

# Phylogenetic study of endophytic fungi associated with *Centella asiatica* from Bengkulu and Malaysian accessions based on the ITS rDNA sequence

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**Abstract.** Radiastuti N, Bahalwan HA, Susilowati DN. 2019. Phylogenetic study of endophytic fungi associated with *Centella asiatica* from Bengkulu and Malaysian accessions based on the ITS rDNA sequence. *Biodiversitas* 20: 1248-1258. *Centella asiatica* is one of the medicinal plants which is known to be symbiotic with various endophytic fungi. The purpose of this research was to determine diversity of culturable fungal endophyte from *C. asiatica*. Identification was conducted using molecular phylogenetic analysis based on the ITS rDNA sequence. The result showed that obtained 145 isolates endophytic fungi (from stolons, leaves, roots, petioles) were grouped into 18 morphotaxa (Bengkulu) and 23 morphotaxa (Malaysian). The fungal endophytic were identified as *Aspergillus austroafricanus*, *Aspergillus oryzae*, *Acrocalymma vagum*, *Ceratobasidium cornigerum*, *Ceratobasidium* sp., *Chaetomium globosum*, *Colletotrichum karstii*, *C. gigasporium*, *C. tabaci*, *Colletotrichum siamense*, *Eutypella* sp., *Fusarium solani*, *F. oxysporum*, *F. falciforme*, *F. keratoplasticum*, *F. striatum*, *Fusarium* sp., *Penicillium capsulatum*, *Phoma multirostrata*, *Perenniporia tephropora*, *Perenniporia* sp., *Phanerochaete chrysosporium*, *Phanerochaete stereoides*, *Phyllosticta capitalensis*, *Phomopsis asparagi*, *Peroneutypa scoparia*, *Phialemoniopsis* sp., *Mycochaetophora gentinae*, *Talaromyces* sp., *Earliella scabrosa*, and *Trichaptum* sp. based on molecular phylogenetic analysis. *Fusarium* (*Nectriaceae*) were the most found of fungal endophytes in both *C. asiatica* Bengkulu and Malaysian accession. The majority strain are including *Ascomycetes* and *Basidiomycetes*. The identification of endophytic fungi from medicinal plants is needed as a preliminary study to determine the potential of endophytic fungi producing bioactive compounds.

**Keywords:** *Centella asiatica*, endophytic fungi, phylogenetic tree

## INTRODUCTION

*Centella asiatica* (L.) Urb. (Apiaceae) (English: centella or Asiatic pennywort, Indonesia: pegagan) is an important medicinal plant in Indonesia and Southeast Asia. This plant is distributed in Indian subcontinent, Southeast Asia, East Asia, West Asia (Saudi Arabia and Yemen), Africa, and Australia (Gupta 2013). This species is frequently found in grassland (along riversides) and in cultivated lands (Cook 1996), as well as in ponds and streamsides, evergreen forests, fields, garden, and flooded agricultural fields. The centella extract has been widely known to cure various diseases, such as skin disorders, gastric ulcers, asthma, hemorrhoids, dysentery and tuberculosis (Zahara et al. 2014; Radji et al. 2015). Several studies noted that medicinal properties of medicinal plants are related to the endophytic microbes, especially endophytic fungi, reside within the medicinal plants (Jia et al. 2016; Kaur et al. 2014).

Endophytic fungi live inter- or intracellularly in the plant without causing symptoms of the disease under normal circumstances (Hyde and Soytong 2008; Delaye et al. 2013). Some endophytic fungi have specific relationships with host plants that can significantly affect the production of medicinal compounds from their host (Jia et al. 2016). For example, an endophytic fungus *Penicillium* sp. from *C.*

*asiatica* produced a high antioxidant activity, similar to its host, with IC<sub>50</sub> value 54.72 ± 2.19 µg/mL (Devi and Prabakan 2014). Similar results were also reported from the study of the endophytic fungi from cinchona, where the endophytic fungi produced similar compound with their hosts (Radiastuti et al. 2015; Hidayat et al. 2016)

Studies on the endophytic fungi from *C. asiatica* have mainly been focused on the diversity and analysis of the phytochemical contents (Joshi and Chaturvedi 2013; Devi and Prabakaran 2014; Nalini et al. 2014; Lulasto 2015). A previous diversity study of the endophytic fungi from *C. asiatica* was based on the morphological data which is not suitable to reveal the true diversity of the endophytic fungal community associated with *C. asiatica*. A study on the endophytic fungal diversity of *C. asiatica* using molecular analysis was only conducted on the leaves part (Rakotoniriana et al. 2008). A few species of the endophytic fungi associated with *C. asiatica* was reported Indonesia, such as *Colletotrichum* spp., *Fusarium* spp., and *Phoma* spp. (Hasyati et al. 2017). Therefore, it is necessary to conduct molecular analysis of the diversity of fungal endophytes associated with various organs of *C. asiatica* to reveal more accurate data on the diversity of these fungal group. This study was conducted to determine the diversity of endophytic fungi of *C. asiatica* (Bengkulu and Malaysian accession in Indonesia) by using ITS rDNA sequences.

## MATERIALS AND METHODS

### Fungal sources and morphotyping

A total of 145 endophytic fungal cultures isolated from various organs of *C. asiatica* were obtained from Biogen Culture Collection (Biogen CC) at Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor, Indonesia. Of them, 85 and 60 cultures were isolated from Malaysian and Bengkulu accessions, respectively. The fungal isolates from the Malaysian accession composed of 24 isolates from leaves, 17 isolates from roots, 11 isolates from petioles, and 33 isolates obtained from the stolon. While from Bengkulu accession composed of 11 isolates from the leaves, 20 isolates from the roots, 6 isolates from the petioles, and 23 isolates from the stolons. The fungal isolates from Malaysian and Bengkulu accessions belong to 23 and 18 morphotypes, respectively. These morphotypes were subjected for further molecular analysis.

