Surveillance of ectoparasitic fungi *Laboulbeniopsis termitarius* thaxt and *Antennopsis gallica* buchli and heim on subterranean termite *reticulitermes* spp. in Japan

I Guswenrivo1*, D Tarmadi1, H Sato2, I Fujimoto3 and T Yoshimura3

1Research Center for Biomaterials, Indonesian Institute of Sciences (LIPI), Jl. Raya Bogor KM.46, Cibinong, Bogor, West Java 16911, Indonesia
2Forest and Forestry Product Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687 Japan
3Laboratory of Innovative Humano-habitability, Research Institute for Sustainable Humanosphere (RISH), Kyoto University, Gokasho Uji, Kyoto 611-0011, Japan

*E-mail: Ikhsan.guswenrivo@biomaterial.lipi.go.id*

**Abstract.** Ectoparasitic fungi grow on the body surface of termites, and they might effect on behavior, reproductive, and survival of host termites. There are two common species of ectoparasitic fungi found on termite: *Laboulbeniopsis gallica* Thaxt and *Antennopsis gallica* Buchli and Heim. In this study, the distribution of *L. termitarius* and *A. gallica* in *Reticulitermes* spp. colonies in Japan was observed. Meanwhile, the infection rate and strength of *L. termitarius* and *A. gallica* were discussed with references to the effects of environmental factors at the collections sites. In a total of 63 colonies of *Reticulitermes* spp. were collected from seventeen locations (from Hokkaido Prefecture to Okinawa Prefecture) in Japan. Five hundred workers and twenty soldiers from each colony were examined individually to see the infection of *L*. *termitarius* and *A. gallica*. The survey showed that *L. termitarius* distributed in whole Japan and *A. gallica* had a little bit restricted distribution. The infection rate of workers of *Reticulitermes* spp. varied among all locations: 0.10 – 16.10% for *L. termitarius* and 0 – 66.40% for *A. gallica*. No infected soldiers were observed. The negative relationship between temperature and infection rate was speculated in both fungi. Rearing the colonies in the laboratory might result in the spreading of the fungi in the colonies. Both fungi grew on any body parts of the termites. The trials for isolation and cultivation of *Laboulbeniopsis termitarius* and *A. gallica* with eight media did not succeed under the laboratory conditions.

1. Introduction

Termites are associated with fungi in many ways, such as symbiotic mutualism and pathogenic relationship. Symbiotic mutualism is the relationship where both termite and fungi get benefit [1–4]. In a pathogenic relationship, termite gets negative effects from the presence of fungi, and it
can threaten the survival, behavior, and reproductive of a termite [5–9]. On the other hand, the pathogenic fungi have been utilized as biological agents against termite pests [10]. More than 20 species of entomopathogenic fungi have been tested for their pathogenicity, but none of them are ectoparasitics [9, 10]. Termite ectoparasitic fungi consist of 22 species from 9 genera, where the majority of the fungi are species of Laboulbeniales (Ascomycota) [11].

Most of the ectoparasitic fungi have a small body size, barely longer than termite setae, and they attach to the body surface of termite. The ectoparasitic fungi Laboulbeniopsis termitarius Thaxter and Antennopsis gallica Buchli and Heim are most widely distributed species and were firstly described in 1914 and 1951, respectively [12, 13]. Geographical distributions of L. termitarius and A. gallica cover the tropical areas up to temperate regions. Thaxter firstly discovered the genus Laboulbeniopsis on the tips of Eutermes moiro from Grand Entang, Grenada, British West Indies [12], and the distribution of the genus covers America, Europe, and Asia [11]. We recently have found L. termitarius from Reticulitermes spp. in Japan [14]. Meanwhile, A. gallica was described firstly on Reticulitermes lucifungus Rossi in Southern France [15]. The following years, Antennopsis gallica was then discovered in North America and northern Florida [16] and Canada [17]. The latest discovery of A. gallica, as the first record in Kyoto, Japan, has been reported [18]. The fungus has been found from Reticulitermes speratus (Kolbe), the most widely distributed common species, and one of the most economically important wood pest insects in Japan. However, there has been no national survey on the distribution of ectoparasitic fungi in Japan so far.

Ectoparasitic fungi grow and attach on the termite cuticle, and they might interrupt the moulting process and termite behavior. Certain ectoparasitic fungi were suspected of causing the death of termite colonies. Lenz and Kombrough [19] discovered that termites infected by ectoparasitic fungus Termitaria sp. tended to have lower survival than healthy termites. Similarly, Nasutitermes corniger Motschulsky and N. ephratae Holmgren in Panama infected by Mattirolella crustosa Thaxter died rapidly in comparing with non-infected individuals [20]. Buchli [15, 21, 22] reported that termites health condition became worse by the presence of A. gallica, and they died when the infection rate was high. He concluded that it was due to the adhesive compounds produced by A. gallica attached to the host, which interfered termite movement and feeding. The information of ectoparasitic fungi has lead us to the idea that we can use the fungi as biological agents against termites. Concludingly, the mortality of termite infected by ectoparasitic fungi might be due to a combination of fungal toxin, physical obstruction of blood circulation, nutrient depletion, and invasion of organs [23]. Culturing the fungi in the laboratory is indispensable in order to understand the effect of ectoparasitic fungi on termite activity. This research was carried out to see the distribution and abundance of two species of ectoparasitic fungi, L. termitarius, and A. gallica in Japan together with trials on isolation and cultivation of the fungi in the laboratory.

