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5 Morphological and molecular identifications of three native arbuscular mycorrhizal fungi isolated from the rhizosphere of *Elaeis guineensis* and *Jatropha curcas* in Indonesia

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Abstract. Rini MV, Yelli F, Tambunan DL, Damayanti I. 2021. Morphological and molecular identifications of three native arbuscular mycorrhizal fungi isolated from the rhizosphere of *Elaeis guineensis* and *Jatropha curcas* in Indonesia. *Biodiversitas* 22: 4940-4947. Molecular analysis has been widely used to provide more accurate identification within arbuscular mycorrhizal fungi (AMF) species than identification based on morphology. However, morphological analysis is essential for a basic preliminary of classification studies. Therefore, a study is needed to complete the identification of AMF isolates through morphological and molecular analyses. This research used three AMF isolates, namely MV 5, MV 17, and MV 18, which were isolated from Indonesian agricultural land. Spore-based taxonomy (shape, size, color, ornamentation, PVLG, and Melzer's reaction) and fungal colonization on roots of maize trap plants were employed for the morphological studies. AMF species identification was performed using molecular analysis through nested-Polymerase Chain Reaction (PCR) to amplify a fragment of SSU rRNA followed by sequencing and phylogenetic tree construction. Morphological analysis showed that MV 5 had spores borne from the neck of the sporiferous sacculus, MV 17 was found to have a bulbous suspensor without a germination shield, and MV 18 had spores borne from subtending hyphae. The SSUR rRNA analysis revealed that MV 5, MV 15, and MV 18 were identified as *Acaulospora longula*, *Gigaspora margarita*, and *Glomus etunicatum*, respectively. Both morphological and molecular methods demonstrated reliable and consistent results that complement AMF taxonomy studies.

Keywords: AMF structure, arbuscular mycorrhizal fungi, molecular, morphology

Abbreviations: AMF: Arbuscular mycorrhizal fungi; SSU rRNA: Small subunit rRNA, ITS: Internal transcribed spacer

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are one of the mycorrhizal types which can form internal and external hyphae structures that are beneficial for plants because they can improve the nutritional status of the plant and soil characteristics (physical, chemical, and biological) (Smith and Smith 2012; Bücking and Kafle 2015; Chen et al. 2018). AMF enhances nutrient uptake and allows plants to be more tolerant against drought, salinity, and other environmental stress (Syafuddin et al. 2016; Duc et al. 2018; Hu et al. 2021). Moreover, AMF is capable of protecting roots from pathogenic attack within the soil, improving plant growth and yield, and fixed solubilizing phosphate in soil (Prasetya and Anderson 2011; Rini et al. 2020).

The effectiveness of AMF is influenced by the types of the host plant and mycorrhizae (Ijdo et al. 2011; Selvakumar et al. 2018; Melo et al. 2019). To successfully obtain higher effectiveness of AMF application, the utilized species needs to be determined. Therefore, identification of AMF species is a crucial step prior to field application, especially in the application of different species within the same genus of AMF. A study by Rias et al. (2014) used

three species of *Glomus* sp. (MV 4, MV 11, and MV 13 on oil palm (*Elaeis guineensis* Jacq.) seedlings and reported that MV 4 and MV 13 isolates showed a good ability to enhance the growth of oil palm seedlings, while MV 11 did not show any benefits to the plant. It suggests that three different species of *Glomus* sp. have different performances to the plant. Relying solely on genus data is insufficient to find the best host for AMF isolates. Hence, molecular analysis is very important for determining AMF species and plays a crucial role in maximizing their potential for agricultural applications.

Spore morphological characters were used to identify arbuscular mycorrhizal fungi before the molecular approaches were widely employed. The AMF was divided into three families (*Acaulosporaceae*, *Gigasporaceae*, and *Glomeraceae*), six genera (*Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis*, and *Scutellospora*), order Glomerales by Morton and Benny (1990), and placed in Phylum Zygomycota (Schenk and Perez 1990; Morton and Benny 1990; Oehl et al. 2011). This method is commonly used because it is easy, fast, cost-effective, and can identify up to the genus level. However, it is not easy to identify up to the species level as spore morphology cannot be relied on to identify many AMF species with similar features. For

example, an observation of a very thin wall layer needs a spore to be in good condition when mounted onto object-glass, which is difficult to do, especially for the big-size spore.