### Isolation and extraction of DNA

The isolation and extraction of DNA of the fungal endophytes were conducted according to the protocol described by Hidayat et al. 2016.

### DNA amplification and sequencing

The DNA amplification was performed by Polymerase Chain Reaction (PCR) method in a 25 µL reaction mixture consisting of nuclease-free water 8.5 µL, Go Taq green master mix 12.5 µL, 1 µL primer ITS5 (forward) 5'-TCCTCCGCTTATTGATATGC-3' and ITS4 (reverse) (5'-TCCGTAGGTGAACCTGCGC-3') (White et al. 1990), and 2 µL of DNA template. The PCR conditions used in this study were set as follows: 5 minutes at 94°C for initial denaturation, followed by 35 cycles of 30 seconds at 94°C for denaturation, 30 seconds at 52°C for annealing process, 30 seconds at 72°C for extension and 7 minutes at 72°C for the final extension. The product of PCR amplification was electrophoresed in a 1.5% (w/v) agarose gel amended with 1% red gel and was immersed in 1x TAE buffer. The electrophoresis condition was set at 110V for 30 min. A 1 µL of DNA marker (1000 bp) was used in the gel. DNA visualization was conducted by Gel Doc™ XR (BioRad, USA). The PCR products were sent to First Base (Malaysia) for sequencing.

### Phylogenetic analysis

The phylogenetic analysis was performed using the Maximum Parsimony (MP) method in the PAUP 4.0.b10 program (Swofford 2002). The heuristic method using tree bisection reconstruction (TBR) with the addition of 1000 random sequence algorithms was performed to obtain the optimal tree. The Tree Length (TL), Consistency Index (CI), Retention Index (RI), Related Consistency Index (RC), and Homoplasy Index (HI) were calculated. Internal branch strength of the phylogenetic tree in MP analysis was tested by Bootstrap (BS) analysis using 1000 replications. Bootstrap (BS) value > 50% was displayed on the phylogenetic tree.

## RESULTS AND DISCUSSION

### Phylogenetic analyses

#### Malaysian accession

A dataset generated from the phylogenetic analysis of Malaysian accession contains 125 sequences including *Rhizopus oryzae* CBS 11207 (NR103595) and *Rhizopus oryzae* strain 783018 (GU594768). This dataset composed of 891 total characters, which included 222 constant characters, 89 uninformative characters, and 580 informative characters (CI = 0.404, RI = 0.760, RC = 0.347, HI = 0.596). The phylogenetic tree showed that four sequences of the fungal endophytes belong to Basidiomycota, and 19 sequences belong to Ascomycota (Fig. 1). All of four Basidiomycota sequences (MM1, MM6, MM12, and MM21) belong to *Agaricomycetes*. The MM1 sequence nested in the clade containing unidentified *Ceratobasidium* sp. strain R113 and *Ceratobasidium* sp. strain AG A with a high BS (86%). These isolates also formed a sister clade with *C. ramicola* strain CBS 758.79 with a 100% BS. These results showed that MM1 belong to *Ceratobasidium* sp. The MM6 sequence was determined as *Earliella scabrosa*. due to nesting with unidentified *E. scabrosa* strain BRFM 1106 with a high BS (100%). The MM21 sequence was determined as *Perenniporia* sp. due to form a sister clade with *P. corticola* strain SKB1 and SK13, *P. tephroppora* strain D2 with a 100% BS. While MM12 sequence was determined as *Phanerochaete stereoides* due to a monophyletic with *P. stereoides* strain VPCI 2073 12 with 60% BS.

In the Ascomycota clade, MM7 sequence nested in the *Guignardia* clade and formed a monophyletic clade with *G. mangiferae* strain CBS 101228 and its asexual state *Phyllosticta capitalensis* strain CBS 117118 with 100% BS. Therefore, this sequence was determined as *P. capitalensis* (*Phyllosticta* is an accepted name in this group). The MM13, MM15, and MM16 sequences nested in the *Eurotiomycetes* clades. The isolates MM13 can only be determined as *Aspergillus oryzae* due to nesting in the same clade with several sequences of *Aspergillus* species such as *A. oryzae* strain CBS 466 91, *A. oryzae* type strain NRRL 447, *A. flavus* type strain ATCC 16883 (52% BS). The isolates MM15 was determined as *Penicillium capsulatum* due to formed a monophyletic with the *P. capsulatum* strain species CBS 274 58 and the strain type NRRL 2056 (71%) in the *Penicillium* clade. While MM16 sequence was determined as *Talaromyces* sp. because did not form a monophyletic clade with any *Talaromyces* species in the *Talaromyces* clade.

The *Sordariomycetes* clade consisted of six subclades (Fig. 1). In the first subclade, MM2 was determined as *Colletotrichum karstii* due to form a monophyletic clade with *C. karstii* strain CPC 26233 and type strain CBS 132134 (75% BS). The MM9 sequence formed a monophyletic with *C. siamense* strain PHL12 and CBS 112985 strain (93% BS), while MM18 and MM23 sequences formed a closely related to *C. tabaci* type strain CPC 18945 and strain N150 with a 76% BS. The identity of MM9 formed a monophyletic with *Colletotrichum siamense* strain PPHL12 with 93%BS. In addition, MM14

sequence was determined as *C. gigasporum* due to form a monophyletic group with *C. gigasporum* strain CBS 125730 and CBS strain 101881 with a high level of bootstrap support (100%). In the second subclade, MM3 and MM17 sequences formed a monophyletic with *Fusarium solani* strain GA12 and strain CPC 27200 with 95% bootstrap value. Therefore, the MM3 and MM17 sequences were identified as *F. solani* and MM20 formed a monophyletic with 69% BS. However, MM4 and MM22 sequences were tentatively determined as *Fusarium* sp. because did not form a monophyletic clade with a single representative *Fusarium* sequences in this subclade. In the third subclade, MM19 sequence was identified as *Chaetomium globosum* due to nesting in the monophyletic clade with *C. globosum* strain ATCC 6250 and type strain CBS 160 62 (100% BS). In the fourth subclade, MM11 sequence was determined as *Phomopsis asparagi* as this sequence formed a monophyletic clade with *P. asparagi* strain HL57 and B144 with a high BS (70%). Sequence of MM8 was determined as *Phialemoniopsis* due to form a clade with *P. hongkongensis* strain FMR 6321 and HKU39, *P. curvata* strain UTHSC 04 956 (86%). The MM5 sequence was also tentatively determined as *Etyrella* sp. due to form a monophyletic with *Etyrella* sp. MEF14 (100% BS), while MM10 sequence was identified as *Peroneutypa scoparia* because this sequence formed a monophyletic clade with *P. scoparia* MFLUCC 11 0615 (89%).

