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Arbuscular Mycorrhizal Association with Rattan Species of the Belum-Temengor Forest Complex, Perak, Malaysia

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ABSTRACT

Rattan (*Calamus* spp.) is a high commercial value palm from the subfamily Calamoideae the primary source for cane in the well-developed rattan industry. Most studies on rattan have focused on its biodiversity, distribution, quality, strength, physical, mechanical, and morphological properties and genetics. Still, only a few have investigated the association of rattan with arbuscular mycorrhizal (AM) fungi. These mycorrhizal fungi are well known to play essential functions as promoting plant growth, maintaining plant community biodiversity and nutrient cycles in soil. This study aims to identify the established AM fungi community and their ecological interactions with *Calamus* spp. in the Belum-Temengor Forest Complex, Perak, Malaysia. *Calamus* spp. roots and their rhizospheric soil samples were collected from six sampling sites in the Belum-Temengor Forest Complex, one of the oldest rainforests in the world. The degree of mycorrhizal colonisation in *Calamus* spp. was evaluated using the grid lines method. At the same time, the AM fungi spore diversity in the rhizospheric soils were isolated using the wet sieving method and identified taxonomically analysed into different genera. *Calamus insignis* showed the highest degree of mycorrhizal colonisation amongst all the *Calamus* spp. present on the sampling sites. The AM fungi spores isolated from the rhizospheric soil from Belum-Temengor Forest Complex belonged to the genera *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, and *Scutellospora*. *Glomus* was the most frequently found genus in all the sampling sites. This study is the first record of the AM fungal diversity found in the Belum-Temengor Forest Complex.

Keywords: *Calamus* spp., *Glomus* spp., mycorrhiza, rainforest, rhizosphere

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INTRODUCTION

Rattans originated from Southeast Asia are the primary source for cane in the well-developed rattan industry. Rattan belongs to the subfamily Calamoideae, has spiny stems and scaly fruits (Stiegel et al., 2011), and is economically and ecologically essential in Asian rainforests (Gentry, 1991). The state of Perak has 17.9 %; the second-highest percentage of whole rattan group in the Permanent Reserved Forests, Peninsular Malaysia, based on the Third National Forest Inventory (NFI-3), behind Pahang with 37.2% (Chin et al., 1994). Certain rattan species' stem flexibility has made them useful for matting, binding, and furniture production (Dransfield, 1992). It is one of the most popular materials for handicrafts besides being used as a construction material, such as temporary building or construction for fishing traps, baskets, mats, and ornaments (Afentina et al., 2020).

Rattans are important to tropical primary and secondary rainforests, but their evolution, diversity and phylogenetic relationships remain poorly understood (Dransfield, 1992). One rattan species, *Calamus manan*, locally known as Rotan Manau, has substantial commercial value because it has a large diameter stem (approximately up to 8 cm without sheath), able to grow up to 100 m length (Kusuma et al., 2011), and it is widely distributed in Southern Thailand, Malaysia, Sumatera, and Kalimantan (Mohamad, 1993). However, due to its high commercial value and despite its broad distribution range, Rotan Manau has been excessively harvested from nature (Mohamad, 1993), which resulted in severe population depletion; therefore, this species has been designated as 'vulnerable.' Another factor contributing to the severe population depletion of Rotan Manau is habitat destruction and degradation (Baillie et al., 2004). In general, rattan is facing challenges in which the resources produced by these species are under serious threat by the transformation of forests to agricultural and other land uses, as well as excessive exploitation of the remaining stocks in the forests (Hirschberger, 2011). The novelty of this paper is the association of indigenous mycorrhizal fungi with local specific rattans in Perak, Malaysia, for example, Rotan Manau. Besides having a high economic value, rattan is associated with mycorrhizal, which plays an important role in promoting rattan plant growth and health. Therefore, it is necessary to isolate and identify the indigenous mycorrhizal fungi associated with the indigenous Malaysian rattan, for example, Rotan Manau.

Rattans have been studied in various research areas, such as clarification of their quality, strength, physical, mechanical, and morphological properties, efficiency in using different species of rattan (Isnard, 2006; Isnard & Rowe, 2007; Mathew & Bhat, 1997; Sudarmonowati et al., 2004), their genetics (Ramesha et al., 2007; Sarmah et al., 2007; Sudarmonowati et al., 2004), and conservation (Lyngdoh et al., 2005). However, most rattan studies mainly focused on the distribution, diversity, and commercial value, but only a few have investigated their association with arbuscular mycorrhizal

(AM) fungi. Arbuscular mycorrhizal fungi are an essential component in the soil ecosystem, as they form a mutualistic symbiosis with more than 80% of terrestrial plant roots (Brundrett & Tedersoo, 2018; Heijden et al., 2015; S. E. Smith & Read, 2008). The AM symbiosis is believed to be fundamental for land colonisation by plants (Brundrett & Tedersoo, 2018). It is found in almost all plant species, including those with commercial value (de Moura et al., 2019). Furthermore, the AM fungi are the fungi from the phylum Glomeromycota, and they form a mutualistic symbiosis with the majority of vascular plants and some non-vascular plants (Peterson et al., 2004).

