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INSECT COMMUNITY ASSOCIATED WITH *Ganoderma* BASIDIOCARPS IN OIL PALM PLANTATIONS OF SABAH

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ABSTRACT

Ganoderma *Basal Stem Rot (BSR) is currently one of the most important diseases in oil palm. The disease transmission is predominantly through root-to-root contact in soil. However, the role of* Ganoderma *basidiospores remains ambiguous in the transmission process despite millions being produced during active phase of fungus. Five plantations in Sabah with varying incidences of* Ganoderma *BSR (6.0% - 9.8%) were selected for the study. A total of 543 basidiocarps were collected and assessed. At least 55 arthropods were associated with* Ganoderma *basidiocarps. Among these arthropods, 8 species were found carrying the basidiospores internally or externally. Handsome fungus beetles,* Eumorphus *spp. were found to be strongly associated as vector of* Ganoderma *disease, but this requires further investigation. This study could potentially provide an insight on the sporadic spread of BSR which was not conceivable via root-to-root contact.*

Keywords: basal stem rot, insect vector, upper stem rot.

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INTRODUCTION

Malaysia is among the two major palm oil suppliers across the world, which signifies the importance of oil palm to Malaysia's economy. The oil palm industry plays a crucial role in the agricultural and economic development of the country (Parveez *et al.,* 2020). Malaysia has entered the 4th generation planting and has celebrated its centennial anniversary after its humble introduction in 1917. After decades of cultivation, the country's golden crop is seriously threatened by *Ganoderma* basal stem rot (BSR) infection, prevalent in all oil palm areas regardless of the soil type. The disease has no immediate curative control and has been managed to remain below the economic threshold through a combination of integrated approaches: Cultural, biological, and chemical (Nur-Rashyeda *et al.*, 2022).

Three *Ganoderma* species have been reported to cause *Ganoderma* BSR and upper stem rot (USR) in oil palm, namely: (1) *G. boninense*, (2) *G. zonatum*, and (3) *G. miniatocinctum* (Idris *et al.,* 1999; Rees *et al.,* 2007). The primary transmission of the disease is through root-to-root contact with the fungal inoculum present in the field, while the basidiospores have implications for the distribution and genetic diversity of *Ganoderma* sp. (Rees *et al.*, 2007). *Ganoderma* is a white rot fungus that produces basidiocarps, which, upon maturity, release millions of basidiospores in the field. The disease epidemiology validates the role of root-to-root contact in the disease's spread in the field, but the role of spores remains ambiguous. Despite the ambiguity of their role, basidiospores are believed to be the primary source of inoculum (Pilotti *et al.*, 2018). Basidiospores are able to germinate and grow on different parts of oil palm including the cut surfaces of fronds, peduncles, and stems (Rees *et al*. 2012). However, the high level of *G. boninense* heterogeneity in oil palm plantations based on previous genetic studies has generated arguments for root-to-root contact as the main method of BSR disease spread (Miller *et al.*, 1999; Pilotti *et al.*, 2004; Pilotti, 2005). Additionally, the occurrence of USR in oil palm plantations has driven the hypothesis that insects are possible vectors of *Ganoderma* disease spread through the dispersal

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of its airborne basidiospores. Oil palms infected with USR differ in their decaying location, whereby it is situated on the upper section of the trunk and the basidiocarps emerge approximately 1-2 m above ground level (Rees *et al.*, 2012). In addition, the sporadic BSR incidences in the field further validate the need to determine the contribution of insects as *Ganoderma* vectors, despite the claim that previous crops were a source of disease inoculum initiating a new infection (Turner, 1965).

Oil palm plantation is a monocrop habitat that host a diverse community of insects that vary in their roles in the ecosystem, ranging from beneficial ones such as parasitoids and pollinators to damaging insects such as the bagworm and rhinoceros beetle that feed on plants. The presence of insects in the oil palm plantation is signified as part of the food web, with different feeding systems and ingestions depending on their mouthparts and physiological adaptations (Yadav, 2003). Regardless of their roles, insects have their own unique, specific niche in the oil palm ecosystem including the association of insects and microorganisms that form a range of symbiotic and pathogenic relationships (Wielkopolan *et al.*, 2021). Thus, insects could be considered as both pest and reservoir, as well as vector of plant pathogen. To date, more than 700 plant diseases are known to be vector-borne threats to food health and security worldwide (Fletcher and Wayadande, 2002; Hammond and Bedendo, 2005; Hogenhout *et al.*, 2008; Weintraub and Beanland, 2006). Vector density, feeding activity on the host, vector longevity during pre- and post-infection, incubation period, and vector competency are some of the factors affecting vectorial capacity that determine the effectiveness of vector-borne pathogens (Chuche *et al.*, 2017).

Ganoderma basidiocarp could provide food and breeding sites for several insect species which subsequently contribute to the biodiversity richness and composition of insects. This has been implied in other studies where Polyporacea fungi were used as breeding and hosting sites for fungivorous insects (Fäldt *et al.*, 1999; Jonsell and Nordlander, 1995; Yamashita *et al.*, 2015). However, there has been no publication or report on the diversity of the insects cohabiting with the basidiocarps in the oil palm plantations specifically.