Furthermore, most spores from the field are not in good condition and are difficult to identify based on their morphology because some spores are mostly found in damaged conditions, and their traits are separated from the spore (Krüger et al. 2012; Souza 2015). The major taxonomic change in AMF occurs as soon as molecular phylogenetic tools have become available based on the nuclear ribosomal RNA (rRNA) gene regions as a marker of AMF (Krüger et al. 2012; Redecker et al. 2013). Molecular identification provides more accurate, highly specific, and fast results. Its application to a specific region in fungal DNA allows species identification by using well-developed techniques, including polymerase chain reaction (PCR) (Oehl et al. 2011; Redecker et al. 2013; Souza 2015).

The current modern classification of Glomeromycota is based on a consensus of regions spanning ribosomal RNA genes: 18S (SSU), ITS1-5.8S-ITS2 (ITS), and/or 28S (LSU) which was proposed by Redecker et al. (2013). SSU rRNA gene has been the most chosen in AMF identification due to the availability of primer pairs and has a highly conserved gene (Victorino et al. 2020). Consequently, we selected the 18S rRNA gene for molecular identification of AMF in the study. Although molecular analysis provides precise and specific results, spore morphology is still an important stage for AMF identification to complete the results of molecular identification (Covacevich et al. 2021). In addition, AMF morphology analysis is also essential for basic science purposes, especially when using AMF to be developed into biofertilizers which requires detailed traits information. The morphological analysis allows identifying the characteristics of AMF spores, while molecular analysis identifies the species and taxonomy position. Thus, both methods are necessary to create a complete and successful taxonomy of AMF.

In this study, we identified three native AMF spores (MV 5; MV17; MV18) from the rhizosphere of oil palm (*E. guineensis* Jacq.) and *Jatropha curcas* L. plant in

Indonesia based on spore morphology and 18S rRNA gene sequences of fungi as molecular analysis to identify the AMF species.

MATERIALS AND METHODS

Morphological analysis

Three AMF isolates were used in this study. MV 5 was isolated from the oil palm rhizosphere in North Sumatera, Indonesia ($-8^{\circ}17'49.4''N$ $113^{\circ}34'00.6''E$) while MV 17 and MV 18 were isolated from *Jatropha* rhizosphere in East Java, Indonesia ($3^{\circ}24'00.6''N$ $98^{\circ}42'45.6''E$). All three isolates were preserved in the Plantation Production Laboratory, Lampung University, Indonesia (Figure 1). Since 2011, these three isolates have been used in the greenhouse of Faculty of Agriculture, University of Lampung and have promoted crop growth (Rini et al. 2017; Rini et al. 2020) and produced high spore density. Morphological identification of AMF was carried out by isolating spores from MV 5, MV 17, and MV 18 using wet-sieving and decanting methods on various sieve sizes, i.e., 45 μm , 90 μm , 150 μm , 250 μm , and 500 μm . Spores were transferred to the Petri dishes and observed under a stereomicroscope with a magnification of 45x for identification of spore morphology, i.e., color, shape, bulbous suspensor, germination shield, etc. Spores were then smeared with PVLG and Melzer reagent on an object glass and crushed gently to prevent damage to the spore. The spore reaction to Melzer reagent was recorded using a stereomicroscope (Schenck and Perez 1990; Brundrett et al. 1996; INVAM 2019). The spores of MV 5, MV 17, and MV 18 were also inoculated on the root of three-day germinated maize seeds and then planted in a plastic pot with a volume of 750 mL containing sterilized river sand. The planted pot was kept for 1 month in the greenhouse and watered every day and fertilized with 20 mL of 2 g L⁻¹ urea solution. After one month, the maize roots were cut and stained with trypan blue dye and the AMF structures inside the roots were observed under a compound microscope.

