGGCCCCCCC CCGGTTCGCT GCCAATCGAC GCTGATTCAC GCTGATTCACA GTCCAATGGAC GTTCAAAGAC GTTCACAAGCGCT GACAATGGAC GTTCACAAGCGCT GACAATGGAC GTTCACAAGCGCT GACAATGGAC GTTCACAAGCAC GTTCACAAGCAC AAAATGCACCAA GTGATCCCCG GTCAAAGCAC AAAACTTC GATAAGCAAT	AGTIGTACT GATCTAACGC GATCTAACGC GATCTAACGC GGATCGAACGAC CTCTACTCAACGA GCTTGCTC CGGCTCACGG ACTTCCACTA ACATACCACA GACATACCAAT GACATCCAACA GTAATGTGAA GCACTCTCAGGAT CACTACCACA CACCACGAGAC CATCAGACATCA CATCAGACATCA TCGATAGGAT TCGATAGGAT TCATAACGAC GGACAGCACTC CATCAGACATCA TCATAACGCT CGATAGGAT TCATAACGAC TCGACAGCAC TCGACAGCAC TCGACAGCAC TCGACAGCAC TCGACAGCAC TCGACAGCAC TCGACAGCC TCGACAGCC TCGACAGCC TCGACAGCC TCGACAGCC TCGACAGCC TCGACAGCC TCCGACAGCC TCCGACAGCC TCCGACAGCC TCCGACAGCC TCCGACACCC TCCGACACCC TCCGACACCC TCCGACACCC TCCGACACCC TCCGACACCC TCCGACACCC TCCGACACCC TCCGACACCC TCCGACACCC TCCGACACCC TCCGACACCC TCCGACACCC TCCGACACCCC TCCGACCCC TCCGACCCC TCCGACCCC TCCGACCCC TCCGACCCC TCCGACCC TCCG TCCGC TCCG TCCG TCCG TCC TCC	GGGCGTACGT ATCTATATAC ANTGCGCTACA ATGTGCGTCCT ACGGGTCCT TGTGATAATT TGTTGCCTCG AAAGTCAAGT TGTTGCCTCG AAAGTCTCT TGCGGAGACCC CGGCGAGCCC CGGCGAGCCC CGCAGACCC AAAGCTAATTG TATTACAGCA ACTGATATACAGCA ATGACTAATTG TGCTTAGGTAATA ATAACTAATAT TATAAGTAATA
5. 6. 7. 9.	кN3-3 КN4-1 ВЈ3-1 LP1-3	81 121 121 121 121 121 121 121 1	ARACCARCAGA ARACCTACAGA ARACTTCACC ARACTACTACAG ARACTACTACAG ARACTACTACAG ARACTACTACAG ARACTACAGA CARATARTAC CONTRACTACAGA CARATARACT TCAATAAGC TCAATAAGC TCAATAAGC TCAATAAGA CARACTACAGA CAGTACATCAGA TCAATACCCA TCAATACCCA TCCATCCGAGA CAGTACACCA CAGTACATCACA ARACACTCAGA ARACCATCAAT	Inclusion Anageditication Anageditication Anageditication Contractore Contract	CACEGGCTT AGTATCAGAA AGTATCAGAA TTGGCTTCGGA TCACTGAATC CTGTTGGACT TGGACTTGGA AGCTTTGGAC AGCTTGGAATC CAACTCCCAA AACCACAA AAAACCGGA AAAAACGGA AAAACCACAA AAACCACAA AAACCACAA AACCACAA CTAACCGCC CAATCACCGC CAATCACCGC GGAAGCTCC CAACTCGTG TGTATAGAAC TAGATCCTA AGGAACTCT AGGACTACC CACTGGCTGCAC CACTGGCT CACCTGCGCC	Internection Grant and a Contraction Arccantration Garactartac Garactartac Garactartac Garactartac Accounter Accounter Accounter Accounter Antantona Antocanta Accounter Contractartac Garactartac Cacantroca Cacantroca Cacantroca Cacantroca Cacantroca Cacantroca Cacantroca Garactartaca Garactartaca Catantroca Cacantroca Garactartaca Cacantroca Garactartaca Cacantroca Garactartaca Catantroca Catantroca Catantroca Catantroca Catantroca Catantroca Catantroca Catantroca Catantroca Catantroca Catantroca Catantroca Catantroca Catantroca Catantraca	AGTIGTACT GATCTAAGC GATCTAAGC CGATCTAAGC CGACGCACC CCCACCAACTA GGCTGCTCC CGCCTCAGC ACCTCCACCAA ACATACCAAT GAGGACCCCT ACTTCCAACA GTAATGTGAA GTATTCGGAT CGCACGAGACCCCA CA CACTTCGGAGAC CCACTGAAAC GACCAGGAGACCCCA CA CAGGACACGA CATCAGAACGA CAACGACGAA CGATCAGGAGAT CGACAGCGAA CGACAACGGA CGCAGAAGCGA TCATAGGCTT CACAACGGA CGCAGAGCGC TCAATGCACG CGCAAGCGCA CGCAGAGCCCA	GGGCCTACGT ATCTATATAC ANTECCATATAC ANTECCATATAC ANTECCATCA ACGGTTCTT TGTGCCTCG ATGTCATAGGA CTTAAGCATA TGTTGCCTCG AAACTCTCTT TGCGAGATCC CGGCCATCC CGGCCATCC CGGCCATCC CGGCAGCCC CTTCCGTAGTA ACTTCTGAATCT TTATTACACC CCCAGAGCCT ACCAGCATCT TCCTTAGTATATA ATAACTATAT TTACTAACAAT