Therefore, the aim of this study was to conduct a generic census on the diversity of the insect community surrounding the *Ganoderma* infected palms and the community of insects cohabiting within *Ganoderma* basidiocarps (fruiting bodies). This was followed by insect species identification using key taxonomic characteristics, basidiospore carrying assessment, and finally, establishing the association potential of the insects as vectors in spreading the disease in oil palm.

MATERIALS AND METHODS

Study Sites

Five oil palm plantations located in Sabah were selected for the insect sampling, focusing on the *Ganoderma* infected palms. The *Ganoderma* disease incidence was determined by ground census and labelling of infected palms. The percentage of disease incidence was calculated by the number of infected palms (basidiocarps presence) over total palms of 5 ha planting area. The percentage of disease incidence was between 6.0% and 9.8% across five sampling sites: Kota Marudu, Beaufort, Tawau, Kunak, and Lahad Datu (*Figure 1*).

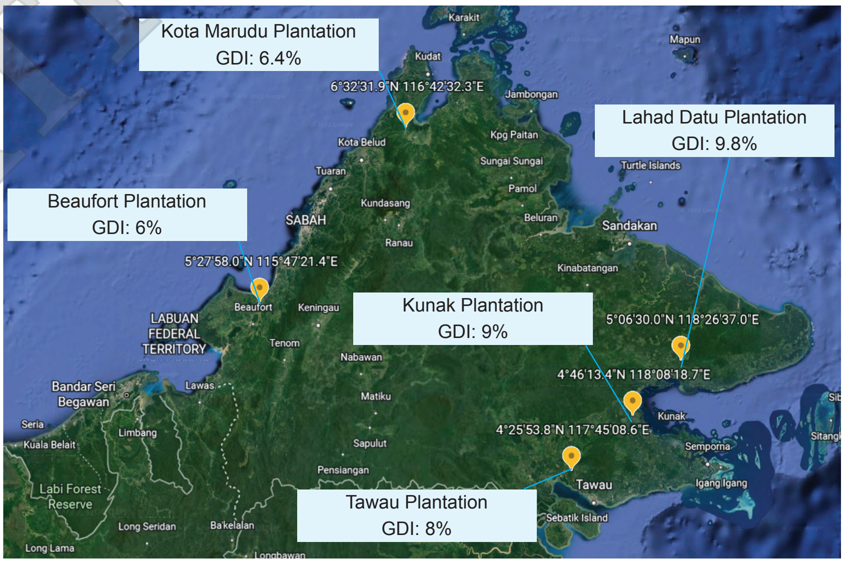


Figure 1. The selected five study sites with varying Ganoderma *disease incidences (GDI) of basal stem rot (BSR) and upper stem rot (USR) in Sabah.*

Insect Species Richness Surrounding *Ganoderma* Basidiocarp

The *Ganoderma* infected palms with the presence of basidiocarps were labelled prior to the sampling activity. Only BSR infected palms were selected in the study as shown in *Figure 2*. Three *Ganoderma* basidiocarps, irrespective of its stage of maturity, were collected from the infected palms, placed into a sterile plastic container, and brought to the laboratory. A total of 543 basidiocarps of *Ganoderma* were collected for the insect enumeration. All insects either in contact with or surrounding the palms were handpicked, and the basidiocarps were placed in a small container and labeled accordingly. The basidiocarps were broken into pieces using hand pressure, and all the insects inside the brackets were carefully removed using soft forceps to ensure the retrieved insects were not physically injured. These insects were classified taxonomically before being subjected to enumeration. The insect classification was based on published taxonomic key such as Zettler *et al.* (2016) for identification to orders, Hölldobler and Wilson (1990) for ants, Strohecker (1949; 1968) for *Eumorphus* spp.

Presence and Viability of *Ganoderma* Basidiospore

Externally. The sampled insects from the basidiocarp were brought to the laboratory for further examination for the presence of *Ganoderma* basidiospores. The insects were dipped into 5 mL sterile distilled water and the suspensions was observed under microscope. The detection of basidiospores was carried out according to the morphological characteristics stated by Rees *et al.* (2012) *i.e.* basidiospore dimensions were 9.8 × 4.5 µm (mean), reddish brown, narrow ellipsoid with a visible hilar appendage, and often containing a large vacuole. Each sampled insect was dipped into 1 mL of sterile distilled water with 0.02% Tween 80®.