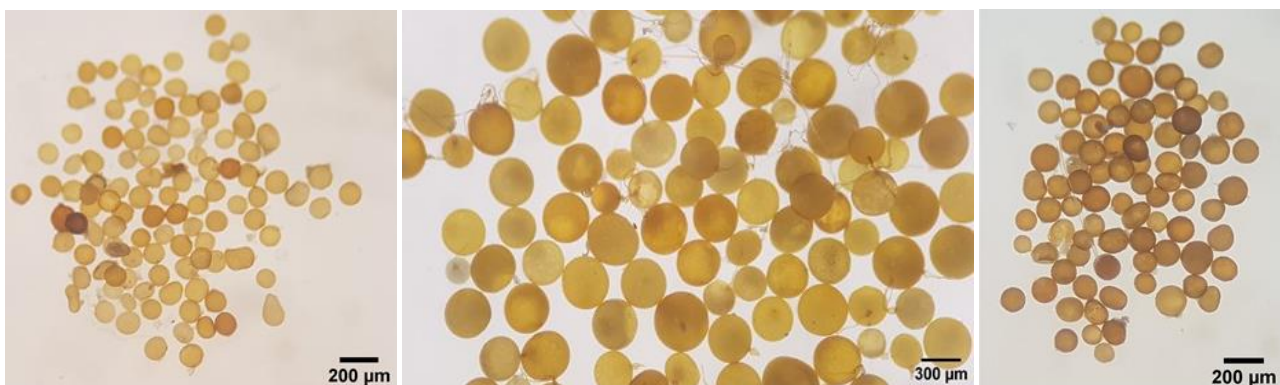


Figure 1. The AMF spores from the Plantation Production Laboratory, Lampung University, Indonesia. A. MV 5; B. MV 17; C. MV 18. Observation under stereomicroscopes with a magnification of 45x

Molecular analysis

Spore isolation

AMF spores were isolated using wet sieving and decanting methods. Ten healthy spores were selected and placed in a Petri dish containing 30 mL of 0.05% Tween 20, then treated in an ultrasonic cleaner (Elmasonic S 60 [H]) for 10 seconds and repeated three times. One of the best spores was transferred into sterile distilled water in a Petri dish and treated with ultrasonic cleaner (Elmasonic S 60 [H]) for 10 seconds. The process was repeated thrice to ensure that the obtained spore was clean and dirt-free (Brundrett et al. 1996; Schwarzott and Schüßler 2001).

The free-debris spores were put into a 0.2 mL sterile microtube. Next, 20 µL Instagene Matrix (BIORAD) was added to each spore in a microtube and crushed with sterile blunt pipette tips. The samples were then processed in Thermal Cycler, which was heated at 56°C for 30 min followed by 10 min at 95°C. After that, the samples were spun down. The 15 µL supernatant was taken and transferred into a new sterile microtube and used as the template for DNA analysis (modified Schwarzott and Schüßler 2001; Redecker 2020).

DNA extraction

PCR amplification

Polymerase chain reaction (PCR) was performed using a SensoQuest (SenseoQuest GmbH, Germany) thermal cycler machine to amplify partial 18S rRNA fragment of AM fungi by nested PCR (Lee et al. 2008). The first PCR was conducted using universal primers NS1 (5'-GTA GTC TA TGC TTG TCT C-3') and reverse primer NS4 (5'-CTT CCG TCA ATT CCT TTA AG-3') with 25 µL of total volume reaction. The PCR reaction consisted of 1 µL of the DNA template, 12.5 µL of MyTaq™ Red Mix (Bioline, USA), 1.25 µL of 10 µM NS1 primer, 1.25 µL of 10 µM NS4 primer, and was adjusted to the volume reaction to 25 µL by adding 9 µL of sterilized distilled water.

PCR amplification was achieved with one cycle of an initial denaturation at 95°C for 4 minutes followed by 30 cycles containing: denaturation at 95°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 1 min, and one cycle for a final extension at 72°C for 5 min. The PCR product of the first PCR was used as the DNA

template for the second PCR using AMF specific primers AML1 (5'-ATC AAC TTT CGA TGG TAG GAT AGA-3') and AML2 (5'-GAA CCC AAA CAC TTT GGT TTC C-3') for 32 cycles, one cycle for an initial denaturation at 95°C for 5 min followed by 30 cycles containing denaturation at 95°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 1 min and one cycle for a final extension at 72°C for 5 min. The PCR products were further analyzed in 0,8% agarose gel for electrophoresis (Invitrogen, USA). After that, 1 µL (10 mg/mL) of Ethidium Bromide was added, homogenized, and poured into the gel tank. The process was employed at 55 V for 60 min, and the result was visualized under a DigiDoc UV trans-illuminator (UVP, USA). The secondary PCR products were sent to 1st Base, Malaysia, for sequencing (Lee et al. 2008).