2. Materials and Methods

2.1. Sampling and preparation for observation

Colonies of Reticulitermes spp. were collected from 17 locations in Japan: Hokkaido 3 colonies, Akita 4 colonies, Niigata 5 colonies, Miyagi 5 colonies, Ibaraki 5 colonies, Toyama 5 colonies, Gifu 3 colonies, Aichi 2 colonies, Shizuoka 2 colonies, Kyoto 3 colonies, Wakayama 4 colonies, Okayama 3 colonies, Tokushima 4 colonies, Yamaguchi 5 colonies, Oita 4 colonies, Kagoshima 3 colonies and Okinawa 3 colonies. Sampling locations, season, and temperatures are summarized in Table 1. The colonies of termite were collected from dead tree branches on the forest ground, and were wrapped with paper and kept inside plastic bags, then brought back to the laboratory. The weather conditions during sampling were measured. Five hundred workers and twenty soldiers were chilled by a refrigerator and examined for infection by ectoparasitic fungus by observing them individually under a dissecting microscope (S8APO, Leica, Wetzlar, Germany). The numbers of thalli per individual termite and the infected body parts were recorded.
2.2. Morphological identification

The infected termites were separated and kept at 4°C before identification. Mounting samples were prepared for morphological identification. Fungus thalli were removed from the termite body using an entomological pin (Dumont 5ST 11252-00, British Columbia, Canada) and embedded in lactophenol solution (phenol 10g, lactic acid 10g, glycerin 20g, and distilled water 10g). The fungus was observed using a light microscope (BX51, Olympus, Tokyo, Japan) and a digital microscope (VHX-5000, Keyence, Osaka, Japan) for the identification process. Standard keys were used to identify the fungi and consulted to experts for confirmation.

2.3. Isolation and laboratory cultivation of the fungi

Infected termites were separated, and the fungus thallus was removed aseptically and placed on media. Eight different media (7 commercial and 1 artificial) were prepared: water agar (WA), potato dextrose agar (PDA), L-broth agar (LBA), molisch agar (MA), brain heart infusion (BHI) oatmeal agar (OA), cornmeal agar (CA) and termite agar (TA). Termite agar (TA) medium was prepared by mixing 2% of termite (wet weight) and 2% agar in distilled water. Two ppm streptomycin was added in all the media as an antibiotic agent to control bacteria. Inoculation was conducted with two ways: a) Fungi were directly inoculated on the surface of the media, and b) fungi were inoculated on sterile termite alate wing on the surface of the media. The fungi were incubated in the Deterioration Organism Laboratory (DOL), Research Institute for Sustainable Humanosphere (RISH), Kyoto University under the dark condition with 26±1°C and 60±5% relative humidity. The growth of fungi was observed every two days for the first two weeks, and every week up to 3 months.

2.4. Statistical analysis

The data from each observation were statistically analyzed. The analysis of variance was used to evaluate the significant difference between factors and levels. A two-factor analysis of variance (ANOVA) was used to test the influence of sampling locations, and infected body part of termite into a number of the thallus. The comparison of the means was done by using Tukey HSD post hoc test to identify which groups were significantly different from other groups at 95% confidence level.

3. Results

3.1. Distribution of ectoparasitic fungi

A total of 63 Reticulitermes spp. colonies were collected from 17 locations over Japan. The samples were collected in different season: 1 sample in winter (Hokkaido), 5 samples in spring (Ibaraki, Gifu, Yamaguchi, Oita, and Kagoshima), 4 samples in summer (Akita, Toyama, Tokushima, and Okinawa), and 7 samples in autumn (Niigata, Sendai, Nagoya, Shizuoka, Kyoto, Wakayama, and Okayama) (Table 1). Termite colonies collected from Hokkaido and Okinawa had been kept in the lab over six months before observation. The results showed that Reticulitermes spp. in Japan were widely infected by L. termitarius and A. gallica. Both species were found most of the sampling locations (Figure 1). Laboulbeniopsis termitarius was widely distributed (Hokkaido, Akita, Niigata, Miyagi, Ibaraki, Toyama, Gifu, Aichi, Shizuoka, Kyoto, Wakayama, Okayama, Yamaguchi, Oita, Kagoshima and Okinawa) around Japan, while A. gallica was not found in termite colonies in Akita, Gifu, Shizuoka, Yamaguchi, and Kagoshima. Termite colonies were infected by one or two species of ectoparasitic fungi at the same time.
Table 1. Season and temperature of sampling locations at the collection period (source: Japan Meteorological Agency, http://www.jma.go.jp/jma/indexe.html).