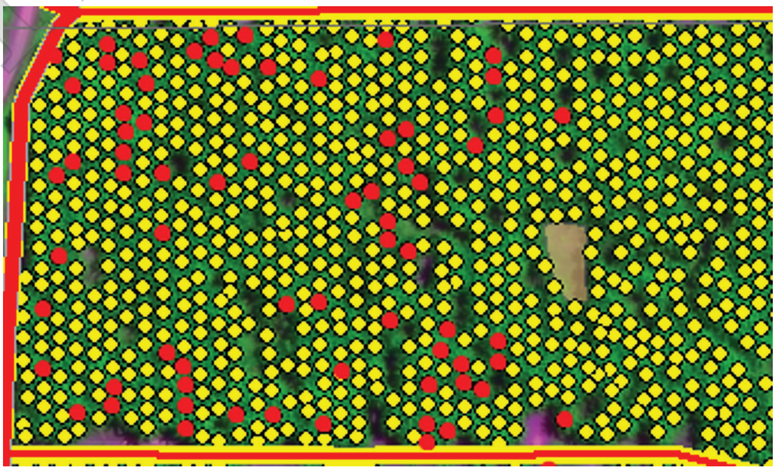
Internally. Insects were removed from the suspension, blotted carefully using sterile tissue, and left to dry for 10 min. The insects were dissected using a sterile dissecting scalpel to expose the inner section of the bodies, followed by dipping into 1 mL of sterile distilled water.

All the resulting suspension were then placed on a glass slide and observed under a Leica DM750 compound microscope to examine the presence of *Ganoderma* basidiospores. This method was adapted from Dhileepan (1992) for examining the pollen carrying capacity of *Elaeidobius kamerunicus*. Any positive presence of basidiospores was quantified using a hemocytometer. The suspension was then immediately spread onto potato dextrose agar (PDA) and *Ganoderma* selective medium (GSM) prepared as previously described in Ariffin and Idris (1992) to observe the identity and viability of the basidiospores.

RESULTS AND DISCUSSION

Species Richness of the Arthropods Associated to *Ganoderma* Basidiocarps

After sampling and data collection from five different plantations in Sabah, 53 species of arthropods were found. Most of the infected palms in the sampled estates had been dominated by Tenebrionids followed by Formicids, as shown in *Table 1*. Some of the insects' pictures are shown in *Figure 3*. Non-insect arthropods were mainly decomposers, and predatory spiders feeding on the abundance of insects located at the base of palm. It was also noted that the presence of *Ganoderma* basidiocarps may have increased the species



Note: Red circle- infected palm.

Figure 2. The map of the oil palm plantation in Lahad Datu selected for insect sampling.

richness of the palm base due to the colonisation by fungivorous insects for breeding and feeding sites. The largest insect order associated with *Ganoderma* basidiocarps was Coleoptera. According to research undertaken in temperate and boreal climates, many Coleoptera and Diptera employ enormous fungal fruiting bodies as both food and habitat (Hammond and Lawrence, 1989; Hanski, 1989; Yamashita and Hijii, 2003). Research on host use patterns of fungivorous insects shows monophagy and oligophagy are commonly seen in Coleoptera that feed on bracket fungi (Nordlander, 2004; Orledge and Reynolds, 2005; Paviour-Smith, 1960), whereas many Diptera that feed on agaric fungi are observed as polyphagy (Jaenike, 1978; Yamashita and Hijii, 2007). Meanwhile, the high biodiversity value (HBV) is a riparian area initiated by MPOB to conserve the flora and fauna along the gazetted river inside the plantations (*Figure 4*). Therefore, there is a possibility that the high number of species recorded in Lahad Datu was influenced by the species migration from this area. There are some common insects found associated with *Ganoderma* in this study which also found in the riparian area. *Dolichoderus* sp., *Anoplolepis* sp., *Camponotus* sp., *Oecophylla smaragdina*, *Polyrhachis furcata* were previously found in the riparian zones of Sungai Sepetang, Sungai Rembau and Sungai Chukai in Peninsular Malaysia (Abdullah *et al*., 2019) but these are ants commonly present in the ecosystems. Pitfall trapping in primary and secondary forest of Danum Valley Field Centre and Sungai Bole found abundance of *Onthophagus* spp. which is also commonly known as dung beetles (Holloway *et al*., 1992). Another insect collection study from bracket fungi of Borneon tropical forest also found insect species from the following genera: Physodera, Tachys, Cis, Eumorphus, Sepedophilus, Staphylinidae, Amarygmus, Pentaphylus and Platydema (Yamashita *et al*., 2015). All of these beetles could also be found associated with the *Ganoderma* basidiocarps in this study.

It was also noted that Tenebrionids were commonly found in most of the plantations (>70%). Generally, Tenebrionid pests feed on plant materials including decaying matter, wood, leaf litter, pollen, and fungal and algal matters (Darya *et al*., 2016). This is explained by the decaying process caused by *Ganoderma* sp. within the palm base, which may have served as a feeding substrate for this pest. Tenebrionid species commonly reside in primary forests and are easily affected by continuous forest development (Grimm and Schawaller, 2021). Thus, the abundance and presence of Darkling beetles in the oil palm planting area is predictable, while the presence of *Ganoderma* and the decaying process subsequently attracted those other associated insects.