Sequence analysis

Sequence editing was performed using CodonCode Aligner software (CodonCode Corporation, Dedham, USA) to ensure the accuracy of generated DNA. Every DNA sequence was assigned to a particular taxon by comparing it with the nucleotide sequences in GenBank database NCBI using Basic Local Alignment Search Tools (BLAST) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Sequence alignment was performed using the ClustalW program (Thompson et al. 1994) embedded in MEGAX (Tamura et al. 2013; Kumar et al. 2018). Based on the aligned sequences, a phylogenetic tree was reconstructed using MEGAX with neighbor-joining (NJ) algorithms. *Paraglomus occultum* (C. Walker) J.B. Morton & D. Redecker was used as an outgroup.

RESULTS AND DISCUSSION

Morphology identification

Results showed that the identification of AMF could be successfully performed by morphological characters. Results showed that MV 5, MV 17, and MV 18 belonged to the genus of *Acaulospora*, *Gigaspora*, and *Glomus*, respectively (Table 1). All isolates were able to infect maize roots indicated by the presence of mycorrhiza structures, namely hyphae, vesicles, and auxiliary cells.

Table 1. Morphological characters of MV 5, MV 17, and MV 18 isolates.

Taxonomic criteria	Description		
	MV 5	MV 17	MV 18
Isolate			
Shape	Globose	Globose, subglobose	Globose, subglobose
Size	>45 µm – <250 µm	>90 µm – <500 µm	>45 µm – <250 µm
Color*	Brownish-yellow (0-60-10)	Brownish-yellow (0-90-0)	yellow (20-80-20) to brown (10-100-0)
Sporocarp	Absent	Absent	Absent
Sporiferous Saccule	Present [#]	Absent	Absent
Bulbose suspensor (BS)	Absent	Present	Absent
Auxiliary Cells (AC)	Absent	Present	Absent
Germination Shield	Absent	Absent	Absent
Melzer's reaction	Positive (Inner wall of spore is darker than the outer wall)	Positive (the whole spore color changed)	Negative
Genus	<i>Acaulospora</i> sp.	<i>Gigaspora</i> sp.	<i>Glomus</i> sp.

Note: *) % CYM according to Brundrett et al. (1996); [#]) Spore was located next to the neck of *Sporiferous saccule*

Based on the morphological characters, MV 5 spores can pass through a 250 μm sieve and can be collected at 45 μm , 90 μm , and 150 μm sieves during the wet sieving process. It has a brownish yellow color (0-60-10), and a sporiferous saccule lies on the spore's wall (Figure 2.A, a typical spore of the genus *Acaulospora* (Budi and Dewi 2016; Walker et al. 2018). In the Melzer solution, the spores gave a positive reaction which was indicated by a change in the spore color where the inner wall of the MV 5 spore turned out to be darker than the outer wall (Figure 2.B). This reaction is similar to the one described by Schenk and Perez (1990) in that the genus *Acaulospora* has a darker color in the inner wall than the outer wall after being treated with Melzer solution. Spores of MV 5 were also successfully colonized the maize roots system as hyphae, and vesicle structures were observed after root staining in Trypan Blue dye. Hyphae and vesicles are globose to oval in shape (Figure 3). The internal hyphae of mycorrhiza from the genus *Acaulospora* form vesicles within the root cortex (Oehl et al. 2011; Walker et al. 2018).

AMF colonization begins with the pre-infection stage, where the spores germinate into a symbiotic phase to form an appressorium (Souza 2015). The infection stage begins when the appressoria penetrate the plant roots and form internal hyphae, finely branched hyphae called arbuscular inside the cortical cell. Arbuscular hyphae are highly branched and form tree-like structures that serve as sites for nutrient and carbon exchange between the fungus and host (Smith and Read 2008; Schüßler and Walker 2011). However, arbuscular does not last long because it will degrade and provide P nutrients to the host plant (Brundrett et al. 1996; Smith and Read 2008; Souza 2015). Therefore, we cannot observe this structure in this study.