AM fungi play essential roles, and fundamental functions of several ecosystems (de Moura et al., 2019) processes ranging from maintaining plant biodiversity, plant development and growth, nutrient cycling, organic matter decomposition, absorption of water and nutrients, phosphate solubilisation, soil aggregation (Andrade Júnior et al., 2018; Silva-Flores et al., 2019; Wicaksono et al., 2018), and improving plant tolerance to different abiotic and biotic stresses (Ait-El-Mokhtar et al., 2019; Meddich et al., 2015). However, despite the contributions showed by AM fungi to plant communities, there is data paucity to elucidate rattans' ability to form a mutualistic symbiosis with AM fungi. Therefore, it is essential to isolate and identify the indigenous AM fungi associated with rattan trees in the Perak reserve forest, Malaysia and understand the symbiotic dynamics between rattans and AM fungi communities in their natural growing environment. Isolation, identification, and propagation of the AM fungi from the rhizosphere of wild rattans are important because it will help understand the symbiotic dynamic of rattan and AM communities on how AM help to enhance rattan growth and health. This knowledge will be fundamental for rattan management, cultivation, productivity, and reduction of production costs in the future. This study was conducted to identify the established AMF community and their ecological interactions with *Calamus* spp. in the Belum-Temengor Forest Complex, Malaysia.

MATERIALS AND METHODS

Study Sites and Sampling

Soil and roots samples of rattan species were collected from Temengor Forest Reserve and Royal Belum State Park, in the Belum-Temengor Forest Complex located in the Northern region of Malaysia, Hulu Perak, Perak. This forest complex is managed under the Perak State Parks Corporation. The temperature of the Belum-Temengor Forest Complex ranges from 20°C to 35°C depending on the time of year, and the mean monthly rainfall (annual) of the Belum-Temengor Forest Complex is 1,500–2,000 mm (Malaysian Meteorological Department [MET], n.d.). The sampling sites at Temengor Forest Reserve were Pulau Perhilitan (N 05°28'13.2" E 101°20'34.3"), Sungai No. 2 (N 05°26'48.5'' E 101°21'38.1''), and Sungai Rokan (N 05°29'47.1" E 101°18'14.8"). Meanwhile, the sampling sites at Royal Belum State Park were Sungai Kejar (N 05°48'28.4"

E 101°25'31.3''), Sungai Papan (N 05°37'54.7" E 101°24'11.8"), and Sungai Kooi (N 05°39'21.3'' E 101°24'18.6''). Root samples and 100 g of soils from the rhizosphere area were collected in three replicates for each rattan tree per site. These sampling sites were chosen due to various rattan species' availability and closely related soil types: clay loam, silty clay, silty clay loam, and sandy clay loam. The soil texture of these sampling sites was determined using the ball and ribbon test (Whiting et al., 2015) and the Feel Method as detailed by Thien (1979).

The sampling was conducted in a 50 m × 20 m plot for each site. Once the rattan species were identified, the root systems of *Calamus* spp. were shovelled carefully to ensure that the fine hairy roots collected did not mix up with other plant roots. Only the confirmed roots attached to the base of rattan were collected to prevent any mix up of roots that are not from rattan. The soil around the rattan's rhizosphere was collected to study the arbuscular mycorrhizal (AM) fungi diversity. The collected root samples were cleaned, cleared, and stored in 50% ethanol (Brundett et al., 1996) before being processed further at the Mycorrhiza Laboratory, School of Biological Sciences, Universiti Sains Malaysia.

Soil Analyses

For moisture content analysis, a 5 g sub-sample of soil from each sampling location was weighted, oven-dried overnight, and the final weight was determined. First, the soil pH was measured using a digital benchtop automatic pH meter; 5 g of dry soil was weighed and mixed with distilled water. Then, the pH meter probe was placed, and the reading was repeated thrice before obtaining the average reading. Next, a digital soil thermometer was used to measure the soil temperature on-site for each location. The probe of the digital soil thermometer was pushed 5 cm vertically into the ground, and the reading was repeated thrice before recording the average reading (modified from GLOBE, 2014).

Arbuscular Mycorrhizal Fungi Spore Isolation and Identification

The spores of AM fungi were isolated using wet-sieving, centrifugation, and filtration methods as described by Brundett et al. (1996) and were identified in Mycorrhiza Laboratory, School of Biological Sciences, Universiti Sains Malaysia. Approximately 100 g of collected soil samples were weighed based on a sampling location and mixed with water. The soil samples were sieved through three different sizes of sieves: 250 µm, 75 µm, and 45 µm were collected and transferred separately into 50 mL centrifugation tubes for the centrifugation process. The soil samples were centrifuged for 5 minutes at 2,000 rotations per minute (rpm) to separate the spores from the soil. The debris with supernatant was discarded, and the pellet was kept for the second centrifugation. After that, the pellet was suspended in 50% sucrose and was centrifuged for 1 minute at 2,000 rpm. A vacuum pump containing filter paper was used to filter the supernatant of

the 50% sucrose. The collected spores from all sieves were counted and collected under a stereomicroscope (Olympus Research Stereomicroscope System SZX16, Olympus, Japan) for the isolation and identification process.