When comparing the presence from other insect orders associated with *Ganoderma* basidiocarp, there is a higher possibility of Coleopterans as disease vectors due to the fact that some of them complete their life cycle during the course of feeding, breeding, and living inside the basidiocarps. Komonen (2003) suggested that fruiting bodies of wood-decaying macrofungi can be considered hotspots of insect diversity after concluding that the insect communities occurring within are rich in species, many of which show preference for a specific fungal host. Previous studies reported that fungal odour have a role in inviting insects living on fungi (Bengtsson *et al*., 1991; Jonsell; Fäldt *et al*., 1999; Nordlander, 1995 and Pacioni *et al*., 1991) or on decayed substrates of the infection (Honda *et al*., 1988; Pfeil and Mumma, 1993; Phelan and Lin, 1991; Pierce *et al*., 1991). Jonsell and Norlander (2004) even concluded that more than half (53%) of the insect species breeding in bracket fungi appeared to be monophagous.

Interestingly, insects such as *Cis* sp., *Eledona* sp. and *Pentaphyllus* sp. were found to bore tunnels inside the *Ganoderma* basidiocarps while feeding on the fungi. Fäldt *et al.* stated in (1999) that many insects that develop under the bark of logs are frequently found on the hymenium of fruiting bodies, where they feed on spores. The patchy distribution of *Ganoderma* basidiocarps may not serve as a predictable breeding or feeding site for insects. The Ciidae family, also called the minute tree fungus beetle, is well known for infesting Polyporacea fungi such as *Fomes fomentarius* and *Ganoderma lucidum*. This could account for the greater diversity of insects in *Ganoderma* infected areas, which draws fungivorous insects.

Among the Coleopterans, *Eumorphus minor* (*Figure 5a*), was found in colony on the surface of *Ganoderma* basidiocarps. This species was identified based on the taxonomic key by Strohecker (1949; 1968). The features of this species matched the taxonomic key of having an elytral margin narrow along the shoulder, an epipleuron, a dark suture, an elytral surface dull and purplish brown, and bicolored femora. Unlike the other Coleopterans mentioned, *Eumorphus* spp. or handsome fungus beetles do not reside entirely inside the basidiocarp. They were observed to intermittently move in and out of the basidiocarps of *Ganoderma* despite colonising and feeding on the basidiocarps. Endomychids behave like any other mycophagous beetles, wandering regularly onto and even nibble on any substrate, including plant tissue (Shockley *et al*., 2009). There are also reports on *Eumorphus quadriguttatus* (Illiger) infesting betel pepper plants, *Piper betel* (L.) (Piperaceae) (Mondal *et al*., 2003). These beetles gradually leave the fruiting bodies upon afternoon to reach darker places around the palm base, usually between the silts of the oil palm roots. This observation shows that handsome fungus beetle prefers dark and moist places for shelter and only emerges for feeding.

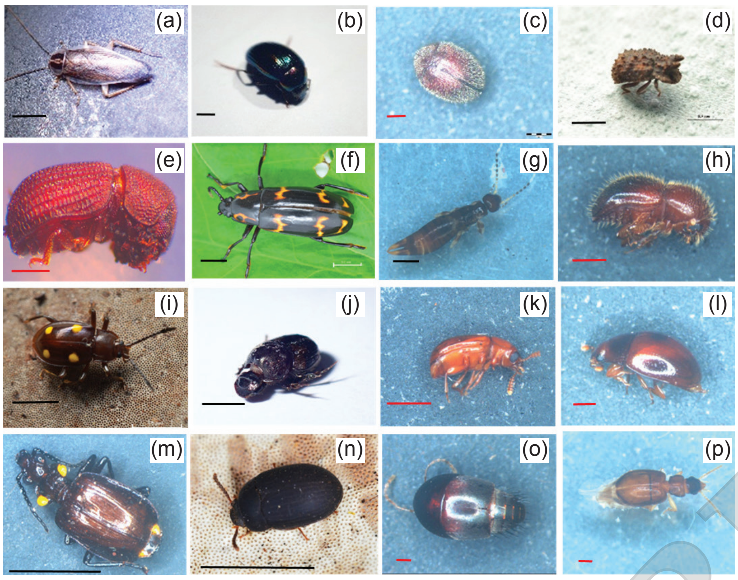
TABLE 1. DIVERSITY OF ARTHROPODS COLLECTED FROM FIVE SAMPLING ESTATES IN SABAH AND TOXONOMIC CLASSIFICATIONS