Besides arbuscular, vesicles are also formed in the cells of the root cortex and have a function as a storage organ. Vesicles are transforming structures of hyphae within the root cortex cells, in which terminal hyphae swell and grow (Redecker et al. 2013; Müller et al. 2017). Vesicles contain glycogen and lipids as a source of energy for fungi (Keymer et al. 2017). As the infection stage occurs, the hyphae grow extensively outside the root, known as external hyphae, forming a network that is responsible for nutrient uptake and transporting them from the soil to the roots (Walker et al. 2018).

In the wet sieving method, the spore of MV 17 (*Gigaspora*) can pass through the 500 μm sieve and be found in 90 μm , 150 μm , and 250 μm sieves. It indicated that the spore size of MV17 was in the range of >90 - <500 μm . Arbuscular mycorrhizae of the genus *Gigaspora* has a relatively large spore size compared to other genera. The approximate size of the *Gigaspora* spore was reported to be around 125 μm to 600 μm (Oehl et al. 2008; Morton and Msiska 2010). The size of the spores changes with age. The young spores are small and gradually enlarge until they reach their maximum size when the spores are mature (Souza 2015).

The MV 17 spore characters were presented in Table 1 to correspond to its common characteristics, with yellow, greenish-yellow, brownish yellow to yellowish-brown

(Morton and Msiska 2010; Souza 2015). Spores of this genus (*Gigaspora*) have a special character, such as bulbous suspensor (Figure 4.A) without germination shields (Walker et al. 2018). This special character was observed in the MV 17 isolate which leads us to conclude that this isolate belongs to the genus *Gigaspora*. According to Oehl et al. (2008), spores of the genus *Gigaspora* are developed by forming small spheres on bulbous suspensors. Afterward, the small spheres develop until they reach their maximum size and eventually form spores called azygospores (Morton and Benny 1990; Schenk and Perez 1990; Souza 2015). In addition, the spore of MV 17 gave a positive reaction to the Melzer solution, where the entire spore color changed to a darker color (Figure 4.B). The Melzer results also corresponded with other studies where the positive reaction occurred in all spores (Oehl et al. 2008; Souza 2015).

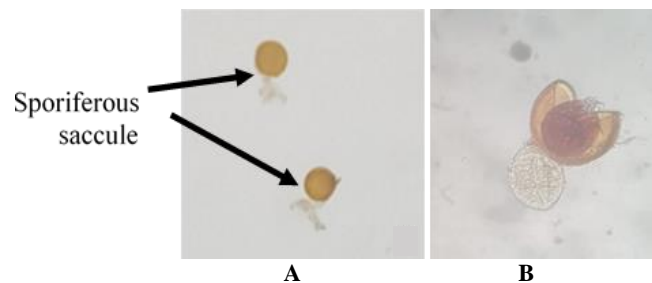


Figure 2. Spore of MV 5 showed sporiferous saccule (A) and MV5 spore in Melzer solution (B).

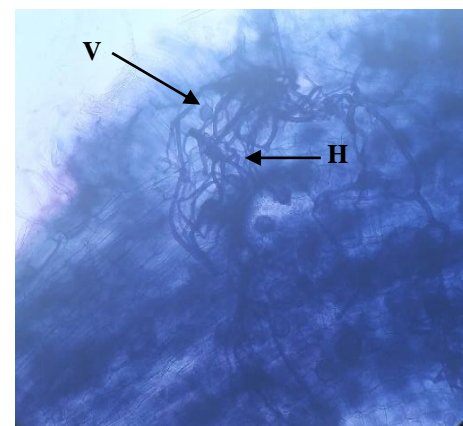


Figure 3. Colonization of maize root by MV 5, the presence of hyphae (H), and vesicles (V)

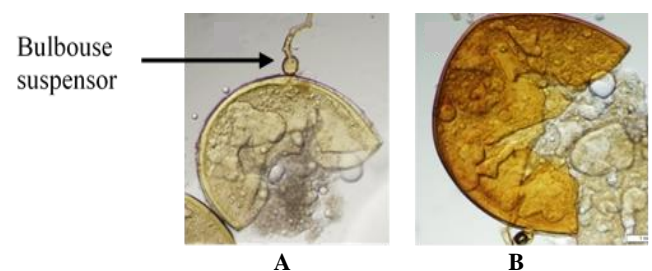


Figure 4. Spore of MV 17 isolate in PVLG solution showing bulbous suspensor (A) and spore's color in Melzer solution (B)