The filter paper containing soil supernatant placed in the Petri dish was observed under a stereomicroscope (Olympus Research Stereomicroscope System SZX16, Olympus, Japan) to separate, count, and collect the spores from all sieves under 400x magnification. The AM fungi spores were taxonomically analysed into different genera based on their phenotypic characteristics, such as shape, size, arrangement, and colour (Oehl et al., 2011). The spores were then mounted on slides with Melzer reagent (1.5 g potassium iodide (KI), 0.5 g iodine crystals (I), and 20 g chloral hydrate ($C_2H_3Cl_3O_2$) were added to 20 mL distilled water and mixed until dissolved.) and observed under an Olympus BX41 Phase Contrast and Darkfield Microscope (Olympus, Japan). The spores' physical morphology image was captured using a camera attached to Olympus BX41 equipped with the software Cell A and saved. The AM fungi spores were taxonomically analysed into different genera based on their phenotypic characteristics, such as shape, size, arrangement, and colour (Oehl et al., 2011) according to the identification manual of VA mycorrhizal fungi (Schenk & Perez, 1990) and the identification keys from International Culture Collection of Vascular-Arbuscular Mycorrhizal Fungi website (INVAM biogeographical database: http//: invam.caf.wvu.edu).

Staining Mycorrhizal Roots and Determination of Root Colonisation Degree

For the staining process, the roots in 15% potassium hydroxide (KOH) (Sigma-Aldrich, Malaysia) were autoclaved for 20 minutes at 121°C and washed in 2% hydrochloric acid (HCL) (Sigma-Aldrich, Malaysia) for a few minutes, leading to the neutralisation of the roots. The roots then were kept in a universal bottle containing 0.05% Trypan blue (Sigma-Aldrich, Malaysia) with lactoglycerol (TBLG) and left overnight (Brundett et al., 1996; Schenk & Perez, 1990). Next, the stained roots were transferred to the universal bottles containing lactoglycerol [lactic acid:glycerol:water, 1:1:1 (volume/volume/volume)], which functioned to store the stained roots for further observation. The stained roots were randomly selected and observed under the stereomicroscope (Olympus Research Stereomicroscope System SZX16, Olympus, Japan) and were smashed carefully to locate the presence of vesicles and hyphae. The most abundant mycorrhizal area in the root was identified under a stereomicroscope (Olympus Research Stereomicroscope System SZX16, Olympus, Japan), selected, and cut before being transferred permanently on a glass slide to be observed further under Olympus BX41 Phase Contrast and Darkfield Microscope (Olympus, Japan) at a magnification of 20× and 40×. The best images showing mycorrhizal colonisation were captured using a camera attached to Olympus BX41 (Olympus, Japan) equipped with the software Cell A and saved.

The degree of AM fungi roots colonisation was calculated using the gridline intersection method (Brundett et al., 1996). The most common mycorrhizal colonisation degree measuring method utilised was the gridline intersect method (McGonigle et al., 1990). The 50 segments of cleared and stained roots (1 g) were spread out evenly on a 9 cm x 9 cm Petri dish with grid lines marked on the bottom of the dish with 1 cm × 1 cm squares. The vertical and horizontal grid lines were scanned under a stereomicroscope (Olympus Research Stereomicroscope System SZX16, Olympus, Japan). The presence and absence of AM infection were recorded at each point where the roots intersected a line. The root segments were re-spread and re-examined three times. The fraction of root length mycorrhizal and total root length was calculated using the derived conversion factor (Giovannetti & Mosse, 1980).

Data Analysis

Statistical analyses were performed using the Minitab 19 Statistical Software (Minitab, 2019), and each data point represented the mean of different groups. One-way analysis of variance (ANOVA) and Tukey test were used to examine the differences among AM root colonisation degree data. *P* values less than 0.05 were considered statistically significant.

RESULTS

Soil Moisture, pH, Temperature, and Physical Characteristics

Moisture, pH, temperature, and physical characteristics of soil collected from each sampling site were measured, and Table 1 shows the recorded measurement.