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 151

152 153 154 steps.

155

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. 156 157 158 159

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

|

N		Result Links					
IN	Sample <del>s</del>	Description	Max	Total	Query	E	Dan Lland
0.		Description	Score	score	cover	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,H	KJ780757.	1,KF1518	51.1,KT844	687.1,KF	850375.1,L
		C431580.1,MK775821.1,KM388611.1,HQ891300.1,	LC31241	3.1			
	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,M	AT136544	.1,MK267	768.1,MG2	41533.1, <b>F</b>	Y105711.
		1,KT900123.1,KT900118.1,KT282395.1,KM982986	.1				
	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,F	KT273380	.1,KT273	379.1,KT27	3370.1,KI	[273364.1,
		KR155105.1,MH053154.1,KT956127.1	r				1
	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,N	AH292842	2.1,LC152	416.1,HQ6	)7958.1,M	IK450628.1
		,MH292844.1,KM613137.1,MH865978.1,KX443215	5.1,KX443	211.1			
	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%
	1	Lantinus saugmogulus voucher WADD 24	1 1 1 0 0	1100	0.00/	0.0	1 1 ( )( )0/

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

	1		1	1	r	1	
		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,K	T273380.	1,KT2733	70.1,KT95	5127.1,KT	273373.1,
		KT273379.1,KT273364.1,JN253598.1,MH053154.1,	KP28348	4.1			*
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,N	AT549849	.1,MN817	705.1,MN8	317704.1,N	MT477707.
		1,MT477704.1,MT466521.1,MT447544.1,MT43400	5.1,MT37	8328.1			
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,K	Y105746	.1,KY105	745.1,KY1(	)5736.1,H	M802133.1,
		<u>NR</u> 111353.1,EF153624.1,NR 073242.1,MT482659.	1,MT4826	558.1			
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(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,K	Y105736	.1,HM802	133.1,NR		
		111353.1,NR073242.1,KY105746.1,MT482659.1,M	T136544.	1,MK6059	936.1,MK26	57768.1	

Based on the nucleotide BLAST searches (Table 3), several of the coprophilous fungal samples could be identified a

the species level. These samples were (1) KN1-1, identical to Ceriporia lacerata; (2) KN1-2, identical to Trichosporo

asahii; and (3) KN3-1 and KN3-3, identical to Lentinus squarrosulus. Samples that could not be identified at the specie

level because they exhibit similarities with several species within a genus were (1) KN4-1, which probably belongs to th

genus Fusarium; (2) KN3-2, which probably belongs to the genus Aspergillus; and (3) BJ3-1 and LP3-1, which probably

Nucleotide BLAST searches against a more specific database, such as Fusarium ID, are needed for the KN4-1

belong to the genus Trichosporon,

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.