No.	Scientific name	Common name	MPOB Phase 2 Lahad Datu	Mawao Estate Beaufort	Langkon Estate Kota Marudu	Madai Estate Kunak	Merotai Besar Estate Tawau	Class	Order / Sub-order	Family / Superfamily
1	*Amarygmus* sp.	Darkling beetle	+	+	+	+	+	Insecta	Coleoptera	Tenebrionidae
2	*Amphicrossus* sp.	Sap beetle	+	-	-	-	-	Insecta	Coleoptera	Nitidulidae
3	*Anoplolepis gracilipes*	Red ant	+	+	+	+	+	Insecta	Hymenoptera	Formicidae
4	*Blattella asahinai*	Asian cockroach	+	+	+	+	+	Insecta	Blattodea	unidentified
5	*Bolitotherus cornutus*	Forked fungus beetle	+	+	+	+	+	Insecta	Coleoptera	Tenebrionidae
6	*Camponotus* sp.	Black carpenter ant	+	+	+	+	+	Insecta	Hymenoptera	Formicidae
7	*Camponotus* sp.	Carpenter ant 2	+	+	+	+	+	Insecta	Hymenoptera	Formicidae
8	*Cis* sp.	Minute tree fungus beetle	+	+	+	+	+	Insecta	Coleoptera	Ciidae
9	*Coptotermes kalshoveni*	Termite	-	+	-	-	-	Insecta	Isoptera	Rhinotermitidae
10	*Dolichodores* sp.	Black ant	+	+	+	+	+	Insecta	Hymenoptera	Formicidae
11	*Eledona* sp.	unidentified	+	+	+	+	+	Insecta	Coleoptera	Tenebrionidae
12	*Encaustes* sp.	Pleasing fungus beetle	+	-	-	-	-	Insecta	Coleoptera	Erotylidae
13	*Euborellia annulipes*	Earwig	+	+	+	+	+	Insecta	Dermaptera	Forficulidae
14	*Eumorphus dilatatus tarittus*	Handsome fungus beetle	+	+	-	-	+	Insecta	Coleoptera	Endomychidae
15	*Eumorphus austerus*	Four spotted handsome fungus beetle	+	+	+	+	+	Insecta	Coleoptera	Endomychidae
16	*Eumorphus minor*	Handsome fungus beetle	+	+	-	-	-	Insecta	Coleoptera	Endomychidae
17	*Eumorphus quadrinotatus*	Handsome fungus beetle	+	+	-	-	-	Insecta	Coleoptera	Endomychidae
18	*Eumorphus dihaani*	Handsome fungus beetle	+	-	-	-	-	Insecta	Coleoptera	Endomychidae
19	*Eumorphus micans*	Handsome fungus beetle	+	-	-	-	-	Insecta	Coleoptera	Endomychidae
20	*Lioptera* sp.	Pleasing fungus beetle	+	-	-	-	-	Insecta	Coleoptera	Erotylidae
21	*Mycomya rondani*	Fungus gnat	-	+	+	-	-	Insecta	Diptera	Mycetophyllidae
22	*Oecophylla smaragdina*	Weaver ant	+	+	+	+	+	Insecta	Hymenoptera	Formicidae
23	*Onthophagus* sp.	Dung beetle	+	-	-	-	-	Insecta	Coleoptera	Scarabaeidae
24	*Oryctes rhinoceros*	Rhinoceros beetle	-	-	+	-	-	Insecta	Coleoptera	Scarabaeidae
25	*Pentaphyllus* sp.	unidentified	+	+	+	+	+	Insecta	Coleoptera	Tenebrionidae
26	*Phalacrus* sp.	unidentified	+	-	-	-	-	Insecta	Coleoptera	Phalacridae
27	*Physodera chalceres*	Ground beetle	+	-	-	-	-	Insecta	Coleoptera	Carabidae

TABLE 1. DIVERSITY OF ARTHROPODS COLLECTED FROM FIVE SAMPLING ESTATES IN SABAH AND TOXONOMIC CLASSIFICATIONS (continued)

No.	Scientific name	Common name	MPOB Phase 2 Lahad Datu	Mawao Estate Beaufort	Langkon Estate Kota Marudu	Madai Estate Kunak	Merotai Besar Estate Tawau	Class	Order / Sub-order	Family / Superfamily
28	*Platydema ruficorne*	Darkling beetle	+	-	-	-	-	Insecta	Coleoptera	Tenebrionidae
29	*Polyrachis* sp.	Ants	+	+	+	+	+	Insecta	Hymenoptera	Formicidae
30	*Porcellionides pruinosus*	Woodlice	+	+	+	+	+	Malacostraca	Isopoda	Porcellionidae
31	*Sepedophilus* sp.	Crab like -rove beetle	+	+	+	-	-	Insecta	Coleoptera	Staphylinidae
32	*Sepedophilus* sp.	Rove beetle	+	+	+	+	+	Insecta	Coleoptera	Staphylinidae
33	*Solenopsis* sp.	Fire ant	+	+	+	+	+	Insecta	Hymenoptera	Formicidae
34	*Tachy* sp.	Ground beetle	+	-	-	-	-	Insecta	Coleoptera	Carabidae
35	*Trigoniulus corallinus*	Red garden millipade	+	+	+	+	+	Diplopoda	Spirobolida	unidentified
36	unidentified 1	unidentified	+	-	-	-	-	Insecta	Coleoptera	Curculionidae
37	unidentified 2	Cricket	+	+	+	+	+	Insecta	Orthoptera	unidentified
38	unidentified 3	Flat bug 1	+	+	+	+	+	Insecta	Heteroptera	Aradidae
39	unidentified 4	Darkling beetle 1	+	-	-	-	-	Insecta	Coleoptera	Tenebrionidae
40	unidentified 5	Flat bug 2	+	+	+	+	+	Insecta	Heteroptera	Aradidae
41	unidentified 6	Leiochrinini beetle	+	-	-	-	-	Insecta	Coleoptera	Tenebrionidae
42	unidentified 7	Damsel bug	+	+	+	+	+	Insecta	Hemiptera	Nabidae
43	unidentified 8	Palm weevil	+	+	-	-	-	Insecta	Coleoptera	Curculionidae
44	unidentified 9	Brown darkling beetle	+	-	-	-	-	Insecta	Coleoptera	Tenebrionidae
45	unidentified 10	Scorpion	+	+	+	+	+	Arachnida	unidentified	unidentified
46	unidentified 11	Spider 1	+	+	+	-	-	Arachnida	unidentified	unidentified
47	unidentified 12	Spider 2	+	+	+	-	-	Arachnida	unidentified	unidentified
48	unidentified 13	Sap beetle 1	+	-	-	-	-	Insecta	Coleoptera	Nitidulidae
49	unidentified 14	Sap beetle 2	+	-	-	-	-	Insecta	Coleoptera	Nitidulidae
50	unidentified 15	Flat bug 3 (black)	+	+	-	-	-	Insecta	Heteroptera	Aradidae
51	unidentified 16	Sap beetle 4	+	-	-	-	-	Insecta	Coleoptera	Nitidulidae
52	unidentified 17	Ground beetle	+	-	-	-	-	Insecta	Coleoptera	Carabidae
53	unidentified 18	Ant 1	+	+	+	+	+	Insecta	Hymenoptera	Formicidae
54	unidentified 19	Ant 2	+	+	+	+	+	Insecta	Hymenoptera	Formicidae
55	*Xylosandrus carrassiuculus*	Asian ambrosia beetle	-	-	+	-	-	Insecta	Coleoptera	Curculionidae