Arbuscular Mycorrhizal Colonisation

The percentage of the mean degree of arbuscular mycorrhizal colonisation in

Table 1
Moisture, pH, temperature, and physical characteristics of soil collected from six sampling sites of Calamus *spp. from Temengor Forest Reserve and Royal Belum State Park in the Belum-Temengor Forest Complex*

Site	Soil moisture (%)	Soil pH	Soil temperature (°C)	[3]Forming ball	[3]Forming ribbon	[3]Soil feel	Type of soil
[1]Pulau Perhilitan	31.58	6.4	25.4	Yes	Yes	No noticeable feel	Clay loam
[1]Sungai No.2	30.72	6.58	24.6	Yes	Yes	Smooth	Silty clay loam
[1]Sungai Rokan	41.84	6.58	24.5	Yes	Yes	Smooth	Silty clay
[2]Sungai Kejar	21.86	6.03	23.5	Yes	Yes	No noticeable feel	Clay loam
[2]Sungai Papan	23.69	6.21	24.1	Yes	Yes	No noticeable feel	Clay loam
[2]Sungai Kooi	21.8	6.82	24.5	Yes	Yes	Gritty	Sandy clay loam

Note. [1]Temengor Forest Reserve, [2]Royal Belum State Park, [3]Characterised based on a ball and ribbon test (Whiting et al., 2015), and Feel Method as detailed by Thien (1979)

Calamus spp. roots were significantly different (one-way ANOVA test, $p < 0.0001$) among the rattan species (Figure 1). *Calamus insignis* showed the highest percentage of AM colonisation degree, whereas *Calamus viridispinus* recorded the lowest percentage degree of AM colonisation compared to other *Calamus* species (Figure 1). Optical microscopy images revealed the structure of arbuscular mycorrhizal fungi, likely arbuscules, vesicles, and hyphal coils in the *Calamus* spp. roots (Figure 2 and Figure 3). *Paris* type of arbuscules was present in the *Calamus* spp. roots (Figure 2a and Figure 3c). The *Paris*-type is characterised by extensive intracellular hyphal coils and arbusculate coils in the root cortex (Cavagnaro et al., 2001).

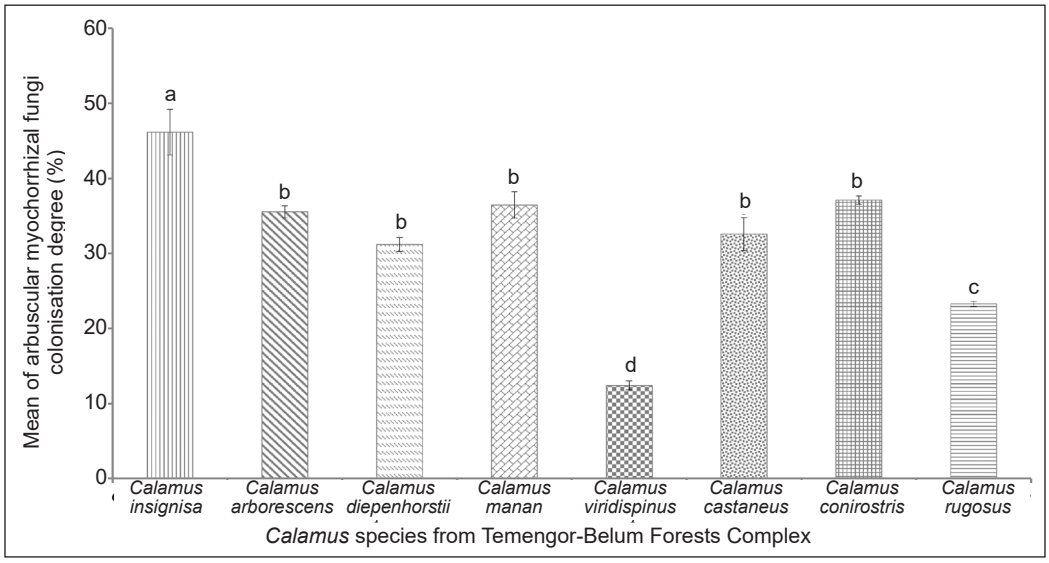

Figure 1. Degree of arbuscular mycorrhizal colonisation in *Calamus* spp. from Temengor Forest Reserve and Royal Belum State Park in the Belum-Temengor Forest Complex
Note. Bars represent mean value ± SE (n = 57). Bars with different alphabetical letters indicate the Tukey's test varies significantly at level $p < 0.05$

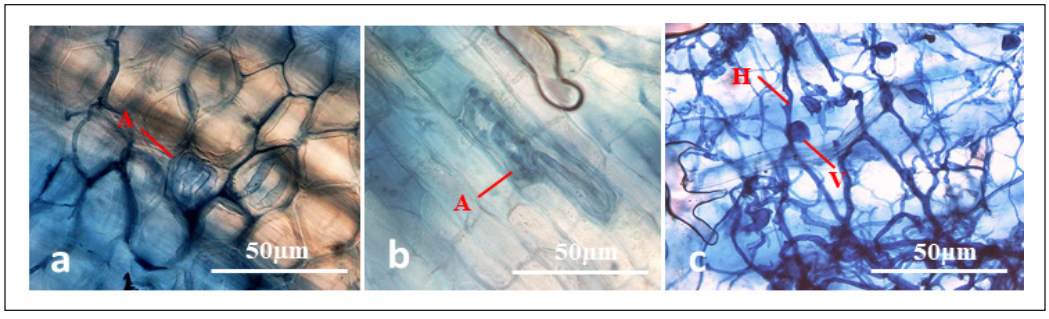

Figure 2. Mycorrhizal structures in the root of *Calamus* spp. from Temengor Forest Reserve in the Belum-Temengor Forest Complex (a-c) were observed under Olympus BX41 Phase Contrast and Darkfield Microscope. Optical microscopy images of *Calamus* spp. roots showed structures of arbuscules (A), vesicle (V), and hyphae (H). Bar scales = 50 µm