#### Bengkulu accession

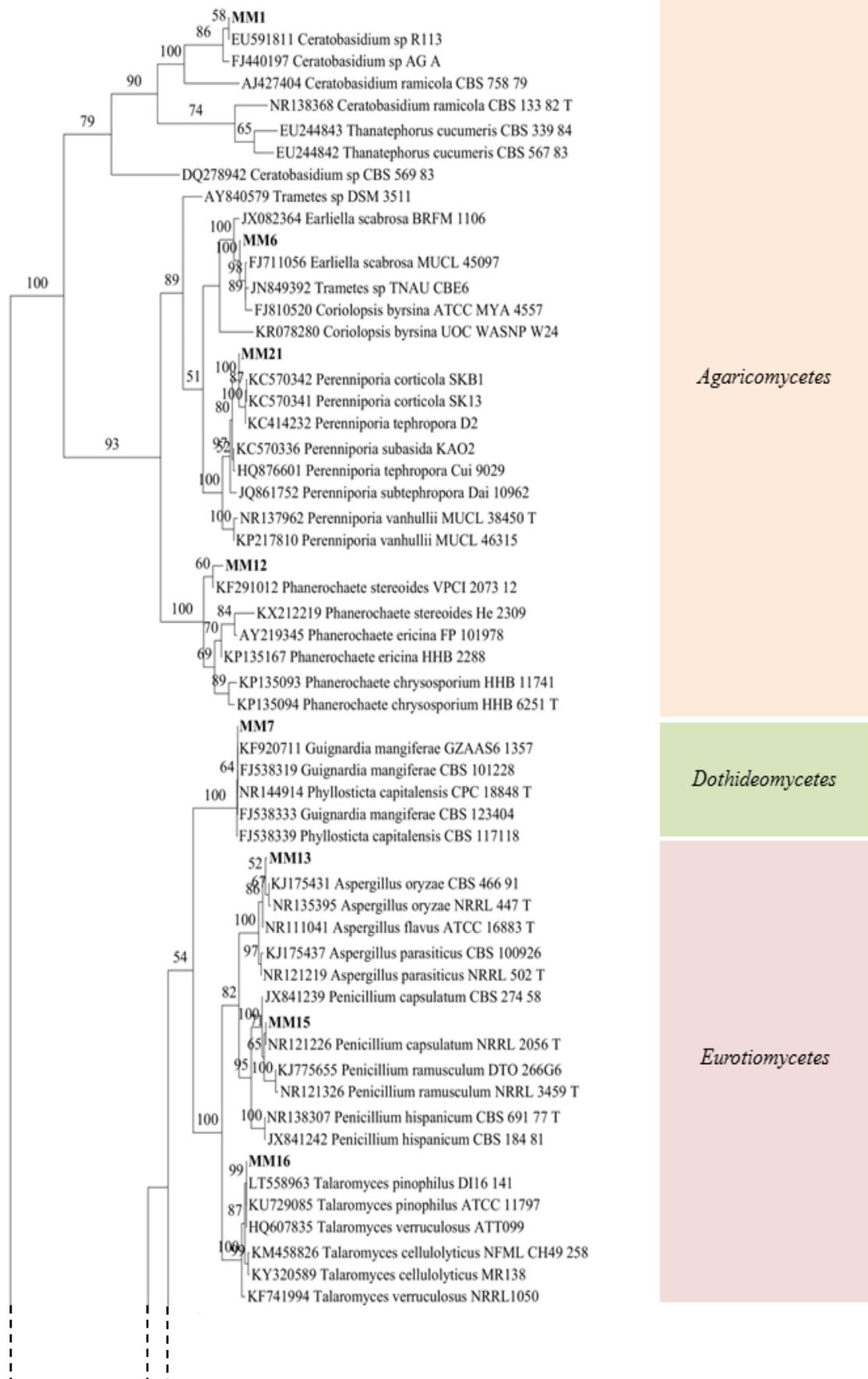
The MP analysis of endophytic fungal sequences from Bengkulu accession comprised of 120 sequences with 925 total characters of which 278 characters were constant, 94 characters was uninformative, and 553 informative characters (CI = 0.397, RI = 0.857, RC = 0.349, HI = 0.603). The phylogenetic tree showed that members of the fungal sequences from Bengkulu accession divided into Basidiomycota clade (4 sequences) and Ascomycota clade (14 sequences) (Fig. 2). All of the sequences in the Basidiomycota clade belong to Agaricomycetes, while members of the Ascomycota clade consisted of Sordariomycetes (11 sequences), Eurotiomycetes (1 sequence), Dothideomycetes (2 sequences) (Fig. 2). In the Agaricomycetes clade, MB2 sequence was determined as *Phanerochaete chrysosporium* due to nesting in the same clade with *P. chrysosporium* strain H008 with a high BS (100%), and this clade is sister clade with *P. chrycocporium* HHB type strain 6251. The sequence of MB11 was tentatively identified as *Trichaptum* sp. because this sequence formed a monophyletic with unidentified *Trichaptum* sp. E7082 strain and *Trichaptum* sp. E7083 strain (83% BS). The species identity of MB5 sequence was determined as *Perenniporia tephropora* as this sequence nested in the same clade with *P. tephropora* species strains A1S3 D98 and *P. tephropora* strains Cui 9029 with a low BS. While MB20 sequence was determined as *Ceratobasidium comigerum* because it formed a monophyletic clade with *C. cornigerum* strain XSD (100% BS).