| No. | Sampling location | Season | Sampling date   | Temperature of the sampling date |
|-----|-------------------|--------|-----------------|----------------------------------|
|     | Prefecture | City/Town |             | average | Max | Min |
| 1   | Hokkaido      | Sapporo | Winter       | 20 Dec 2014 | -1.3 | 3.7  | -4.3 |
| 2   | Akita         | Noshiro | Summer       | 17-18 June 2015 | 19.9 | 24.4 | 15.9 |
| 3   | Niigata       | Niigata | Autumn       | 15 Oct 2015 | 15.8 | 19.9 | 11.5 |
| 4   | Miyagi        | Sendai  | Autumn       | 16 Oct 2015 | 15.5 | 20.4 | 11.0 |
| 5   | Ibaraki       | Shirosato | Spring    | 25 May 2015 | 18.8 | 24.3 | 13.8 |
| 6   | Toyama        | Imizu   | Summer       | 23 Aug 2015 | 26.7 | 30.6 | 23.3 |
| 7   | Gifu          | Ena     | Spring       | 11 May 2015 | 21.3 | 27.2 | 16.2 |
| 8   | Aichi         | Nagoya  | Autumn       | 15 Sept 2015 | 23.1 | 27.5 | 19.8 |
| 9   | Shizuoka      | Shizuoka | Autumn    | 4 Sept 2015 | 23.3 | 27   | 20.4 |
| 10  | Kyoto         | Uji     | Autumn       | 5 Sept 2015 | 22.9 | 27.5 | 19.5 |
| 11  | Wakayama      | Kozagawa | Autumn    | 15 Sept 2015 | 23.4 | 27   | 20.3 |
| 12  | Okayama       | Maniwa  | Autumn       | 8 Sept 2015 | 19.0 | 24.4 | 15.1 |
| 13  | Tokushima     | Tokushima | Summer   | 2 June 2015 | 22.4 | 26.1 | 19.3 |
| 14  | Yamaguchi     | Yamaguchi | Spring   | 25-30 May 2015 | 19.8 | 26.4 | 13.9 |
| 15  | Oita          | Bungotakada | Spring   | 20-23 May 2015 | 20.1 | 25.0 | 15.5 |
| 16  | Kagoshima     | Hioki   | Spring      | 21 Apr 2015 | 18.8 | 23.1 | 15.2 |
| 17  | Okinawa       | Miyako  | Summer      | 13 Aug 2014 | 28.7 | 31.4 | 26.7 |
Figure 1. The distribution of ectoparasitic fungi \textit{L. termitarius} and \textit{A. gallica} on \textit{Reticulitermes} spp. from 17 locations: (1) Hokkaido, (2) Akita, (3) Niigata, (4) Miyagi, (5) Ibaraki, (6) Toyama, (7) Gifu, (8) Aichi, (9) Shizuoka, (10) Kyoto, (11) Wakayama, (12) Okayama, (13) Tokushima, (14) Yamaguchi, (15) Oita, (16) Kagoshima and (17) Okinawa.

* Only \textit{L. termitarius} was found in the sampling location

** Both \textit{L. termitarius} and \textit{A. gallica} were detected in the location

3.2. \textit{Laboulbeniopsis termitarius} Thaxt.

The morphological observation showed that the average length of \textit{L. termitarius} was 98.90 μm, which was small and hardly seen by naked eyes. This fungus had three main body structures, namely: foot cell (F), stalk (St) and sporogonium (S) (Figure 2). The foot cell had 26.20 μm long on average, and was characterized from the doughy brown color and had a convex shape with dark black flat base attached to termite cuticle.
6

Figure 2. A: *Reticulitermes speratus* infected by *Laboulbeniopsis termitarius* (red circle). B: Thallus of *L. termitarius* attach to termite antenna (red circle). C: *Laboulbeniopsis termitarius* construct with three major body structures: foot cell (F), stalk (St) and sporongonium (S)

The foot cell adjoining with the stalk had two cells, narrower and elongated tube shape. The length of the stalk was 45.66 μm on average. The sporogonium was 52.53 μm in length on average, link on the top of the thalli, had an elongated shape, slightly bulging with the apical ring. The fungal spore came out from the apical ring when it reached the maturity stage. The morphological identification of the fungi from 17 locations has high similarity with the first previous report [14].

3.3. *Antennopsis gallica* Buchli and Heim

Similar to *L. termitarius*, the morphological observation of *A. gallica* on *Reticulitermes* spp. from 17 locations in Japan showed the high similarity to the first finding of the fungi [13] and the fungi description on Japanese termite [18]. It was 139.28 μm in length on average and was slightly longer than *L. termitarius*. Similar to *L. termitarius*, *A. gallica* had three main body parts: holdfast (H), conidiophores (C) and conidial head (Ch) (Figure 3). The holdfast (H) attached to termite cuticle was 20.46 μm in length on average with ellipse shape and consisted of 4 cells. One or two conidiophores (C) were grown from the two center cells of the holdfast, and was 105.14 μm in length on average, and comprised of 18–27 cells. The conidial head (Ch) was 22.81 μm in length on average, comprising 3–10 cells, showing an oval shape at maturity and spores released from the conidial head.
3.4. Infection rate and strength

*Laboulbeniopsis termitarius* was distributed widely in Japan. Worker termites collected from all the 17 location in Japan were infected with *L. termitarius*. On the other hand, no soldier was observed for the fungal infection. The infection rates of the fungus varied from each location: 0.10–16.10% on average (Table 2). The statistically higher infection rates were obtained in Ibaraki (16.10%), Hokkaido (14.13%) and Okinawa (13.93%) colonies, and the Ibaraki colonies showed the statistically highest rate. Statistical analysis showed that sampling location gave significance effect onto fungal infection rate (P>0.05).