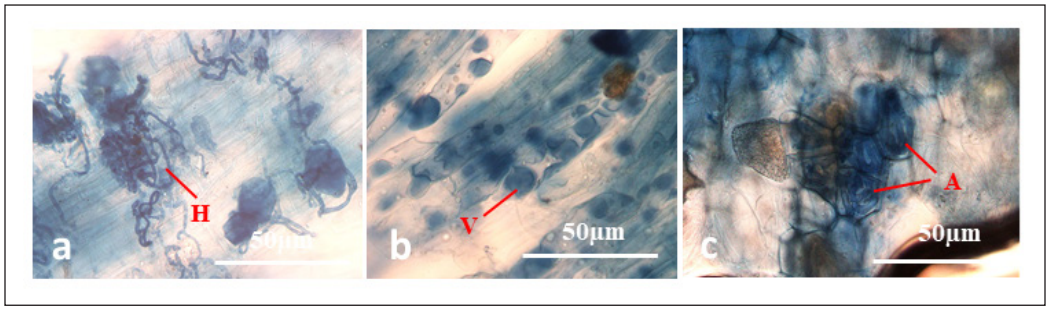

Figure 3. Mycorrhizal structures in the roots of *Calamus* spp. from Royal Belum State Park in the Belum-Temengor Forest Complex (a-c) were observed under Olympus BX41 Phase Contrast and Darkfield Microscope. Optical microscopy images of *Calamus* spp. root showed structures of arbuscules (A), vesicle (V), and hyphae (H). Bar scale = 50 µm

Arbuscular Mycorrhizal Spore Distribution

The arbuscular mycorrhizal fungi spores isolated from the rhizospheric soil samples from Belum-Temengor Forest Complex are *Acaulospora* sp., *Entrophospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. (Figure 4 and Figure 5). Five genera of AM fungi spores were identified from all soil samples from six sampling sites at Temengor Forest Reserve and Royal Belum State Park in the Belum-Temengor Forest Complex (Table 2). Sungai No. 2, located in Temengor Forest Reserve, recorded the presence of all five genera of AM fungi spores, with a total count of AM spores is 226 (Table 2, Figure 5). Meanwhile, Pulau Perhilitan, also located in Temengor Forest Reserve, recorded the presence of only one AM fungi spore genus, *Glomus* sp., with a total count of AM spores is 95 (Table 2, Figure 4). *Glomus* sp. was isolated in every sampling site; meanwhile, *Gigaspora* sp. was isolated only from the soil sample in Temengor: Sungai No. 2 and Sungai Rokan, while *Entrophospora* sp. was isolated only from Sungai No. 2, Temengor and Sungai Kooi, Royal Belum State Park. Total count of arbuscular mycorrhizal spores in Royal Belum state park is higher compared

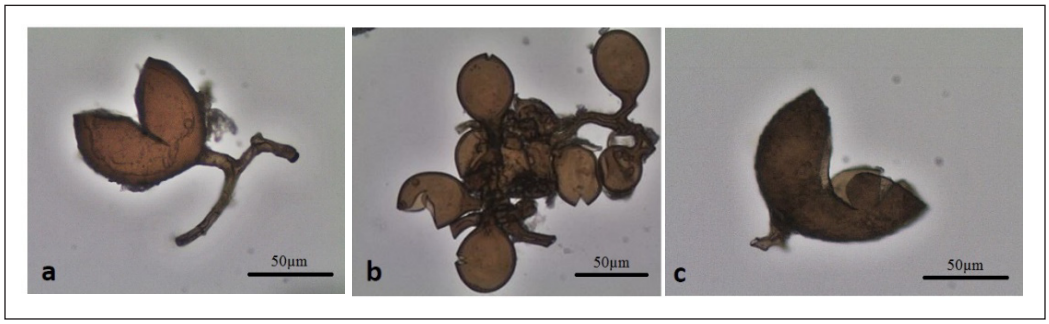

Figure 4. Arbuscular mycorrhizal spores. *Glomus* sp. (a-c) isolated from soil of Pulau Perhilitan, Temengor Forest Reserve in the Belum-Temengor Forest Complex observed under Olympus BX41 Phase Contrast and Darkfield Microscope. Bar scale = 50 µm