174 175

Based on the nucleotide BLAST searches (Table 3) and the resulting phylogenetic trees (Figs. 2-9), several of the 176 coprophilous fungal samples could be identified at the species level. These samples were (1) KN1 1, identical to Ceriporia 177 lacerata; (2) KN1-2, identical to Trichosporon asahii; and (3) KN3-1 and KN3-3, identical to Lentinus squarrosulus. 178 Samples that could not be identified at the species level because they exhibit similarities with several species within a 179 genus were (1) KN4 1, which probably belongs to the genus Fusarium; (2) KN3 2, which probably belongs to the genus 180 Aspergillus; and (3) BJ3 1 and LP3 1, which probably belong to the genus Trichosporon, Further nucleotide BLAST 181 searches against a more specific database, such as Fusarium ID, are needed for the KN4-1 sample (most likely Fusarium). 182 Furthermore, morphological analyses can be performed to complement the obtained molecular data. Molecular 183 identification of coprophilous fungi obtained in Banyumas regency found Ceriporia lacerata, Trichosporon insectorium, 184 and Lentinus squarrosulus at species level and Fusarium sp., Aspergillus sp., and Trichosporon sp. at genus level based on 185 ITS1 and ITS4 in the 16S rRNA gene. According to Stackebrandt and Goebel (1994) the 16S rRNA markers of 186 microorganisms such as fungi tend to be very similar or identical at the species level when the identity exceeds 97.5%, 187 whereas the identity threshold is 95% at the genus level-().

188 The 16S rRNA markers of microorganisms such as fungi tend to be very similar or identical at the species level when 189 the identity exceeds 97.5%, whereas the identity threshold is 95% at the genus level (Stackebrandt and Goebel, 1994).

190 The fungal genera isolated and identified in this study have never been reported as being coprophilic, except for 191 Trichosporon spp., which has been found in chicken manure (Obire et al., 2008), buffalo dung (Lorliam et al., 2013), and 192 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 193 194 specimens in East Kalimantan classified as Ceriporia species, C. inflata and C. lacerata, which were identified based on 195 morphological characteristics and the ITS and nuclear ribosomal large subunit sequences. L. squarrosulus is an edible 196 fungus commonly found growing in the wild on decaying tree trunks during the rainy season. Similar to other macrofungal 197 species, this fungus can grow in a wide variety of substrates and habitats. Many Lentinus species have been reported to 198 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 199 biofilms on the surface of the temple stone destroys the integrity of the substrate material and is a biodeteriogen 200 201 responsible for the destruction of the temple stones over time.

202 To conclude, we have uncovered the existence of coprophilous microscopic fungi occurring in Banyumas District 203 regency in Central Java, Indonesia. At the species level, we identified as Ceriporia- lacerata, Trichosporon insectorium, 204 and LentinusL 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 205 206 human interests.

#### 207

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#### ACKNOWLEDGEMENTS

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

ARIS MUMPUNI<sup>\*</sup>, 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-	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	We choose "widely" to explain how do coprophlous fungi are cosmopolite organisms
2	48-49	textcheck)Confusing statement. Check and rewriteReviewer'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 :	We made correction according to
		Although, the results of the present	of the menuscrimt
		presented mostly either as table of	or the manuscript.
		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	
6	111	(viz [not understood] samples KB2 1	This the complete sentence after
0	111-	(VIZ. [not understood], samples KD2-1, I P1 1 and I P4 1) indicated a pure	correction :
	112	sample free of RNA and protein	
		contamination: a ratio greater than 1.8	Table 1 shows the of genomic DNA
		(viz KN1-1 KN1-2 KN2-1 KN3-1	quantification results for DNA
		(V12., K1(1-1, K1(1-2, K1(2-1, K1(3-1, K1(3-	extracts from the conronhilous
		I P1-2 I P1- 3	fungal isolates. The purity of each
			DNA extract was determined
		Reviewer's comment ·	according to the 260/280 nm
		What is the meaning of these codes?	absorbance ratio Samples KB2-1.
		Authors does not mention anywhere in	LP1-1, and LP4-1 are free of RNA
		the manuscript. It is better to elaborate	and protein contamination as they
		these codes somewhere in the text.	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	We have corrected it into "Sample"
		Reviewer's comment :	
		If it denotes samples, then why KN1-	
		1, KN1-2, etc. are presented singly.	
8	156	The BLAST search results are	We have deleted Figure 2 - 9
		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.	

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

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.



# [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.

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