

SHORT COMMUNICATION

Parasitic *Gregarine blattarum* Found Infecting American Cockroaches, *Periplaneta americana*, in a Population in Pulau Pinang, Malaysia

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Abstrak: Satu kajian ke atas prevalens endoparasit biasa dalam populasi Lipas Amerika liar di Pulau Pinang dilakukan dengan kaedah perangkap di beberapa kawasan pensampelan di pulau tersebut. *Gregarine blattarum* ditemui dalam saluran pencernaan 5 daripada 115, atau 4.35%, dari Lipas Amerika liar, *Periplaneta americana* yang disampel. Ini merupakan penemuan pertama *Gregarine blattarum* di dalam Lipas Amerika tempatan yang dilaporkan di Malaysia.

Kata kunci: Lipas Amerika, Endoparasit, Gregarin

Abstract: A study on the prevalence of a common endoparasite in the wild population of the American cockroach was conducted in Penang Island using a trapping method at several sampling sites on the island. *Gregarine blattarum* was found in the digestive tract in 5 out of 115, or 4.35%, of the wild American cockroaches, *Periplaneta americana*, that were sampled. This is the first report in Malaysia of *Gregarine blattarum* in local American cockroaches.

Keywords: American Cockroach, Endoparasite, Gregarines

Gregarina blattarum von Siebold, originally referred to as *Blatta orientalis* in 1839, has been reported to infect at least six other cockroach species worldwide. However, despite the morphological similarity among these species, Clopton and Gold (1996) have experimentally demonstrated that *G. blattarum* were able to infect laboratory colonies of *Blatella germanica*, while they were unable to infect four other cockroach species in which this gregarine had previously been reported (Smith & Cook 2008). This parasite, which belongs to the Order Eugregarinida (Protista: Apicomplexa), is typically distinguished by their characteristic appearance (Smith & Cook 2008), and this protozoa is also an obligate intestinal parasite in a large number of invertebrates, including annelids, crustaceans, echinoderms, arachnids, pelagic tunicates and insects (Clopton & Gold 1996). Arthropods, such as German cockroaches, *Blatella germanica*, have

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been found to be a specific host for *Gregarine blattarum* (Lopes & Alves 2005). This is the first report of an infection by the *Gregarine* species in the American cockroaches sampled in Penang Island, Malaysia. *Gregarine* sp. infecting local American cockroaches has never been reported or observed in Malaysia previously.

The trapping method was performed according to the method suggested by Kinfu and Erko (2008). Clean and empty jars were internally coated with Vaseline using cotton, while the outside of the jars were wrapped with crumpled paper. A piece of bread was dipped in fermented liquid or beer and was placed inside the trap. The fermented liquid or beer produced a strong smell and attracted more cockroaches to the traps. The purpose of coating the jars with Vaseline is to ensure that the inside wall was slippery so that the trapped cockroaches could not climb out. The outside traps were wrapped with crumpled paper to ensure that a nearby cockroach could easily climb and get into contact with the bait inside the trap. Some of the traps were tied with ropes on both sides to enable them to be connected to a suitable anchor or corner when the traps were placed in sewers, drains, ditches or any other crucial locations. This method ensured that the traps could not be easily removed by locals or other animals and that they could not be moved or be carried by the water flow in drains or in the case of rain. A large hole approximately half of the size of the bottle covering was made for each trap so that cockroaches could be attracted to the baits inside, and at the same time, other larger animals such as rats could not easily fall into the traps. For some traps, double-sided tape was used to stick the bottom of the traps to the floor of the sampling sites. The traps were then brought to the sampling sites and placed at locations with a high potential of encountering cockroaches.

All of the trapped cockroaches from the sampling sites were brought to the laboratory for identification. Identification was made according to the description and the general characteristic appearances of the samples after collection from the sampling sites. Only whole and live samples of the American cockroach species *P. americana* were used for examination; any other species were discarded. The samples were divided according to whether they were adult males, adult females or nymphs. This classification facilitated the next steps of determining which sex and life stage of the cockroaches were the most numerous and how this influenced a higher or lower chance of parasitic infection.

Morphologically, American cockroaches have a shiny red to chocolate brown exoskeleton, and nymphs are uniformly brown in colour and resemble adult cockroaches, except that they are wingless. This species has long and tapered cerci during all life cycle phases and the presence of undefined yellow and brown markings on the pronotum. Male cockroaches have a reproductive system known as a stylus, while female cockroaches have an ovipositor. Nymph cockroaches are wingless and do not have fully developed reproductive systems (Lee & Ng 2009).