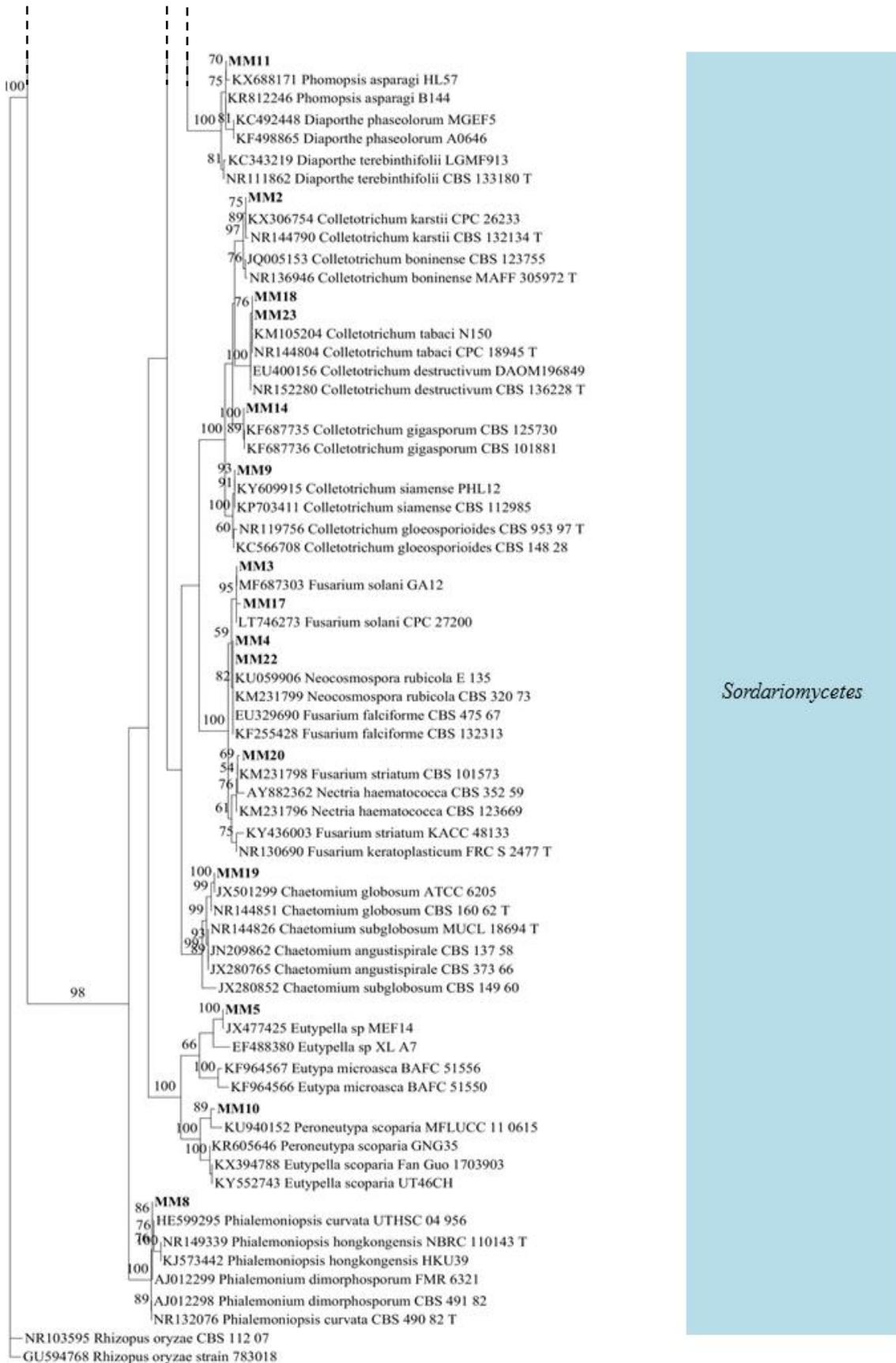
In the Ascomycota clade, the MB19 and MB21 sequences were determined as *Mycochaetophora gentianae* as these sequences formed a sister clade with *M. gentianae* MAFF 241068 and type strain MAF239231, while MB3, MB9, and MB17 sequences belong to *Fusarium oxysporum* due to nesting in the same clade with *F. oxysporum* strains CBS 129.24, *F. oxysporum* strain CPC 27701 and *F. oxysporum* strains CSB F338 with a high bootstrap value (100%). The endophytic fungal sequences of MB7, MB8, and MB10 were determined as *F. falciforme* as these sequences formed a monophyletic clade with *F. falciforme* strains CBS 475.67 and *F. falciforme* strains CBS 132313 with a high BS (74%). The MB12 sequence was determined as *F. keratoplasticum* due to nesting in the same clade with three sequences of *F. keratoplasticum*, viz, *F. keratoplasticum* IHEM 19026 MITS 1840, *F. keratoplasticum* type strain FRC S 2477, and *F. keratoplasticum* strains CBS 139461 with 70% BS. In the *Colletotrichum* clade, the MB14 and MB18 sequences were determined as *Colletotrichum tabaci* as both sequences formed a monophyletic clade with *C. tabaci* type strain CPC 18945 and *C. tabaci* N150 strains with low BS. In the *Eurotiomycetes* clade, the MB1 sequence was determined as *Aspergillus austroafricanus* due to this sequence formed a monophyletic clade with *A. austroafricanus* type strain NRRL 233 with low BS. In the *Dothideomycetes* clade, the MB4 sequence was determined as *Acrocalymma vagum* as this sequence formed a monophyletic clade with *A. vagum* strain CPC 24222 (100% BS), while the MB16 sequence was determined as *Phoma multirostrata* because this sequence formed a monophyletic clade with three sequences of *P. multirostrata* with a high bootstrap support (90%).