Note: + : present; - : absent; ---- grey highlights in the table indicates the presence of insect species in all five sampling sites in Sabah.



Note: (a) *Blattella asahinai*; (b) *Amarygmini* sp.; (c) *Amphicrossus* sp.; (d) *Bolitotherus cornutus*; (e) *Eledona* sp.; (f) *Encaustes* sp.; (g) *Euborellia annulipes*; (h) *Xylosandrus crassiusculus*; (i) *Eumorphrus micans*; (j) *Onthopagus* sp.; (k) *Penthaphyllus* sp.; (l) *Phalacrus* sp. (*Phlacridae*); (m) *Physodera chalceres*; (n) *Platydema ruficorne*; (o) *Sepedophilus* sp.; (p) *Tachys* sp.
Scale Bars: ——— : 0.5 cm ——— : 0.5 mm

Figure 3. Close up of the insect community captured from the five study sites in Sabah.

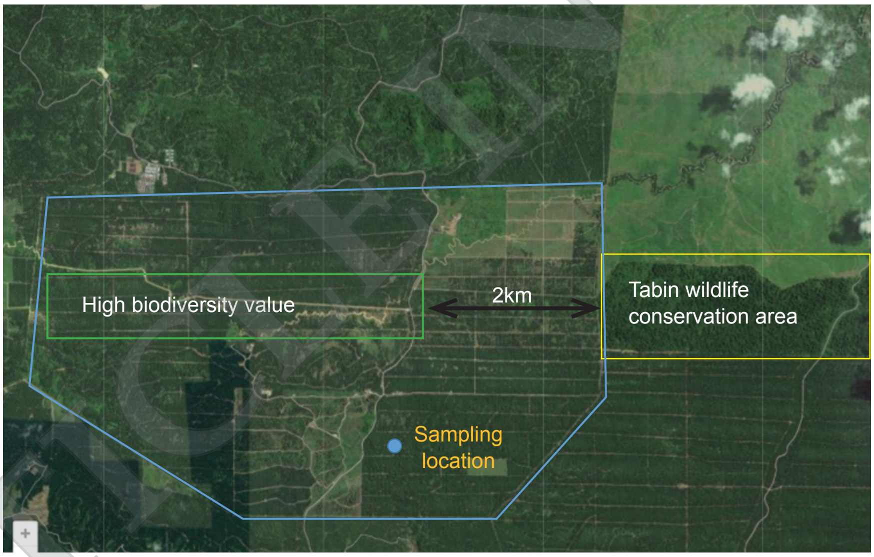


Figure 4. Sampling area in Lahad Datu (study site) bordered by Tabin Wildlife Conservation Area and High Biodiversity Area.

Previously, *Episcapha quadrimaculata* was reported by Idris (2013) to be associated as a vector of *Ganoderma* BSR dispersal in Peninsular Malaysia due to its common presence in the basidiocarps. This species belongs to Erotylidae family or commonly known as pleasing fungus beetles, a different family than handsome fungus beetle which is Endomychidae. Erotylidae were generally synonym to the bracket fungus pest. *Megalodacne* spp. also an Erotylids was reported to infest bracket fungi found on dead trees and stumps (Skelley, 1999). Contrary to the findings of Idris (2013), *E. quadrimaculata* was not detected in Sabah; instead, two other Erotylids: *Lioptera* sp. and *Eucastes* sp. were found in solitude.