Dissection of the cockroaches was performed to determine the presence of parasites inside the digestive tract and the alimentary canal. Before the cockroaches were dissected, the head and both wings were pinned on the dissecting tray using fine pins to ensure that the sample did not move during the

dissection process. The legs were removed using pointed forceps or scissors, and the abdomen was exposed to the upper side. Then, dissection scissors were used to cut the ventral side along the abdomen to remove the exoskeleton. The internal part of the sample was exposed after removing the exoskeleton, and the body fat surrounding the abdominal organs was removed slowly using pointed forceps and sharp needles to expose the internal organs of the cockroaches and to isolate the alimentary canal. Then, the alimentary canal was removed carefully by cutting at the end near the oesophagus and at the other end, near the anus/cloaca, and placed onto a Petri dish. Under the dissecting microscope, the alimentary canal was macerated using sharp scissors and fine needles on the petri dish, which contained 2 ml of normal saline.

Any presences of parasites such as nematodes, helminths, protozoa or the eggs of parasites under the dissecting microscope were recorded. If any endoparasites were found, a pipette was used to transfer them to be observed under the compound microscope for identification. The nematodes found were fixed in 70% alcohol for further identification. For the examination of any presence of ova/cysts/eggs of parasites, 1 ml from the result of internal washing was centrifuged at 2000 rpm for 5 minutes, and the deposit was examined after staining with 1% Lugol's iodine. Parasite identification was based on the morphology and characteristic appearances. The ova and cysts of the parasites were measured and identified using a compound microscope, and the identification was according to the length, width and special characteristics shown (Melvin *et al.* 1964).

A total of 115 samples of cockroaches were collected during the sampling period, and five of them, or 4.35% of the samples, were infected by parasitic gregarines. The cockroaches infected with *G. blattarum* were collected from Gelugor, Bukit Jambul, Bayan Baru, Bayan Lepas, and Batu Lanchang dan Tanjung Bungah, while the samples collected from Universiti Sains Malaysia (USM) Main Campus and Sungai Dua, Penang were uninfected. Most of the gregarines observed were in gamont form (Figure 1). Gregarines have been reported as one of the most diverse groups of organisms and have been found in invertebrate hosts, including cockroaches, dragonflies, beetles and crustaceans (Clopton & Gold 1996; Wise *et al.* 2000; Lopes & Alves 2005; Smith & Cook 2008; Roberts & Javony 2010; Bunker *et al.* 2013). The gregarine-infected invertebrate insects were grouped under the order Eugregarinorida and suborder Septatorina. The gregarines infecting the cell of intestine of American cockroaches were identified as *Gregarina blattarum*.

The gamonts of *G. blattarum* were cylindrical in shape and ranged from 100 μm to 2000 μm in length and from 30 μm to 1500 μm in width. Each of the gamont was attached to the host intestinal epithelium. A pair of gamonts will undergo syzygy and detach from the intestinal epithelium. The association of the two gamonts will then cause the formation of a gametocyst wall, in which the process of gametogenesis, fertilisation and the division of the zygote occurs. Then, the production of an oocyst occurs, which is also known as the process of sporulation. Gametocysts with oocysts encapsulated inside are excreted with the faeces of the cockroach. When other cockroaches consume the infected faeces, they become infected by the oocysts of *G. blattarum*. In the intestine of the

infected host, the oocyst will undergo exsporulation and penetrate epithelial cells as a sporozoite. Then, the sporozoite will grow to become a trophont. The trophont grows into a gamont that attaches to the intestinal epithelium, and the process repeats. According to Lopes and Alves (2005), a gregarine infection in a population of cockroaches can cause mortality, which we also found in this study. Many of the cockroaches sampled that were infected with *G. blattarum* were dead before being dissected.



Figure 1: Gamont of *G. blattarum* is cylindrical in shape. Note the presence of the nucleus (N).

The gregarine life cycle can be divided into four phases: a) excystation, b) infection and development, c) assortment and syzygy and d) mixis and sporogony. Mechanisms enforcing host specificity may operate during any part of this life cycle phase (Clopton & Gold 1996), and the oocysts in the environment or from the cadaver of the infected host can be accidentally consumed by American cockroaches. However, some of the cockroaches infected with *Gregarine* sp. were alive and appeared well during trapping and collecting, probably due to the low number of gamonts found in the digestive tract. According to Lopes and Alves (2005), gregarines were also found in insects with a healthy appearance but in low numbers (1–10 gamonts), demonstrating the chronic aspect of the disease. The occurrence of chronic infections of gregarines in cockroaches has been reported in colonies maintained under laboratory conditions (Clopton 1995; Clopton & Gold 1996).

Since the population of the American cockroaches in the sampling areas normally share the same habitat with numerous other species of cockroaches,

such as German cockroaches (*Blattella germanica*), Lobster cockroaches (*Nauphoeta cinerea*), and Brown-banded cockroaches (*Supella longipalpa*), American cockroaches could have been consuming the contaminated faeces or body of a dead infected host belonging to a different species and were thus infected by a horizontal transmission of the parasite.

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