to Temengor Forest Reserve with 556 spores (Table 2). *Glomus* sp. is a relatively simple structure with globules shape, which develop thickened walls that may be multi-layered, brown, and usually with subtending hyphal attachment (Figure 4); *Acaulospora* sp. squashed spore of stained with Melzer's reagent showed orange outer wall later and dark purple stain in the innermost layer, the thin intermediate wall layer can be seen (Figure 5a) and multi-layer walls (Figure 5k). *Gigaspora* sp. is appeared to be a glomoid with a simple single structural wall (Figure 5b-c). *Scutellospora* sp. is formed singly in the soil, formed broadly ellipsoid, subglobose to oblong-shaped spores, the spore colour is brown to dark brown with a multi-layered wall (Figure 5i). Moreover, *Entrophospora* sp. is a spore with cicatrices formed; spore is globose to subglobose and has two walls: an outer spore wall and an inner wall (Figure 5j). *Glomus* sp. was isolated in every sampling site; meanwhile, *Gigaspora* sp. was isolated only from the soil sample in Temengor: Sungai No. 2 and Sungai Rokan, while *Entrophospora* sp. was isolated only from Sungai No. 2, Temengor and Sungai Kooi, Royal Belum State Park. The total count of arbuscular mycorrhizal spores in Royal Belum state park is higher compared to Temengor Forest Reserve, with 556 spores (Table 2).

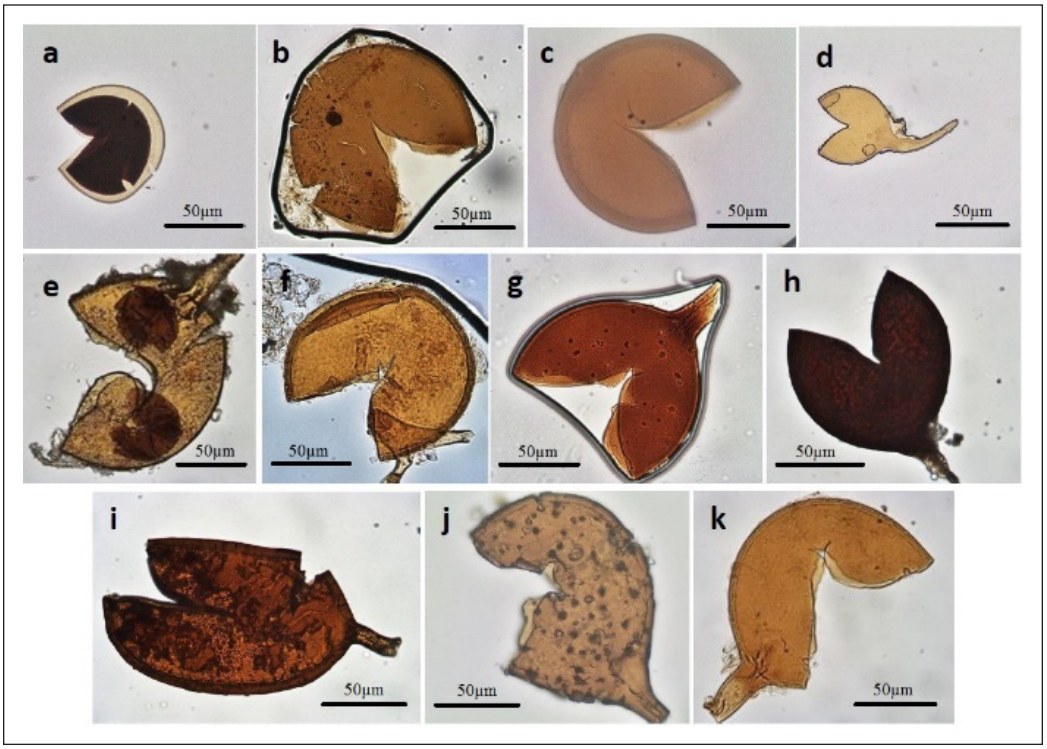

Figure 5. Arbuscular mycorrhizal spores. *Acaulospora* sp. (a), *Gigaspora* sp. (b-c), *Glomus* sp. (d-h), *Scutellospora* sp. (i), *Entrophospora* sp. (j), and *Acaulospora* sp. (k) observed under Olympus BX41 Phase Contrast and Darkfield Microscope. These spores are isolated from the soil of *Calamus arborescens* at Sungai No. 2, Temengor Forest Reserve in the Belum-Temengor Forest Complex. Bar scale = 50 µm

Table 2
The number of Calamus *spp. presence, mean degree of arbuscular mycorrhizal (AM) fungi colonisation (%), the total count of AM spores in 100 g soil and AM spore diversity and distribution from six sampling sites of* Calamus *spp. from Temengor Forest Reserve and Royal Belum State Park in the Belum-Temengor Forest Complex*

Sampling sites	Number *Calamus* sp. present per site	Mean degree of AM fungi colonisation (%) per site	Total count of AM spores (per 100 g soil)	Types of AM spore
[1]Pulau Perhilitan	2	46.18	95	*Glomus* sp.
[1]Sungai No. 2	2	34.93	226	*Acaulospora* sp., *Entrophospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp.
[1]Sungai Rokan	2	29.94	146	*Acaulospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp.
[2]Sungai Kejar	2	40.27	157	*Acaulospora* sp., *Glomus* sp., and *Scutellospora* sp.
[2]Sungai Papan	3	31.96	154	*Acaulospora* sp., *Glomus* sp., and *Scutellospora* sp.
[2]Sungai Kooi	3	27.01	245	*Acaulospora* sp., *Entrophospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp.