The infection strength characterized by the thallus number grown per termite was classified into five groups: 1–5; 6–10; 11–15; 16–20; and >20. The results showed that the infection strength of *L. termitarius* was relatively low, and the major groups were 1–5 and 6–10 in all the sampling locations (Figure 4A). As shown in Figure 5A, in *L. termitarius*, the higher infection rates gave a higher thallus number per termite with the linear correlation ($R^2 = 0.9665$). The fungus was found in every body part of termites: from antenna, mandible, thorax, abdomen, and legs. Legs and abdomen were significantly preferable for *L. termitarius* to grow, followed by abdomen, antennae, thorax, head, and mandible (Table 3).
Table 2. The infection rates of *Laboulbeniopsis termitarius* and *Antennopsis gallica* on *Reticulitermes* spp. in Japan

| No. | Location | Season       | No. of colonies | *Laboulbeniopsis termitarius* | *Antennopsis gallica* |
|-----|----------|--------------|-----------------|------------------------------|------------------------|
|     |          |              | Infected colonies | Infection rate (%)*** | Infected colonies | Infection rate (%)*** |
| 1   | Hokkaido | Winter**     | 3/3             | 14.13 (cd)                  | 3/3                    | 66.40 (b)              |
| 2   | Akita    | Summer       | 4/4             | 7.04 (bc)                   | 0/4                    | 2.16 (a)               |
| 3   | Niigata  | Autumn       | 5/5             | 5.87 (ab)                   | 5/5                    | 0.00 (a)               |
| 4   | Sendai   | Autumn       | 3/5             | 4.40 (ab)                   | 2/5                    | 0.00 (a)               |
| 5   | Ibaraki  | Spring       | 3/5             | 16.10 (d)                  | 1/5                    | 1.60 (a)               |
| 6   | Toyama   | Summer       | 3/5             | 0.13 (a)                    | 3/5                    | 0.00 (a)               |
| 7   | Gifu     | Spring       | 3/3             | 3.55 (a)                    | 0/3                    | 0.00 (a)               |
| 8   | Nagoya   | Autumn       | 2/2             | 0.64 (a)                    | 2/2                    | 4.72 (a)               |
| 9   | Shizuoka | Autumn       | 1/2             | 0.10 (a)                    | 0/2                    | 0.55 (a)               |
| 10  | Kyoto    | Autumn       | 3/3             | 0.20 (a)                    | 3/3                    | 15.73 (a)              |
| 11  | Wakayama | Autumn       | 1/4             | 5.04 (a)                    | 1/4                    | 5.76 (a)               |
| 12  | Okayama  | Autumn       | 3/3             | 0.40 (a)                    | 3/3                    | 1.04 (a)               |
| 13  | Tokushima| Summer       | 1/4             | 0.80 (a)                    | 1/4                    | 11.10 (a)              |
| 14  | Yamaguchi| Spring       | 5/5             | 0.30 (a)                    | 0/5                    | 0.00 (a)               |
| 15  | Oita     | Spring       | 4/4             | 1.13 (a)                    | 1/4                    | 20.47 (a)              |
| 16  | Kagoshima| Spring       | 1/3             | 0.10 (a)                    | 0/3                    | 0.70 (a)               |
| 17  | Okinawa  | Summer**     | 1/3             | 13.93 (cd)                  | 1/3                    | 12.60 (a)              |

*small letters show the significant difference of infection rates between sampling locations (P>0.05).
**termite colonies had been reared in the lab over six months before observation
***infection rate is an average from colonies observed for each location.
The infection rates of *A. gallica* on *Reticulitermes* spp. varied much wider than those of *L. termitarius*: 0 – 66.40% on average (Table 2). Out of 17 sampling locations, Hokkaido gave a significantly higher infection rate. The most frequent groups of thallus number per termite were similar to *L. termitarius*: 1-5 (Figure 4B). Differ from *L. termitarius*, the correlation between infection rate and thallus number on *A. gallica* had a polynomial trendline ($R^2 = 0.9812$) (Fig. 5B). Some locations showed high infection rates such as Hokkaido (66.40% on average), Kyoto (20.47% on average) and Okinawa (15.73% on average). Similar to the results of *L. termitarius*, *A. gallica* was found in every body part of termites. The significantly higher strength was observed in the abdomen and legs, followed by antennae, head, thorax, and mandible (Table 3).

![Graph](image1.png)

**Figure 4.** The infection strength of ectoparasitic fungi *Laboulbeniopsis termitarius* and *Antennopsis gallica* on termites from 17 sampling locations. Thallus number was grouped into 5: 1–5, 6–10, 11–15, 16–20, and >20.
Figure 5. The relationship between infection rate and the thallus number per individual of *Laboulbeniopsis termitarius* (A) and *Antennopsis gallica* (B).

*Total thallus number from total infected termite per termite colony.