There were six *Eumorphus* spp. discovered on the basidiocarp of *Ganoderma* in MPOB Phase 2 and Mawao Estate, but these species were found in solitude. The six species were *E. minor*, *E. austerus*, *E. dihaani*, *E. micans*, *E. dilatatus tirittus* (*Figure 5c*), and *E. quadrinotatus* (*Figure 5b*) based on Strohecker's descriptions. Only *E. dihaani* and *E. micans* were not found in Mawao estate. The wide spread *Eumorphus* spp. in South East Asia region has led to the assumption that this genus is endemic to this region (Yusup and Eris, 2020).

Note: Notice the distinct four yellow spots on the dorsal view.

Figure 5. Handsome fungus beetles (a) Eumorphus minor *(b)* Eumorphus quadrinotatus, *and (c)* Eumorphus dilatatus turittus.

Interestingly, *E. austerus* samples were occasionally observed to secrete yellow fluids from their femoral apex, which subsequently spread a pungent odour; a similar observation was reported by Brown (1985). And all the *Eumorphus* spp. were confirmed to carry the basidiospores of *Ganoderma,* internally and externally, making them an obvious candidate as vectors for BSR disease transmission. The PDA viability test further validated the cultured basidiospores as viable (*Table 2*). This is the first finding indicating *Eumorphus* spp. as one of the common insects from the *Ganoderma* basidiocarps on oil palm in Malaysian. In a study conducted by Yamashita *et al.* (2015), fungivorous insects' diversity was assessed by collecting brackets of forest-growing fungal species. The study reported that *Eumorphus* spp. had the dominance of presence compared to other families of Coleopteran species within the *Ganoderma* spp. basidiocarps.

Presence and Viability of *Ganoderma* Basidiospores

The list of insects with viable spore germination through the PDA plating is shown in *Table 2* below. *Eumorphus* spp. were carrying *Ganoderma* basidiospores (*Figure 6*) in both internal and external parts of their bodies.

Figure 6. The presence of Ganoderma *basidiospores found inside* Eumorphus aestrus *body observed on a hemocytometer.*

TABLE 2. *Ganoderma* BASIDIOSPORE PRESENCE AND VIABILITY

No.	Scientific name	Common name	Basidiospore		Viability
			External	Internal	
1	*Amarygmus* sp.	Darkling beetle	+	-	/
2	*Amphicrossus* sp.	Sap beetle	-	-	
3	*Anoplolepis gracilipes*	Red ant	-	-	
4	*Blattella asahinai*	Asian cockroach	-	-	
5	*Bolitotherus cornutus*	Forked fungus beetle	+	+	/
6	*Camponotus* sp.	Black carpenter ant	-	-	
7	*Camponotus* sp.	Carpenter ant 2	-	-	
8	*Cis* sp.	Minute tree fungus beetle	+	-	/
9	*Coptotermes kalshoveni*	Termite	-	-	
10	*Dolichodores* sp.	Black ant	-	-	
11	*Eledona* sp.	unidentified	+	-	X
12	*Encaustes* sp.	Pleasing fungus beetle	-	-	
13	*Euborellia annulipes*	Earwigs	-	-	
14	*Eumorphus dilatatus tarittus*	Handsome fungus beetle	+	+	/
15	*Eumorphus austerus*	Four spotted handsome fungus beetle	+	+	/
16	*Eumorphus minor*	Handsome fungus beetle	+	+	/
17	*Eumorphus quadrinotatus*	Handsome fungus beetle	+	+	/
18	*Eumorphus dihaani*	Handsome fungus beetle	+	+	/
19	*Eumorphus micans*	Handsome fungus beetle	+	+	/
20	*Lioptera* sp.	Pleasing fungus beetle	-	-	
21	*Mycomya rondani*	Fungus gnat	-	-	
22	*Oecophylla smaragdina*	Weaver ant	-	-	
23	*Onthophagus* sp.	Dung beetle	-	-	
24	*Oryctes rhinoceros*	Rhinoceros beetle	-	-	
25	*Pentaphyllus* sp.	unidentified	+	-	X
26	*Phalacrus* sp.	unidentified	-	-	
27	*Physodera chalceres*	Ground beetle	-	-	
28	*Platydema ruficorne*	Darkling beetle	+	-	/
29	*Polyrachis* sp.	Ant	-	-	
30	*Porcellionides pruinosus*	Woodlice	+	+	/
31	*Sepedophilus* sp.	Crab like -rove beetle	-	-	
32	*Sepedophilus* sp.	Rove beetle	-	-	
33	*Solenopsis* sp.	Fire ant	-	-	
34	*Tachy* sp.	Ground beetle	-	-	
35	*Trigoniulus corallinus*	Red garden millipade	-	-	
36	unidentified 1	unidentified	-	-	
37	unidentified 2	Cricket	-	-	
38	unidentified 3	Flat bug 1	+	-	/
39	unidentified 4	Darkling beetle 1	+	-	X
40	unidentified 5	Flat bug 2	+	-	/
41	unidentified 6	Leiochrinini beetle	-	-	
42	unidentified 7	Damsel bug	-	-	