Note. [1]Temengor Forest Reserve, [2]Royal Belum State Park

DISCUSSION

Arbuscular Mycorrhizal Colonisation on Forest Rattan Root Samples

Rattans are known as climbing palms and are well used for cane and cane products due to their stem's flexibility. Scanty literature is available on the mycorrhizal study of rattans. The earlier study on AM fungi associated with rattan was conducted by Zakaria (1991), comprising preliminary investigations on growth dependency of *in vitro* micro-propagated *Calamus manan* on the AM fungi before transplanting to the field. Meanwhile, Gong et al. (1994, 1995) studied the colonisation and presence of the AM fungi in the rhizosphere soils of four rattan species (*Daemonorops margaritae*, *Calamus simplicifolius*, *Calamus tetradactylus*, and *Calamus tetradactyloides*). Finally, Marati and Devadiga (2018) studied the colonisation of AM fungi on *Calamus thwaitesii*, *Calamus nagabettai*, and *Calamus prasinus* and reported variation in the spore density and spore diversity in the rhizosphere soils. To our knowledge, the present study is the first attempt to investigate the colonisation of arbuscular mycorrhizal (AM) fungi in the roots of *Calamus insignis*, *Calamus viridispinus*, *Calamus arborescens*, *Calamus diepenhorstii*, *Calamus manan*, *Calamus castaneus*, *Calamus conirostris*, and *Calamus rugosus*. In addition, this study reports the AM and spore diversity in the Belum-Temengor forest complex. The AM fungi are able to form AM symbioses in

exchange for carbon with 80% of terrestrial plants roots and play a crucial role in soil fertility, assist the plant in uptakes and mobilisation of nutrients (e.g., phosphorus and nitrogen) and inducing changes in plant physiology and secondary metabolism (Cervantes-Gámez et al., 2016; Schweiger & Müller, 2015; Wipf et al., 2014), which indirectly makes AM fungi is important for plant health and establishment (Ban et al., 2017).

All *Calamus* spp. roots located in the Belum-Temengor Forest Complex were colonised by the AM fungi with different colonisation degrees (Figure 1). Compared to other species, *Calamus insignis* showed the significantly highest colonisation degree with 46.18%, and the lowest colonisation degree of 12.40% was demonstrated by *C. viridispinus* (Figure 1). It is because the primary root system of the rattan is fibrous, and the roots spread to 1 to 2 m in width and range from 50 to 60 cm depth. This root system is a favourable structure for AM colonisation. Therefore, this might explain why all the *Calamus* spp. found in the samplings site were able to form the AM colonisation. Furthermore, the soil physical characteristics from the sampling sites for both Temengor Forest Reserve and Royal Belum State Park were approximately the same, with soil moisture ranging from 21.80% to 41.84%, soil pH ranging from 6.03 to 6.58, and soil temperature ranging from 23.5°C to 25.4°C (Table 1). These properties provide a suitable condition for AM fungi colonisation and propagation.

The presence of arbuscular mycorrhizal structures like hyphae and vesicles; hyphal swellings in the root cortex contain lipids and cytoplasm (Reddy et al., 2013) arbuscules in the *Calamus* spp. roots indicate the ability of this plant to form the mycorrhizal association (Figure 2 and Figure 3). Arbuscules are important macro-and micronutrient and water exchange sites between the AM fungi and their symbiotic plants (Bonfante & Requena, 2011; Lee et al., 2012; Miransari, 2011). The type of arbuscules was formed in the *Calamus* spp. roots from the Belum-Temengor Forest Complex is the *Paris*-type (Figure 2 and Figure 3). *Paris*-type arbuscules are formed from small branches developed from the hyphal coil (Peterson et al., 2004). The hyphae grow from cell to cell without any intercellular phase, and in the whole cortex cell, the AM fungus forms large coils (Franken, 2010). *Paris*-type arbuscules seem to occur more frequently compared to the *Arum*-type (F. A. Smith & Smith, 1997).

Arbuscular Mycorrhizal Spore Distribution

The AM fungi spores isolated from the soil samples from Belum-Temengor Forest Complex were *Acaulospora* sp., *Entrophospora* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. (Figure 4 to Figure 5). The distribution of AM fungi species depends on the environment, such as the microclimate factor, the biome's location, and the competition between the AM fungi species. The AMF communities are influenced by different soil types (Oehl

et al., 2010; Torrecillas et al., 2014), and the diversity of AM fungi and the types of AM fungi propagules depending on their taxonomic group are affected by soil texture (H. Zhao et al., 2017; Lekberg et al., 2007). From the soil collected from all the six sampling sites in the Belum-Temengor Forest Complex, the AM fungal genus *Glomus* sp. was found in all sites, followed by *Acaulospora* sp. and *Scutellospora* sp., which were equally present in all sites except Pulau Perhilitian, Temengor (Table 2). Meanwhile, *Gigaspora* sp. was isolated from Sungai No. 2 and Sungai Rokan, Temengor and Sungai Kooi, Royal Belum State Park sampling sites (Table 2). *Entrophospora* sp. was isolated only from Sungai No. 2, Temengor and Sungai Kooi, Royal Belum State Park (Table 2).