#### Discussion

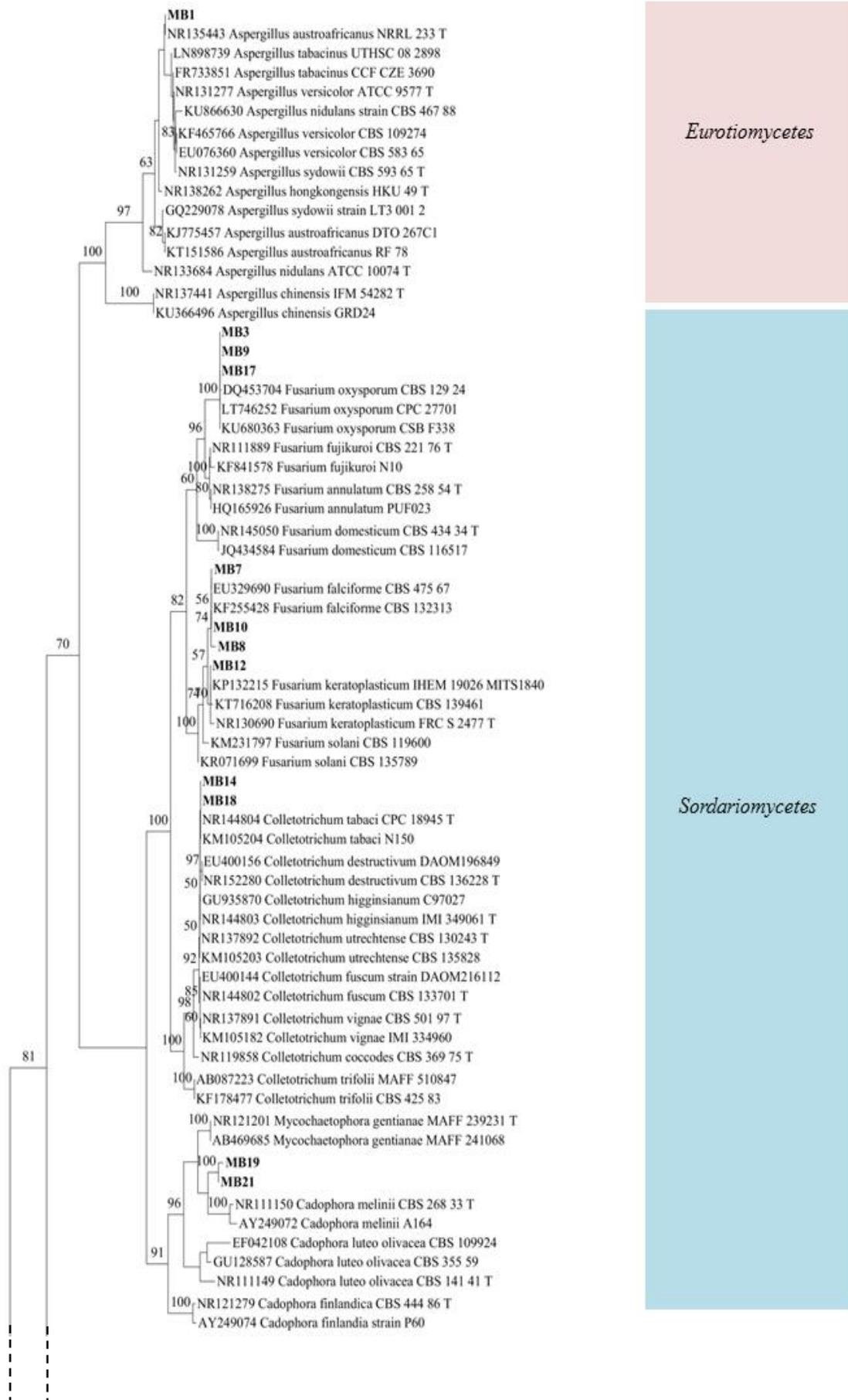
This study found that the highest number of endophytic fungi was mostly obtained from stolons, followed by leaves, roots, petioles from *C. asiatica* of Malaysian accession (table 1), and from *C. asiatica* of Bengkulu accession followed roots, leaves, and petioles (table 2).