TABLE 2. *Ganoderma* BASIDIOSPORE PRESENCE AND VIABILITY (continued)

No.	Scientific name	Common name	Basidiospore		Viability
			External	Internal	
43	unidentified 8	Palm weevil	-	-	
44	unidentified 9	Brown darkling beetle	-	-	
45	unidentified 10	Scorpion	-	-	
46	unidentified 11	Spider 1	-	-	
47	unidentified 12	Spider 2	-	-	
48	unidentified 13	Sap beetle 1	-	-	
49	unidentified 14	Sap beetle 2	-	-	
50	unidentified 15	Flat bug 3 (black)	+	-	/
51	unidentified 16	Sap beetle 4	-	-	
52	unidentified 17	Ground beetle	-	-	
53	unidentified 18	Ant 1	-	-	
54	unidentified 19	Ant 2	-	-	
55	*Xylosandrus carrassiuculus*	Asian ambrosia beetle	-	-	

Note: + : present; - : absent; /: viable X: non-viable

From *Table 2*, there were 17 arthropods which were able to carry basidiospores externally and 8 of them also carried the basidiospores internally. The viability of the basidiospores was determined and 14 of them carried viable basidiospores. Darkling beetles such as *Cis* sp., *Eledona* sp. and *Pentaphyllus* sp. that were feeding on *Ganoderma* fruiting bodies were only found carrying the spore outside (externally) their bodies. *Eumorphus* spp. were often found underneath the fruiting bodies feeding on the basidiospores, which allowed the *Ganoderma* basidiospores to remain intact internally and externally on their bodies. Meanwhile, the darkling beetles, usually present on the top surface of the fruiting bodies, bore into the *Ganoderma* basidiocarps, mining and building tunnels. In Polypores fungi, the basidiospores are released from the underneath structure of the basidiocarp, where the pores are located. The darkling beetles were more often found travelling on the top surface of the basidiocarps, which may have contributed to the fact that they had limited contact with the basidiospores of *Ganoderma*. It has been reported that *Bolitotherus cornutus*, the forked fungus beetle, favours the tissues and spores of bracket fungi, including *Ganoderma applanatum*, *Ganoderma tsugae*, and *Fomes fomentarius* (Liles, 1956; Pace, 1967). We found *Bolitotherus cornutus* to be associated with the basidiocarps of *Ganoderma*, and they were found in all five sampling locations (*Table 1*).

All basidiospores from internal and external collections of the insects were spread on the GSM agar surface, but unfortunately none of the spores were found viable. According to Ariffin *et al.* (2000), GSM was developed to isolate and confirm *Ganoderma* infected tissues but was not tested for spores. Since the insects were directly collected on *Ganoderma* fruiting bodies, the possibility that the basidiospores were not *Ganoderma* was very low. However, the high content of different chemicals will not be conducive to the germination of these basidiospores. The GSM is a mixture of antibiotics, fungicides, and tannic acids that provides the selective growth conditions for infected *Ganoderma* tissues while eliminating and suppressing the growth of other microbes associated with oil palm to achieve a pure culture of *Ganoderma* (Ariffin *et al.*, 2000). The formulation was suitable to directly conduct an *in-situ* plating of infected tissues of *Ganoderma* in the field (Sundram *et al.*, 2015). Meanwhile, fungal spores are robust structures of the fungi kingdom and are well known to withstand harsh environmental conditions for survival (Sephton-Clark *et al.*, 2018). These robust structures will only break their dormancy and begin their germination once exposed to favourable conditions, which may explain their inability to germinate on GSM. Germination in fungal spores is the mechanism that converts the spore from a dormant biological organism to one that is vegetatively compatible and capable of sexual or asexual reproduction. Therefore, a final attempt to recover the viability of the basidiospores was conducted on PDA, and the monokaryons were indeed viable. However, we encountered high levels of cross-contamination (bacteria) because the spores were not subjected to any form of surface sterilisation.

CONCLUSION

The importance of insect transmission of plant diseases has generally been overlooked and greatly underestimated. Insects are capable of facilitating the entry of a pathogen into its plant host through wounds made above ground or below ground of the plant structures. Based on findings in the current study, we could conclude that insect community visiting *Ganoderma* basidiocarps were mainly from Coleoptera and Hymenoptera orders. We also observed that insects were able to carry and move the spores. Although GSM was unable to germinate the basidiospores, it was the PDA spread that confirmed the viability of the spores. This study is the first report that enlists the possible insects as potential vectors for *Ganoderma* BSR disease in oil palm plantations in Sabah. Among the sixteen species of insects detected to have a positive presence of basidiospores, the handsome fungus beetle, *Eumorphus* spp. indeed has the biggest possibility of transmitting the basidiospores. Further investigation should include assays to obtain pure cultures of the basidiospores, followed by identification via molecular tools. Additionally, research should also include insects sampling in the other states of Malaysia for comparison, especially Sarawak, the state with the worst infection by USR. All the possible vectors should be screened against healthy palms to validate initiation of infection due to the role of vectors.

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