**Infection rate per colony.
Table 3. Thallus number of *Laboulbeniosis termitarius* and *Antennopsis gallica* on termite body parts

| No. | Termite Body part | *Laboulbeniopsis termitarius* | *Antennopsis gallica* |
|-----|-------------------|-------------------------------|---------------------|
| 1   | Head              | $8 \pm 12^*$ (a)              | $60 \pm 146.4 \text{ (ab}^{**}$) |
| 2   | Antenna           | $29 \pm 43 \text{ (bc)}$      | $102 \pm 250.8 \text{ (ab)}$ |
| 3   | Thorax            | $13 \pm 25 \text{ (ab)}$      | $60 \pm 183.2 \text{ (ab)}$ |
| 4   | Mandible          | $3 \pm 10 \text{ (a)}$        | $18 \pm 57.59 \text{ (a)}$  |
| 5   | Legs              | $76 \pm 101 \text{ (d)}$      | $129 \pm 284.9 \text{ (b)}$ |
| 6   | Abdomen           | $30 \pm 49 \text{ (c)}$       | $469 \pm 1820 \text{ (c)}$  |

*Average thallus number followed by standard deviation
**Small letters indicate the significant difference

3.5. Isolation and laboratory cultivation of the fungi

Trials for isolation and cultivation of *L. termitarius* and *A. gallica* were conducted in eight artificial media: water agar (WA), potato dextrose agar (PDA), L-broth agar (LBA), molisch agar (MA), brain heart infusion (BHI) oatmeal agar (OA), cornmeal agar (CA) and termite agar (TA). The observation showed that all the media with two different inoculation processes gave negative results. Direct inoculation was contaminated faster than indirect inoculation, and both fungi did not give any sign of growing.

4. Discussion

*Reticulitermes* spp. are the most important wood pests in Japan, and most widely distributed from the northern part of Hokkaido to the southern part of the Ryukyu Islands, Okinawa. They cause damages in houses, books, furniture, and even plantation trees. Like subterranean termites, *Reticulitermes* spp. generally live and build their nests in the fallen logs and branches. They maintain the nest temperature stable and keep the humid environment. These conditions are favorable for fungi to grow and disperse their spores. There are some fungal groups closely associated with termite nests such as *Termitomyces* spp. [24]. Moreover, termite activity may invite and bring some fungi spore back into the colonies. This lead to the finding of fungi inside the termite nest, even those fungi are in dormant condition [25].

On the other hand, there are numbers of ectoparasitic fungi that infest termites. Some species connect the basal cell with termite cuticle, and others are restricted with haustoria system, and only one (*Cordycepioideus* sp.) is mycelioid [11]. Fungal genera *Laboulbenia, Dimeromyces, Cordycepioideus, Laboulbeniopsis, Coreomycetopsis, Ampchoromorpha, Hormiscioideus, Antennopsis, Termitaria,* and *Mattirolla* were found on termite body surface from 8 termite families [11]. Blackwell and Rossi [11] reviewed the distribution of termite ectoparasitic fungi and stated that among all of these species *Laboulbeniopsis termitarius, Antennopsis gallica,* and *Termitaria* sp. were the most common ectoparasitic fungi from temperate to tropical regions.

*Laboulbeniopsis termitarius* was reported from many places with different termite species as its hosts: Florida [26, 27], Georgia [28], Michigan and Louisiana [11] on *R. flavipes*, Florida on *R. virginicus* Banks [26], Italy on *R. lucifugus* Rossi [29], Sierra Leone on *Leptomyxotermes doriae* Silvestri, Indo–China on *Ahmaditermes* sp., Indonesia on *Nasutitermes* sp., and French
Guiana on *Coptotermes crassus* Snyder [11]. While *A. gallica* was found on *Kalotermes flavicolis* Fabricius, *R. virginicus* Banks and *R. flavipes* (Kollar) in Italy, USA, Canada, and Brazil [11, 16, 17, 22, 28, 30]. Discovering *L. termitarius* and *A. gallica* in Japan has extended the geographical distribution with *Reticulitermes* spp. as their host.

The infection strength of ectoparasitic fungi can be measured by the infection rate and thallus number grown on each termite. Blackwell [28] observed that 20–53% of *R. flavipes* was infected by *L. termitarius*, and Kimbrough and Gouger [26] found less than 40% individuals of *R. flavipes* was infected by *L. termitarius*. In this study, the infection rates per colony and thalli numbers per termite of *L. termitarius* and *A. gallica* varied with sampling locations. It might be affected by environmental conditions as well as termite activities. Buchli [15] noticed that the infection rate of *A. gallica* was high on termites collected in winter, and the colony gradually died. The similar pattern was observed in the present result on Hokkaido colonies, showing high infection rates for both fungi (*L. termitarius*: 14.13%, *A. gallica*: 66.40%) after being kept for six months before examination. It might be due to both the low level of termite activity during winter and rearing in the laboratory in a small container. Kambara [41] reported that the activity of *R. speratus* was high at 25–30°C, and it would be decreased over 30°C and below 12°C. The infection rates of the Okinawa colonies collected in summer (average air temperature: 28.7°C) and reared in the laboratory for almost one year were also high (*L. termitarius*: 13.93%, *A. gallica*: 12.60%). In other colonies collected during summer (Akita, Toyama and Tokushima), the infection rates significantly varied. The colonies collected in spring from Kyoto, Ibaraki, and Oita showed high infection rates of *A. gallica* (Kyoto and Oita) and *L. termitarius* (Ibaraki). These results might suggest that both environmental factors such as temperature and rearing in the laboratory have effects on the infection rate by the fungi. In order to conclude this speculation, further study should be carried out by observing termite colonies from the same location with different seasons.