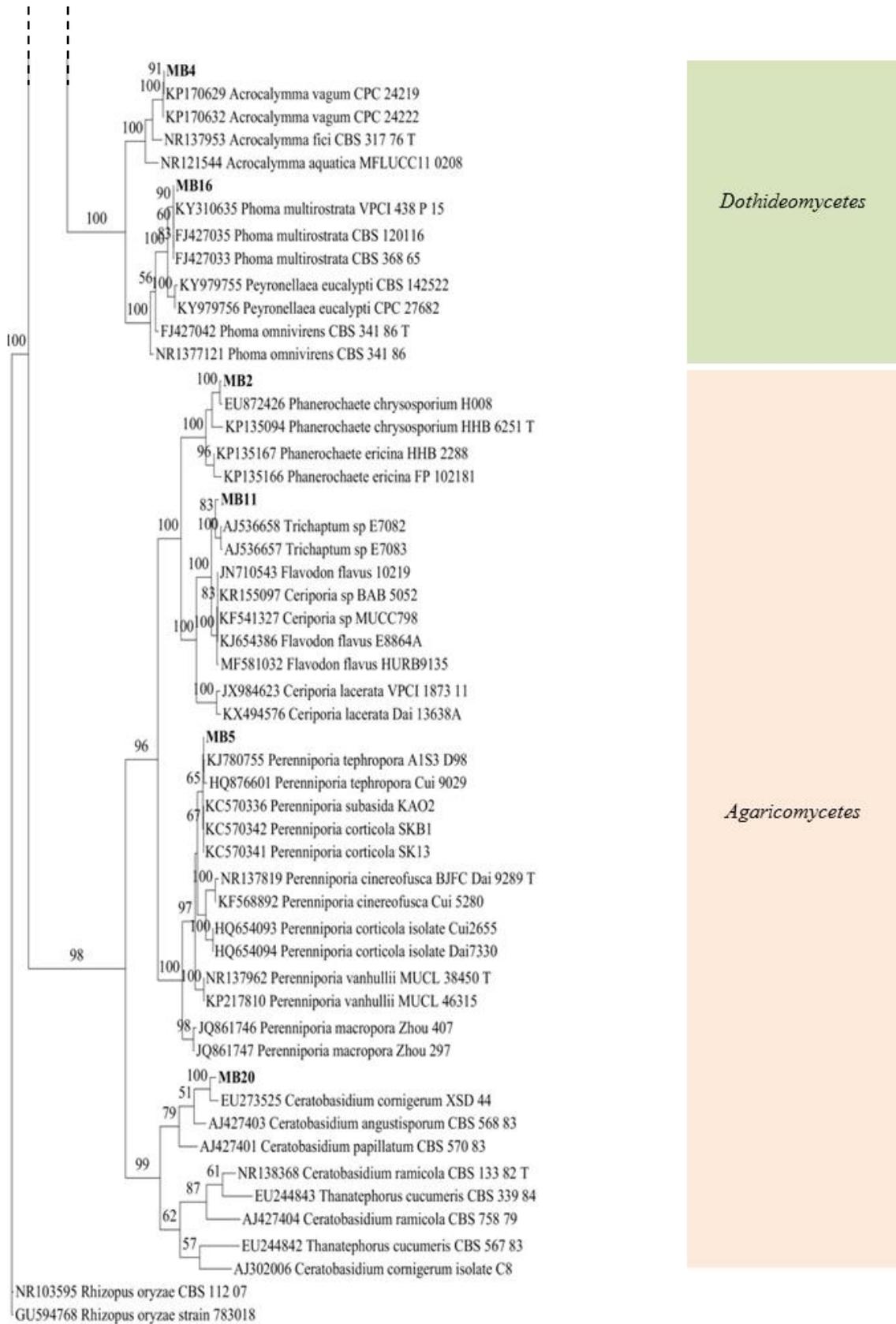
The distribution of endophytic fungi occurs in different host plants and organs of a host plant. Table 1 and Table 2 shows the distribution of the endophytic fungi in the *C. asiatica* plant of Malaysian and Bengkulu accession. The number of endophytic fungi obtained from the stolons was the largest number compared to the number obtained from the other organs. The distribution of endophytic fungi in the host plants can be influenced by several factors, such as the origin of the colonized endophytes and the substances in the plant organ tissues. The distribution of endophytic fungi is possibly related to the ability of each endophytic species to use specific substrates or plant tissues in showing the distribution strategy to obtain resources from the same plants (Jia et al. 2016). Arnold and Lutzoni (2007) suggested that different endophytic composition in different host organs occurred due to the histologic differences and the availability of the plant organ nutrients, in which endophytic fungi colonized. Arnold et al. (2003) stated that different leaves in the same tree may have distinct endophytic colonies.





**Figure 1.** MP tree generated from the ITS rDNA sequences from endophytic fungal isolate morphotypes from *C. asiatica* (Malaysian accession) and related sequences





**Figure 2.** MP tree generated from the ITS rDNA sequences of the endophytic fungi from *C. asiatica* (Bengkulu accession) and related sequences

**Table 1.** Fungal endophytic species distribution in the different organs of *C. asiatica* of Malaysian accession

Taxa	Leaves	Roots	Petioles	Stolons
<b>Sordariomycetes</b>				
<i>Colletotrichum kartsii</i>	1	-	-	-
<i>C. tabaci 1</i>	1	-	-	-
<i>C. tabaci 2</i>	-	4	3	-
<i>C. gigasporum</i>	3	-	-	-
<i>C. siamense</i>	10	-	-	-
<i>Fusarium solani 1</i>	1	-	-	6
<i>F. solani 2</i>	-	-	-	6
<i>F. striatum</i>	-	-	-	2
<i>Fusarium sp. 2</i>	-	-	-	1
<i>Fusarium sp. 3</i>	-	-	1	-
<i>Chaetomium globosum</i>	-	1	-	-
<i>Phomopsis asparagi</i>	1	1	-	-
<i>Phialemoniopsis sp.</i>	-	4	-	-
<i>Eutypella sp.</i>	-	1	-	-
<i>Peroneutypa scoparia</i>	-	1	-	-
<b>Agaricomycetes</b>				
<i>Ceratiobasidium sp.</i>	-	3	-	9
<i>Perenniporia scoparia</i>	4	-	-	-
<i>Phanerochaete steroides</i>	1	-	-	-
<i>Earliella scabrosa</i>	-	-	3	-
<b>Dothidiomycetes</b>				
<i>Phyllosticta capitalensis</i>	-	-	4	-
<b>Eurotiomycetes</b>				
<i>Aspergillus oryzae</i>	-	2	-	-
<i>Penicillium capsulatum</i>	2	-	-	-
<i>Talaromyces sp.</i>	-	-	-	2
<b>Total</b>	<b>24</b>	<b>17</b>	<b>11</b>	<b>26</b>