This study indicated that MV 17 could colonize the root system of maize as hyphae and auxiliary cells were found in maize root (Figure 5). However, the vesicle was not observed. Auxiliary cells are typical characters that are only found in the genus *Gigaspora* and *Scutellospora*. Therefore, they can be used as a trait to identify them (Oehl et al. 2011; Souza 2015). Other studies (Khade 2011; Redecker et al. 2013) also reported that the genus *Gigaspora* formed auxiliary cells in external hyphae but did not form vesicles in the internal hyphae.

Based on the characters of MV 18, it could be identified as *Glomus*. Spores can pass through a 250 μm sieve and be collected from a 45 μm to 150 μm (in the wet sieving process) which was aligned with the spore size of the genus *Glomus* in the range of 20-400 μm (Souza 2015). The reaction with Melzer's reagent gave negative results, which meant no color change when spores were crushed in Melzer solution. MV 18 spore has a globose, subglobose, and range in color from yellow to brown (10-100-0) (Figure 6) (Brundrett et al. 1996). Souza (2015) described that spore from *Glomus* has yellow, brownish yellow, yellowish-brown, brown, old brown, and blackish colors. We also observed that the spore of MV 18 isolate has typical subtending hyphae as it develops from the hyphal terminus and further enlarged to its maximum size, known as chlamydospore (Oehl et al. 2011; Souza 2015). In the root cortex, internal hyphal structures and vesicles were also observed (Figure 7). No auxiliary cell was found (Walker et al. 2018). Hyphae have an important role in the uptake of nutrients and water in the soil and transfer them to plant roots, while vesicle is a mycorrhizal structure emerging from hyphae and serves as a temporary storage organ also observed in MV 18 (*Glomus*) (Redecker et al. 2013). A vesicle has a round shape to oval, thin-walled and balloon-like structures (Souza 2015; Walker et al. 2018).

Molecular identification

Internal transcribed spacer (ITS) sequences have been used extensively to reveal phylogenetic relationships to identify glomalean fungi (Schwarzott and Schüßler 2001; Badotti et al. 2017). However, due to the high degree of ITS variation within AMF species, it is difficult to find distinct features of closely related AMF species (Krüger et al. 2012; Victorino et al. 2020). Therefore, the SSU rRNA gene of AMF has been the best option to use for AMF-specific primers as it is less variability compared to ITS, is highly conserved and has high specificity of the primer combination (Lee et al. 2008; Lekberg et al. 2018; Perez-Lamarque et al. 2020).

The success of PCR amplification was evaluated qualitatively by agarose gel as shown in Figure 8. It showed that all mycorrhiza isolates have a thick and clean band around 800 bp DNA in size. It indicates that the primer used in the PCR process successfully amplified specific DNA sites for these 3 isolates. Lee et al. (2008) reported that AML 1 and AML 2 were primers that amplified the AMF SSU rRNA gene of an 800 bp length sequence. They are highly sensitive detection and specific primers towards AMF, thereby avoiding nonspecific amplification (Lee et al. 2008; Arofatullah et al. 2019; Kobae et al. 2019).

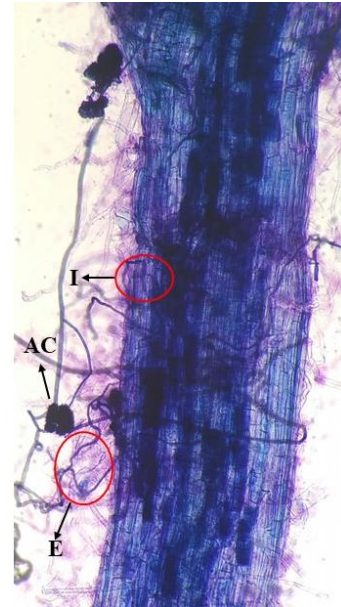


Figure 5. Colonization of maize root by MV 17 showing the presence of auxiliary cells (AC) in external (E) and internal hyphae (I) without vesicle.