Gong et al. (2000) showed that *Glomus*, *Acaulospora*, and *Scutellospora* were the predominant genera found in rattan plantations, and *Glomus* sp. was found in all sampling sites. Lekberg et al. (2007) reported that AM fungi from Glomeraceae species are more prevalent in soils with higher clay content. Mahulette et al. (2021) also reported that soils with clay texture and pH near neutral are dominated by *Glomus* sp. It supports the presence of *Glomus* sp. in all sampling sites due to clay soil texture present in all sampling sites (Table 2) and would also probably because AM fungi from the Glomeraceae family are known to have different propagation strategies (Lekberg et al., 2007), which provided them with a greater adaptation to various soil situation (Vieira et al., 2020). Marati and Devadiga (2018) found that *Glomus* was the dominant genus of the AM fungi in the rhizosphere soils of all rattan trees. Meanwhile, Huang et al. (2020) demonstrated that *Glomus* spp. increased root length, projected area, surface area, and volume, and increased leaf photosynthesis rate, transpiration rate, and stomatal conductivity, reducing intercellular carbon dioxide (CO_2) concentrations and leaf temperature. Marinho et al. (2018) found that *Glomus* and *Acaulospora* were the most representative genera in tropical forests. *Glomus* and *Acaulospora* genera are known to adapt to a different environment (Loss et al., 2009), as evidenced by their global distribution (INVAM biogeographical database: http//: invam.caf.wvu.edu), and they are tolerant to a wide range of pH (Silva et al., 2007). These genera produce numerous small-diameter spores (Dandan & Zhiwei, 2007), which potentially make them the most representative genera in tropical forests. The ability to alter its germination pattern depending on the environment has made *Glomus* sp. the most available genus in the re-established and logged-over forest soil (Ong et al., 2012). Bever et al. (1996) reported that *Glomus* and *Acaulospora* species usually produce more spores than *Gigaspora* and *Scutellospora* species in the same environment due to the difference in development, and they required less time to produce spores (Hart & Reader, 2002; Piotrowski et al., 2004). It indirectly increased the domination of *Glomus* sp. in the soil (Gai et al., 2009) and revealed an excellent adaptation feature, which promotes its ability to survive in disturbed

soils (Muleta et al., 2008). *Glomus* and *Acaulospora* are the AM fungi genera commonly found worldwide in a broad range of ecosystems (Davison et al., 2015).

Hart and Reader (2002) reported AM fungi from Gigasporaceae species to have robust hyphae, and their mycelial growth is higher in sandy soils. It supports the presence of *Gigaspora* sp. in Sungai No. 2 and Sungai Rokan, Temengor and Sungai Kooi, Royal Belum State Park because of the sandy and silty soil textures present only in these locations. The distribution and expansion of AM fungi hyphae are influenced by the size of soil particles in which the mycelial growth is reported to be higher in sandy soils (coarse particles compared to fine particles (Kohler et al., 2016). Sandy soils with a high porosity could stimulate mycorrhizal colonisation by favouring root growth compared to clay soil, restricting root growth due to low porosity (Carrenho et al., 2007). The seasonality, dormancy, edaphic factors, host dependence, age of the host plants, and the sporulation abilities of arbuscular mycorrhizal fungi could also affect the distribution and abundance of AM fungi spores in the soils (Bever et al., 1996; Z. W. Zhao et al., 2003). Ong et al. (2012) reported in their study that *Gigaspora* was found only in the logged-over forest and not in the re-established forest. The broad diversity of plant species in Malaysian forests may contribute to the selection of this host by *Gigaspora* sp. and enhance its survivability (Keen Chubo et al., 2009; Z. W. Zhao et al., 2003).

CONCLUSION

This study revealed the ability of a significant commercial plant, rattans, to form a mutualistic symbiosis with arbuscular mycorrhizal (AM) fungi. *Calamus insignis* showed the highest percentage, whereas *Calamus viridispinus* recorded the lowest percentage of AM colonisation degree compared to other *Calamus* species. Five genera of the AM fungi were isolated from Sungai No. 2 and Sungai Kooi, four genera of AM fungi were isolated from Sungai Rokan, three genera of AM fungi were isolated from Sungai Kejar and Sungai Papan, and one genus of AM fungus was recorded in Pulau Perhilitan, which is a relatively small number of AM genera considering the high plant diversity in the sampling locations. *Glomus* sp. was the main distributed genera, followed by *Acaulospora* sp. and *Scutellospora* sp. Sungai Kooi, Royal Belum State Park, recorded the highest spore density, 245 spores per 100 g of soil. This study has shown that the rattans species in the Belum-Temengor Forest Complex do form the AM association. The rhizospheric soils of these rattans are present with the indigenous AM fungi constitution.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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