**Table 2.** Fungal endophytic species distribution in the different organs of *C. asiatica* of Bengkulu accession

Taxa	Leaves	Roots	Petioles	Stolons
<b>Sordariomycetes</b>				
<i>Colletotrichum tabaci 1</i>	2	-	-	-
<i>C. tabaci 2</i>	2	-	2	2
<i>Fusarium oxysporum 1</i>	-	1	-	-
<i>F. oxysporum 2</i>	-	2	-	6
<i>F. oxysporum 3</i>	-	1	-	1
<i>F. falciforme 1</i>	-	2	-	2
<i>F. falciforme 2</i>	-	6	-	3
<i>F. falciforme 3</i>	1	1	-	1
<i>F. keratoplasticum</i>	-	1	-	2
<i>Mycochaetophora gentinae 1</i>	2	-	-	-
<i>Mycochaetophora gentinae 2</i>	1	-	-	-
<b>Agaricomycete</b>				
<i>Ceratiobasidium cornigerum</i>	-	1	-	9
<i>Phanerochaete chrysosporium</i>	-	-	2	-
<i>Perenniporia tephropora</i>	1	-	-	-
<i>Trichaptum sp.</i>	-	-	-	1
<b>Dothidiomycetes</b>				
<i>Acrocalymma vagum</i>	1	2	-	-
<i>Phoma multirostrata</i>	1	3	-	3
<b>Eurotiomycetes</b>				
<i>Aspergillus austroafricanus</i>	1	-	2	-
<b>Total</b>	<b>12</b>	<b>20</b>	<b>6</b>	<b>30</b>

This study also showed that endophytic fungal community in both Malaysian and Bengkulu accession is dominated by Sordariomycetes taxa (Table 1 and Table 2). Members of Sordariomycetes occupy about 66% and 61% of the endophytic fungal community in Malaysian and Bengkulu accessions, respectively. The phylogenetic tree showed that the tree form four clades of polyphyletic clusters and were divided into 4 classes, namely Eurotiomycetes (13%), Sordariomycetes (66%), Dothideomycetes (4%), and Agaricomycete (17%) from *C. asiatica* of Malaysian accession (Table 1) and fungal endophyte from *C. asiatica* of Bengkulu accession showed that Sordariomycetes (61%), Dothideomycetes (11%) and Eurotiomycetes (6%) classes (Table 2).

Almost all of the total endophytic fungi species found in *C. asiatica* plants in Bengkulu accession are different from those of Malaysian accession. The endophytic fungi of *C. asiatica* of Bengkulu accession could be identified as *Aspergillus austroafricanus*, *Acrocalymma vagum*, *Ceratiobasidium cornigerum*, *Colletotrichum tabaci*, *Fusarium oxysporum*, *F. falciforme*, *F. keratoplasticum*, *Phoma multirostrata*, *Perenniporia tephropora*, *Phanerochaete chrysosporium*, *Mycochaetophora gentinae*, and *Trichaptum sp.* The endophytic fungi of *C. asiatica* of Malaysian accession could be identified as *Aspergillus oryzae*, *Ceratiobasidium sp.*, *Chaetomium globosum*, *Colletotrichum karstii*, *C. gigasporum*, *C. tabaci*, *Colletotrichum siamense*, *Eutypella sp.*, *Fusarium*

*solani*, *F. striatum*, *Fusarium sp.*, *Penicillium capsulatum*, *Phyllosticta capitalensis*, *Perenniporia sp.*, *Peroneutypa scoparia*, *Phanerochaete steroides*, *Phomopsis asparagi*, *Phialemoniopsis sp.*, *Talaromyces sp.* and *Earliella scabrosa*. Based on the genus level, endophytic fungi were identified in both accessions of Pegagan plants, namely *Aspergillus*, *Ceratiobasidium*, *Colletotrichum*, *Fusarium*, *Perenniporia*, and *Phanerochaete*, but on the species level wasn't same between endophytic fungi from *C. asiatica* Malaysian and Bengkulu accession. Therefore, the species found in each Pegagan plant are endophytic fungi specific to each plant.

The most dominant genus of endophytic fungi from *C. asiatica* are *Colletotrichum* (4 species) and *Fusarium* (5 species), because these two genera have capability to be distributed in some organs of plant. This result is in agreement with Arnold and Lutzoni (2007) who noted that the endophytic fungi that are often isolated from tropical plants are *Colletotrichum* and *Fusarium*. Both taxa were also found in Manoko and Boyolali accessions of *C. asiatica* (Hasyiyati et al. 2017) as well as in Indian accession (Gupta and Chaturvedi 2017). Beside *Colletotrichum* and *Fusarium*, in this study found some genera such as *Guignardia*, *Phoma*, *Phomopsis*, *Penicillium* and some of these genera were also found in the same research such as by Ginting (2013) has isolated endophytic fungi from the leaves of *C. asiatica* of Malaysian accession and obtained three species of

*Mycleptodiscus indicus*, *Glomerella cingulata* (teleomorph of *Colletotrichum gloeosporioides*), and *Stagonosporopsis cucurbitacearum*. Devi and Prabakan (2014) found *Penicillium* sp. of local *C. asiatica* from India. Rakotoniriana et al. (2008) have also identified some genera, such as *Guignardia*, *Phoma*, *Phomopsis*, *Leptosphaerulina*, and *Phialophora* from *C. asiatica*, from Mangoro and Madagascar.

Another common fungal endophytes are *Phyllosticta* (sexual state: *Guignardia*), *Phyllosticta* can be found in the *C. asiatica* as an endophyte, or saprophyte or pathogenic in the other plant. The *G. mangiferae* (*P. capitalensis*), a ubiquitous endophyte of woody plants and as pathogen some genus plant e.i *Citrus*, *Mangifera*, *Vitex*, *Artocarpus*, *Musa*, *Psidium*, and *Orchid*. *G. mangiferae* is the same species as *G. endophyllicola* (anamorf: *Phyllosticta modalensis*), based on molecular identification with encoding ITS2 (Baayen et al. 2002). However, subsequent research by Glienke et al. (2011) revealed that *P. capitalensis* has low based sequence homology with referenced isolates, *G. mangiferae*. Hence, it is a strong statement that these two species are distinct species.