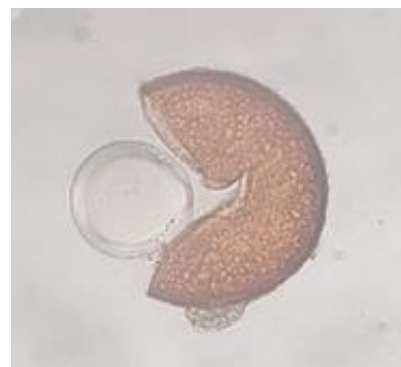


Figure 6. Spore of MV 18 isolate after crushing in PVLG solution.

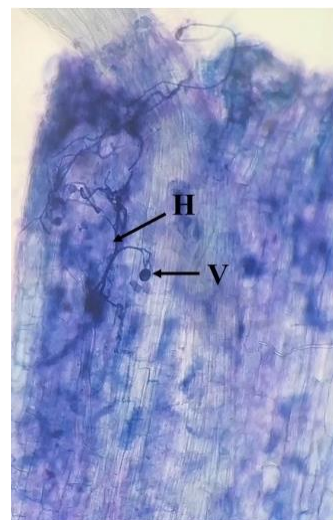


Figure 7. Structure of MV 18 colonization in maize's roots. Internal hyphae (H) and vesicle (V) were observed

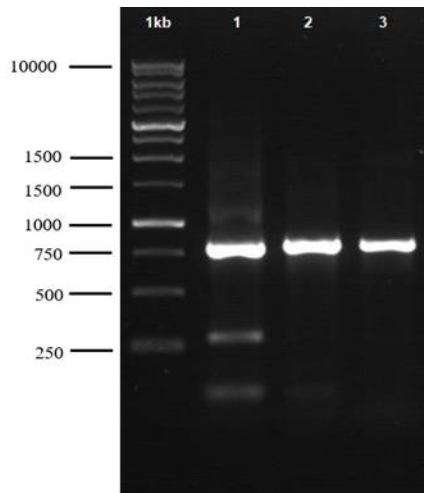


Figure 8. Electrophoresis of PCR product on agarose gel, 1) MV 17 isolate; 2) MV 18 isolate; 3) MV 5 isolate

To evaluate the success rate of species identification, the results of morphological identification were compared to the results of molecular identification using Basic Local Alignment Search Tools (BLAST-n). All AMF isolates had a match between morphological and molecular identification at the species level, with an identity value of 100%. Identity value is the similarity percentage of the input DNA sequence with the DNA sequence in GenBank. A high identity value indicates a high nucleotide sequence match. The alignment of the sequence using BLASTn showed that the MV5 isolate, which was morphologically identified as *Acaulospora* sp., had high similarity (ident:

98,12%) to *Acaulospora longula* Spain & N.C.Schenck with a query cover value of 100%. The MV 17 isolate, which was morphologically identified as *Gigaspora* sp., had high similarity (ident: 99,86%) to *Gigaspora margarita* W.N.Becker & I.R.Hall, and the value of query cover was 100%. The third isolate, MV 18, which was morphologically identified as *Glomus* sp., had the highest similarity (ident: 100%) to *Glomus etunicatum* W.N.Becker & Gerd. (synonym *Claroideoglomus etunicatum* [Schüßler and Walker 2010]), with the query cover value of 100%. The BLAST identification results are shown in Table 2.

The phylogenetic trees were constructed based on multiple sequence alignments from sequences of three AMF isolates and GenBank (NCBI) data sequences using the Kimura 2-parameter method. This method uses transitional and transversion parameters to calculate the percentage difference in genetic distance between samples (Nei and Kumar 2000). The method used is Neighbor-Joining (NJ) with a bootstrap value of 1000x. This method effectively quantifies the nucleic acid differences used in identifying species and assessing similarities. The phylogenetic tree analysis aims to confirm the relationship among isolates in the research sample. The expected phylogenetic tree is the one that can group samples based on taxonomic similarity at the species, genus, and family levels and is confirmed by the high value of bootstrap value in the phylogenetic tree branches. The phylogenetic tree shown in Figure 9 showed that the bootstrap values range from 56-100. The higher the bootstrap value, the less likely the branch tree or taxon will change; thus, the phylogeny has a firm consistency and high confidence level (Prasetya et al. 2011; Sharuddin and Muda 2015).