Grooming is one of the most important termite activities affecting fungal growth. Grooming is the activity that termites clean up themselves and nest-mates. The grooming activity will reduce the infection rate among the termites themselves. This social behavioral facilitates the transmission of ectoparasitic fungi even though grooming also has the benefit in termites for conferring resistance and is an antifungal defense strategy [31–34].

*Laboulbeniopsis termitarius* and *A. gallica* grew over any body parts of termite. It was supported by the results of our previous report that *A. gallica* thalli did not have any specific termite body parts to grow, though thallus numbers varied depending on each body part [18]. Legs, antennae, and abdomen were three body parts with higher thallus numbers of *L. termitarius* and *A. gallica*. Legs contact with soil, the sources of fungi, the thallus numbers would be higher. The antennae were found highly infected too, due to the antennae’s function. Termite antennae have several functions such as sensing of touch, taste, odors (including pheromones), heat and vibration [35], which may lead to fungal infection. It was proved by a previous study by Bao and Yendol [36]. They observed that antennae and legs are the first body parts of termite infected by *Beauveria bassiana* after 10 hours of inoculation, followed by thorax and abdomen [36].

The infection strength of entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* was evaluated by the conidial number grown on its host [37, 38]. The highest number of fungal conidia would be in line with the infection strength and turn out the highest mortality of the host [37, 38]. Differing from entomopathogenic fungi, the infection strength of ectoparasitic fungi *L. termitarius* and *A. gallica* is likely to be considerable by the number of thalli grown on each infected termite. The highest thallus number would increase the possibility of the fungi to germinate and infected other termite in a colony. This assumption was supported by our observation that the highest infection rate of ectoparasitic fungi positively correlates with thallus number of *L. termitarius* and *A. gallica* (Figure 5). As shown in Figure 5, the manipulation and spreading manners of *L. termitarius* and *A. gallica* are likely to be different. In *L. termitarius*, the thallus number of the individual increased with the colony infection rate linearly, meaning that the fungus manipulates and spreads by a simple manner. However, for *A. gallica*, there might be a threshold line of the infection rate for manipulating and spreading, and over the line, the fungus will have a faster infection.

---

7th Sustain Conference in conjunction with 3rd ICGDTR 2018
IOP Publishing
IOP Conf. Series: Earth and Environmental Science 361 (2019) 012030
doi:10.1088/1755-1315/361/1/012030
Buchli [15] mentioned that infected termite colony by *A. gallica* will gradually die by the time, but the effects of ectoparasitic fungi on termite activity remain unclear. Isolation and culturing of the ectoparasitic fungi must be an only possible way to understand the effect of ectoparasitic fungi on termites. Generally, ectoparasitic fungi growing on the body surface of their hosts are believed to take the nutrient through absorption or via contact with living tissues [39, 40]. Ectoparasitic laboulbenialean fungi stick to host cuticle and produce some penetration structures [39, 40]. *Laboulbeniopsis termitarius* was reported to secrete some chemicals to stick with the host through the basal cell, while *A. gallica* constructed a haustorum in order to absorb nutrition from termite cell wall [11]. Incubation condition and media contents will affect on the growth of fungi. Fungi have a wide range of temperature tolerance, but the optimal temperature range for fungi to grow is 20–30°C with high moisture or free water [42].

The media preference has to be synchronized with the nutrient needs by the fungi to grow. Several media such as corn meal, malt extract, potato dextrose agar, maltose, and yeast extract have been used to isolate for most entomopathogenic Hyphomycetes [42, 43]. However, these media did not support the growth of *L. termitarius* and *A. gallica* in the lab conditions. Further research has to be conducted for culturing *L. termitarius* and *A. gallica*. Chitin–based or cuticular lipid-based media might be the options for culturing ectoparasitic fungi *L. termitarius* and *A. gallica*.

5. Conclusion

Distribution of *L. termitarius* and *A. gallica* in *Reticulitermes* spp. colonies in Japan was observed. Meanwhile, the infection rate and strength of *L. termitarius* and *A. gallica* were discussed with references to the effects of environmental factors at the collections sites. In a total of 63 colonies of *Reticulitermes* spp. were collected from seventeen locations (from Hokkaido Prefecture to Okinawa Prefecture) in Japan. Five hundred workers and twenty soldiers from each colony were examined individually to see the infection of *L. termitarius* and *A. gallica*. The survey showed that *L. termitarius* distributed in whole Japan and *A. gallica* had a little bit restricted distribution. The infection rate of workers of *Reticulitermes* spp. varied among all locations: 0.10 – 16.10% for *L. termitarius* and 0 – 66.40% for *A. gallica*. No infected soldiers were observed. The negative relationship between temperature and infection rate was speculated in both fungi. Rearing the colonies in the laboratory might result in the spreading of the fungi in the colonies. Both fungi grew on any body parts of the termites. The trials for isolation and cultivation of *Laboulbeniopsis termitarius* and *A. gallica* with eight media did not succeed under the laboratory conditions.