Many endophytic fungal isolates from *C. asiatica* are unidentified until species level based on the ITS rDNA sequence, therefore, further analysis using additional genes is necessary to determine these taxa until the species level. A number of previous studies had used ITS primer to identify the endophytic fungi, but it proven insufficient for several highly diverse genera. Majority genus *Fusarium* included as species complex, meanwhile, need some gen for the identification for example research of Herron et al. 2015 described eight more species in the fujikuroi complex from stem cancers and branches of *Pinus* plants. According to research of Watanabe et al. 2011, currently, the identification of *Fusarium* involving combination of several gene regions such as the internal transcribed spacer (ITS) and the elongation factor 1 $\alpha$  (EF-1 $\alpha$ ),  $\beta$ -tubulin ( $\beta$ -tub) and aminoacidate reductase (*lys2*) gene regions. *A. austroafricanus* is a new species found, based on phylogenetic with multilocus analysis by Jurjevic et al. (2012) as a part of the *Aspergillus Versicolores* group with closed relatives, namely *A. versicolor*, *A. tabacinus*, *A. fructus*, *A. protuberus*, and *A. amoneus* (Table 2).

**Table 2.** Additional gene regions for the identification of genera found in this study

Genera	Genes/Regions	References
<i>Aspergillus</i>	$\beta$ -tubulin gene (BT2), calmodulin gene (CF), ITS and partial <i>lsu</i> -rDNA (ID), RNA polymerase 2 gene (RPB2),	Jurjevic et al. (2012)
<i>Acrocalymma</i>	ITS regions and LSU gene sequences	Jayasirri et al. (2019)
<i>Acremonium</i>	ITS regions, NL209 , NL912, tubulin gene (TUB1F, TUB2R)	Zhang et al. 2017
<i>Colletotrichum</i>	ITS regions, partial actin gene (ACT), $\beta$ -tubulin gene (TUB2), calmodulin gene (CAL), glutamine synthetase, glyceraldehyde-3-phosphate dehydrogenase genes (GPDH)	Prihastuti et al. (2009)
<i>Chaetomium</i>	ITS regions, partial ribosomal large subunits (LSU rDNA), RNA polymerase II gene (RPB2), $\beta$ -tubulin gene (TUB)	Zhang et al. (2006)
<i>Ceratobasidium</i>	ITS and LSU regions, RNA polymerase II gene (RPB2), Elongation factor $\alpha$ gene (EF- $\alpha$ ), mitochondria gene (ATP6)	Gonzalez et al. (2016)
<i>Eutypella</i>	ITS regions, $\beta$ -tubulin gene (Bt2a, Bt2b)	Mayorquin et al. (2016)
<i>Fusarium</i>	ITS regions, $\beta$ -tubulin gene ( $\beta$ -tub), Elongation factor $\alpha$ gene (EF- $\alpha$ ), aminoacidate reductase gene ( <i>lys2</i> )	Watanabe et al. (2011)
<i>Phyllosticta</i>	ITS regions, partial translation elongation factor 1-alpha gene (TEF1), actin gene (ACT), and glyceraldehyde 3-phosphate dehydrogenase (GPDH) gene regions.	Su and Cai (2012)
<i>Penicillium</i>	ITS regions, BenA, CaM and RPB2 regions	Wang et al. (2017)
<i>Phoma</i>	ITS-rDNA, tubulin gene ( $\beta$ TUB), actin gene (ACT), translation elongation factor 1 alpha gene ( <i>tef1</i> ), Histone protein gene	Rai et al. (2014)
<i>Phomopsis</i>	ITS regions, partial translation elongation factor 1-alpha gene (TEF1), $\beta$ tubulin gene (TUB), histone H3 gene (HIS), calmodulin gene (CAL)	Gomes et al. (2013)
<i>Perenniporia</i>	ITS and LSU-rDNA regions	Zhao and Cui (2017)
<i>Phanerochaete</i>	ITS regions, nLSU, RNA polymerase II largest subunit (RPB1, RPB2)	Floudas and Hibbett (2015)
<i>Phialemonopsis</i>	ITS regions, D1/D2 domains of the 28S rRNA, actin gene, $\beta$ -tubulin gene	Perdomo et al. (2017)
<i>Mycochetophthora</i>	ITS regions, partial translation elongation factor 1- $\alpha$ gene (TEF1), RNA polymerase I, II gene (RPB1, RPB2)	Carlson et al. (2014)
<i>Talaromyces</i>	ITS regions, calmodulin gene (CaM), $\beta$ -tubulin gene (TUB), RNA polymerase II largest subunit gene (RPB2)	Su and Niu (2018)
<i>Trichaptum</i>	ITS regions, IGS1, IGS2, partial translation elongation factor gene (EF1160 F, EF1750 R)	Kauserud and Schumacher (2003)
<i>Earliella</i>	ITS regions, nLSU, partial translation elongation factor 1-alpha gene (TEF1), RNA polymerase I, II gene (RPB1, RPB2)	Justo and Hibbet, 2011

This study found several genera as new records of endophytic fungi associated with *C. asiatica* of Bengkulu and Malaysian accessions such as *Perenniporia*, *Phanerochaete*, *Acrocalymma*, *Mycochaetophora*, *Chaetomium*, *Eutypella*, *Peroneutypa*, *Phialemoniopsis*, *Talaromyces*, and *Earliella*. These fungi are also rarely found as endophytes in other host plants.

Several genera of endophytic fungi from the *C. asiatica* plant of Bengkulu accession was not found in Malaysian accessions namely *Acrocalymma*, *Mycochaetophora*, and *Phoma*. In contrast, endophytic fungi genus were identified in Malaysian accession but not found in Bengkulu accession were *Chaetomium*, *Eutypella*, *Penicillium*, *Peroneutypa*, *Phialemoniopsis*, *Phomopsis*, *Phyllosticta*, *Talaromyces*, and *Trametes*. Endophytic fungi are ubiquitous and occur within different plants in various ecosystems. This study provides information on differences of fungal endophytes diversity, community, composition in different tissues of *C. asiatica*.

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