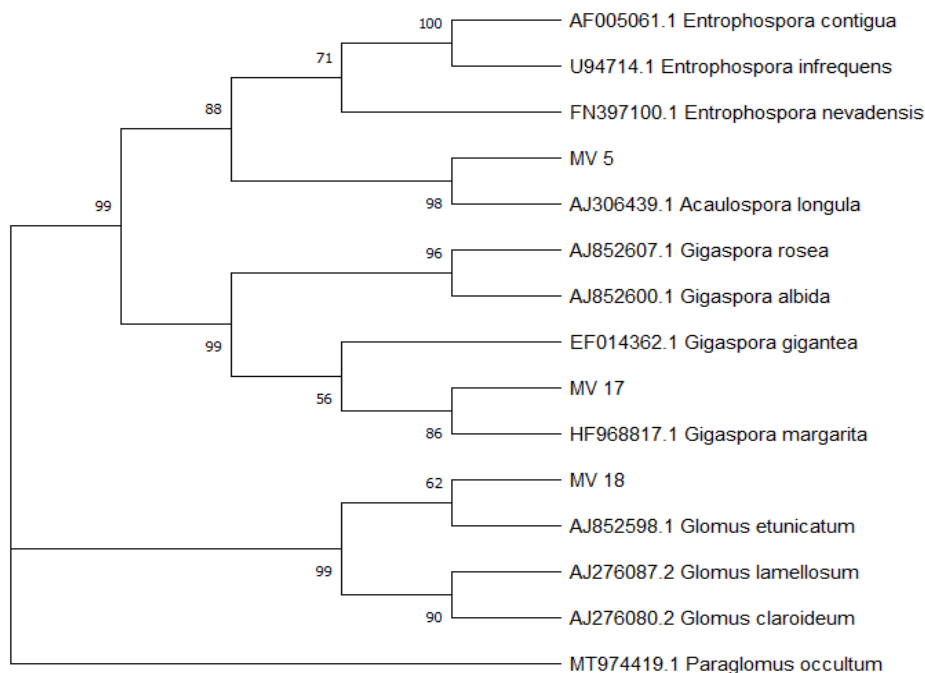


Figure 9. Phylogenetic tree of MV 5, MV 17, and MV 18

Table 2. Blast identification result

Sample	Morphology identification	Molecular identification (BLAST)	% Query cover	% Ident
MV 5	<i>Acaulospora</i> sp.	<i>Acaulospora longula</i>	100	98,12
MV 17	<i>Gigaspora</i> sp.	<i>Gigaspora margarita</i>	100	99,86
MV 18	<i>Glomus</i> sp.	<i>Glomus etunicatum</i>	100	100

The constructed phylogenetic tree separated the three AMF isolates from their outgroup, *P. occultum*. The phylogenetic tree in Figure 9 showed that the MV 5 isolate formed the same cluster as *A. longula* and was on a different clade with the *Entrophospora* genus. The MV 17 isolate formed the same cluster with the *Gigaspora* genus and on the same clade with *G. margarita*. The phylogenetic tree also showed that MV 18 isolate was on the same clade with *G. etunicatum* (synonym *Claroideoglomus etunicatum*).

Identification of the three AMF isolates based on morphology complemented the identification based on molecular (species level) (Table 2). Therefore, we have more detailed, valid data, high confidence, and stronger results by conducting the two methods. Nonomura et al. (2011) stated that combining the results from two methods is the best approach to identify AMF taxonomy.

Based on morphological identification, it can be concluded that MV 5, MV 17, and MV 18 isolates have characteristics belonging to the genera *Acaulospora*, *Gigaspora*, and *Glomus* genus, respectively, while identification based on molecular analysis showed that MV 5, MV 17, and MV 18 isolates are included in the species of *A. longula*, *G. margarita*, and *G. etunicatum* (synonym *Claroideoglomus etunicatum*), respectively. The results of this study would be useful for further research when using these three isolates due to the detailed information on the characteristics and species information.

8 ACKNOWLEDGEMENTS

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