Acknowledgments

We thank Professor Junji Sugiyama and Professor Hiroyuki Yano of the Research Institute for Sustainable Humanosphere (RISH), Kyoto University for the use of the lab equipment during this study. We also thank Dr. Toru Miura (Hokkaido University), Dr. Yasuji Kurimoto (Akita Prefectual University), Dr. Kengo Mikame (Niigata University), Dr. Wakako Ohmura (Forestry and Forest Products Research Institute), Mr. Hiroshi Kurishaki (Toyama Prefectural Agriculture, Forestry and Fisheries Research Center), Mr. Takeshi Katada (Kaneko Construction Co.), Mr. Shigeru Hashimoto (Tokushima Agriculture, Forestry and Fisheries Technology Support Center), Mr. Masao Yamashima (Alpine Enterprise Co.) and Prof. Yoko Takematsu (Yamaguchi University) for providing us termite colonies. This work was financially supported in part by a Monbukagaku Sho (MEXT; Ministry of education, Culture, Sports, Science, and Technology) Scholarship and a Japan Society for the Promotion of Science (JSPS) Kakenhi grant, no. 15H04528.
References

[1] Hyodo F, Inoue T, Azuma JI, Tayasu I and Abe T 2000 Role of the mutualistic fungus in lignin degradation in the fungus-growing termite Macrotermes gilvus (Isoptera: Macrotermitinae). Soil Biology and Biochemistry 32 p 653–658

[2] Aanen DK, Hendrik H, Debets AJM, Kerstes NAG, Hoekstra RF and Boomsma J 2009 High symbiont relatedness stabilizes mutualistic cooperation in fungus-growing termites. Science 326 p 1103–06

[3] Matsuura K, Yashiro T, Shimizu K, Tatsumi S and Tamura T 2009 Cuckoo fungus mimics termite eggs by producing the cellulose digesting enzyme β-glucosidase. Curr. Biol. 19 p 30–36

[4] Rosengaus RB, Traniello JFA and Bulmer MS 2011 Ecology, behavior and evolution of disease resistance in termites ed DE Bignell et al. (Netherland: Springer Science Business Media BV) pp 165–191

[5] Grace JK and Zoberi MH 1992 Experimental evidence for transmission of Beauveria bassiana by Reticulitermes flavipes workers (Isoptera: Rhinotermitidae). Sociobiology 20 p 23–28

[6] Yoshimura T, Tsunoda K, Takahashi M and Katsuda Y 1992 Pathogenicity of an entomogenous fungus, Conidiobolus coronatus Tyrrell and Macleod, to Coptotermes formosanus Shiraki. Japanese Journal of Environmental Entomology and Zoology 4 p 11–16

[7] Yoshimura T and Takahashi M 1998 Termiticidal performance of an entomogenous fungus, Beauveria brongniartii (Saccardo) Petch in laboratory tests. Japanese Journal of Environmental Entomology and Zoology 9 p 16–22

[8] Strack BH 2000 Biological control of termites by the fungal entomopathogen Metarhizium anisopliae. MS Thesis, University of Toronto, Ontario, Canada

[9] Culliney TW and Grace JK 2000 Prospects for the biological control of subterranean termite (Isoptera: Rhinotermitidae), with special reference to Coptotermes formosanus. Bulletin of Entomological Research 90 p 9–21

[10] Chouvenc T, Su N-Y and Grace JK 2011 Fifty years of attempted biological control of termites: Analysis of a failure. Biol. Control 59 p 69–82

[11] Blackwell M and Rossi W 1986 Biogeography of fungal ectoparasites of termites. Mycotaxon 25 p 581–601

[12] Thaxter R 1920 On certain peculiar fungi-parasites of living insects. The Botanical Gazette 58 p 235–253

[13] Heim R and Buchli HHR 1951 Un champignon parasite du terme de Saintogne. Compt. Rend. Acad. Sci. 232 p 277–280

[14] Guswenrivo I, Sato H, Fujimoto I and Yoshimura T 2018 First record of the termite ectoparasite Laboulbeniopsis termitarius Thaxter in Japan. Mycoscience p 1–5

[15] Buchli HHR 1952 Antennopsis gallica, a new parasite on termites. Trans. IXth Int. Congr. Entomol. 1 p 519–524

[16] Gouger RJ and Kimbrough JW 1969 Antennopsis gallica Heim and Buchli (Hyphomycetes: Gloeohaustoriales), an entomogenous fungus on subterranean termites in Florida. J. Invert. Pathol. 13 p 223–228

[17] Myles TG, Strack BH and Forschler B 1998 Distribution and abundance of Antennopsis gallica (Hyphomycetes: Gloeohaustoriales), an ectoparasitic fungus, on the eastern subterranean termite in Canada. J. Invert. Pathol. 72 p 132–137

[18] Guswenrivo I, Sato H, Fujimoto I and Yoshimura T 2017 The first record of Antennopsis gallica Buchli and Heim, an ectoparasitic fungus on the termite Reticulitermes speratus (Kolbe) in Japan. Jpn. J. Environ. Entomol. 28 p 71–77

[19] Lenz M and Kimbrough JW 1982 Effect of species of termitaria (Termitariales, Deuteromycetes) on Australian termites (Isoptera). Botanical gazette 143 p 546–550
[20] Thorne BL and Kimbrough JW 1982. The impact of *Mattirorella crustose* (Termitariales, Deuteromycetes) on species of *Nasutitermes* (Isoptera: Termitidae) in Panama. *Mycologia* 1 p 242–249

[21] Buchli H 1960a L’effet du champignon parasite *Antennopsis gallica* sur les jeunes colonies de termites. *C. R. Hebd. Séances Acad. Science* 250 p 1320–31

[22] Buchli H 1960b Une nouvelle espèce de champignon parasite du genre *Antennopsis* Heim sur les termite de Madagascar. *C. R. Hebd. Séances Acad. Sci.* 250 p 3365–67

[23] Goettel MS and Inglis GD 1997 Fungi: Hyphomycetes in *Field manual of techniques in insect pathology* p 213–249

[24] Rouland-Lefèvre C 2000 Symbiosis with fungi ed T Abe et al. (Dordecht: Kluwer Academic) pp 289–306

[25] Guswenrivo I, Hideyuki N and Chow-Yang Lee 2013 Analysis of Cellulose and Nitrogen Content of Nest Materials of a Higher Termite *Globitermes sulphureus* (Haviland). *Proceeding of Pacific Rim Termite Research Group* 8, Bangkok, Thailand

[26] Kimbrough JW and Gouger RJ 1970 Structure and development of the fungus *Laboulbeniopsis terminarius*. *J. Invertebr. Pathol.* 16 p 205–213

[27] Blackwell M and Kimbrough JW 1976 Ultrastructure of the termite-associated fungus *Laboulbeniopsis terminarius*. *Mycologia* 68 p 541–550

[28] Blackwell M 1980 New records of termite-infesting fungi. *J. Invertebr. Pathol* 35: 101–104

[29] Rossi W and Cesari Rossi MG 1977 Sui funghi parassiti di termiti italiane. *G. Bot. Ital.* 111 p 165–170

[30] Blackwell M and Kimbrough JW 1978 *Hormiscioideus filamentous* gen. et. sp. nov., A termite-infesting fungus from Brazil. *Mycologia* 70 1275–80

[31] Boucias DG, Stokes C, Storey G and Pendland JC 1996 The effects of imidacloprid on the termite *Reticulitermes flavipes* and its interaction with mycopathogen *Beauveria bassiana*. *Pflanzenschutz-Nachr. Bayer* 49 p 103–144

[32] Shimizu S and Yamaji M 2003 Effect of density of the termite, *Reticulitermes speratus* Kolbe (Isoptera: Rhinotermitidae), on susceptibilities to *Metarhizium anisopliae*. *Jpn. J. Appl. Entomol. Zool.* 38 p 125–135

[33] Yanagawa A and Shimizu S 2005 Defense strategy of the termite, *Coptotermes formosanus* Shiraki to entomopathogenic fungi. *Jpn. J. Environ. Entomol. Zool.* 16 p 17–22

[34] Yanagawa A and Shimizu S 2007 Resistance of the termite, *Coptotermes formosanus* Shiraki to *Metarhizium anisopliae* due to grooming. *Biocontrol* 52 p 75–85

[35] Bignel DE, Rosin Y and Lo N 2011 Biology of termite: a modern synthesis. Springer, Berlin Tavares II (1985) Laboulbeniales (Fungi, Ascomycetes). *Mycologia Memoir* 9 p 1–627

[36] Bao LL and Yendol WG 1971 Infection of the eastern subterranean termite *Reticulitermes flavipes* (Kolar) with the fungus *Beauveria bassiana* (Balsamo) Vuill. *Biocontrol* 16 p 343–352

[37] Wraight SP, Carruthers RI, Bradley CA, Jaronski ST, Lacey LA, Wood P and Galaini-Wraight S 1998 Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii*. *J. Invertebr. Pathol.* 71 p 217–226

[38] Wraight SP, Carruthers RI, Jaronki ST, Bradley CA, Garza CJ and Galaini-Wraight S 2000 Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of silverleaf whitefly, *Bemisia argentifolii*. *Biological control* 17 p 103

[39] Benjamin RK 1971 Introduction and supplement to Roland Thaxter’s contribution towards a monograph of the Laboulbeniaceae. *Bibliotheca Mycologica* 30 p 1–55

[40] Tavares II 1985 Laboulbeniales (Fungi, Ascomycetes). *Mycologia Memoir* 9 p 1-627

[41] Kambara K, Ohmura W, Takematsu Y, Yanase Y and Yoshimura T 2017 *The basic knowledges of termite and fungi management* (in Japanese). *Japan Termite Control Association*
[42] Wraight SP, Inglis GD and Goettel MS 2007 Fungi in Field manual of techniques in invertebrate pathology 2nd edition ed. Lacey LA and Kaya HK (Netherland: Springer) p 223–247

[43] Goettel MS 1984 A simple method for mass culturing entomopathogenic Hyphomycete fungi. J. Microbiol. Methods 3 p 15–20