（ 続紙 1 ）

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論文題目	Termite Ectoparasitic Fungi in Japan: Distribution, Prevalence, and Molecular Detection（日本におけるシロアリ外部寄生菌：分布、感染率および分子生物学的検出法）		

（論文内容の要旨）

In the natural environment, subterranean termites live in soil with relatively high humidity. These conditions can expose termites to parasites such as fungi. The relationships between termites and fungi are generally divided into two categories: symbiotic mutualism and pathogenic relationships. Termite-fungus interaction is a topic that has attracted researchers for more than fifty years. Studies have explored both termite-fungus symbiotic mutualism and pathogenic relationships. Extensive research has been carried out to reveal the interaction between termite and fungi, either symbiotic or pathogenic interaction, but none of the researches has focused on ectoparasitic fungi.

There are 22 species of ectoparasitic fungi, obligate parasite to termites, of which *Laboulbeniopsis termitarius* and *Antennopsis gallica* are the most commonly found on termite cuticle. Ectoparasitic fungi have been reported to have the ability to reduce a termite's lifespan. However, the effects of ectoparasitic fungi on termite activity remain unclear due to their inability to grow under laboratory conditions. On the other hand, ectoparasitic fungi can be found in a wide distribution region, from tropical to temperate areas, but none of the reports has mentioned Japan.

Infection by the ectoparasitic fungi *L. termitarius* and *A. gallica* is commonly detected manually under a light microscope with several hundred termites are required when inspecting a colony. By utilizing DNA-based method, the detection of fungal infection would be faster and easier. Polymerase Chain Reaction (PCR) is the most promising method for detecting fungi infection due to its simplicity, specificity, and sensitivity. PCR-based methods targeting different genes have been described for identification of mycotoxigenic fungi and even molecular variations in insect pathogenic fungi.

This study aimed to find *L. termitarius* and *A. gallica* species associated with *Reticulitermes* termites, which are widely distributed in Japan. Furthermore, the PCR-based assay to detect termite-associated ectoparasitic fungi was developed for effective detection of the ectoparasitic fungi together with trials for cultivation of the fungi in the laboratory. Furthermore, as termite colonies are often infected by multiple ectoparasitic fungi, a multiplex PCR assay was further designed to allow simultaneous detection of *L. termitarius* and *A. gallica*.

Chapter 1 introduced the background, objective and outline of the study. Furthermore, the chapter also explained about the ectoparasitic fungi on termite, the fungi distribution status, and short description of DNA-based methods developed for fungi detection.

Chapter 2 described the finding of *L. termitarius* from the body surface of *R. speratus*, collected in Uji, Kyoto Prefecture, Japan. This is the first record of this fungus from Japan. Three to eighteen termite workers from 500 individuals were infected among the eight colonies investigated. From our results, several hundred termite are required for the survey of this ectoparasitic fungus. The temperature effect on the relationship between the infection rate and the host activity was discussed.

Furthermore, the discovering of another ectoparasitic fungus *A. gallica* from *R. speratus* colonies collected from Uji, Kyoto Prefecture, was described in Chapter 3. This is the first record in Japan of this species. The infection rate was 17.8–25.0% in workers and 0–10% in soldiers. On average, five thalli were found per individual termite. The fungus grew on all the surface of the termites and showed no preference for particular body parts.

Chapter 4 discussed the distribution of *L. termitarius* and *A. gallica* in *Reticulitermes* spp. colonies in Japan. Meanwhile, the infection rate and strength of *L. termitarius* and *A. gallica* were discussed with references to effects of environmental factors at the collections sites. A total of 63 colonies of *Reticulitermes* spp. were collected from seventeen locations (from Hokkaido Prefecture to Okinawa Prefecture) around Japan. The survey suggested that *L. termitarius* distributed in whole area of Japan and that *A. gallica* had a little bit restricted distribution. The infection rate of workers of *Reticulitermes* spp. varied among all locations: 0.1–16.1% for *L. termitarius* and 0–66.4% for *A. gallica*. No infected soldiers were observed in both fungi. 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. The both fungi were observed on any body parts of the termites. The trials for isolation and cultivation of *L. termitarius* and *A. gallica* with eight media did not succeed under the laboratory conditions.

Visual observation under a dissecting microscope is a common method for screening such fungi, it generally requires a large number of termites and is thus very time consuming. Therefore, in Chapter 5, we developed a fast, efficient protocol to detect fungi infection on the termite *R. speratus*. Species-specific primers were designed based on sequence data, and amplified using a number of universal fungi primer pairs that targeted partial sequences of the 18s rRNA gene of the two fungi. To detect these fungi in a robust yet economic manner, a multiplex nested PCR assay using species-specific primers was then developed. The results suggested that both fungi were successfully detected, even in cases where *L. termitarius* was at low titer (e.g., a single thallus per termite). The new

method described here is recommended for future surveys of these two fungi, as it is more sensitive, species-specific, and faster than visual observation, and is likely to facilitate a better understanding of these fungi and their dynamics in host populations.

In the last chapter, Chapter 6, the general conclusion was provided to summarize all the results and findings in this study.

Overall, the two fungi *L. termitarius* and *A. gallica* were found in *Reticulitermes* termites in Japan as the first records. They were distributed almost all through the country and only workers were infected. The fungi did not have any preferences to the body parts of termites, being found in all the body surface of termites. Unfortunately, the artificial cultivation of the fungi with a variety of media did not succeed under the laboratory conditions. A multiplex nested PCR assay using species-specific primers was developed, and the assay was likely to facilitate a better understanding of these fungi and their dynamics in host populations.

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（続紙 ２ ）

（論文審査の結果の要旨）

世界的な環境意識の高まりおよび住宅における省エネルギー化の進展は、シロアリ防除におけるレス・ケミカル化、ケミカル・フリー化の流れを加速しつつある。昆虫寄生菌を用いたシロアリの生物的防除は過去50年以上に渡って研究が行われてきたが、近年再注目を集めつつある。しかしながら、これらの研究は全て昆虫の体内へ発芽・侵入する内部寄生菌を対象としており、外部寄生菌を用いたシロアリ防除についてはこれまで検討されていない。本論文は、シロアリ外部寄生菌を用いたシロアリ防除法の開発を最終目的とし、その基礎的検討として日本におけるシロアリ外部寄生菌の分布および感染率等の詳細な調査を実施するとともに、その新規検出法として分子生物学的方法を提案したものであり、特に評価すべき点として以下の３点を挙げることができる。

１．日本に広く分布するヤマトシロアリ職蟻から、*Laboulbeniopsis termitarius* および*Antennopsis gallica*という世界的に広く報告されているシロアリ外部寄生菌を日本で初めて記載し、これら２種の世界での分布について新たな知見を加えた。また、得られた感染率の低さから、これら菌類の調査には最低数百頭のシロアリ個体が必要であることを明らかにした。

２．北海道から沖縄までの17地点から得た計63個のヤマトシロアリ属シロアリコロニーを用い、両種の感染の有無、感染率、および体表部位毎の菌体数を詳細に調査し、前者が日本全土に広く分布すること、後者が一部分布していない地域があること、コロニーによって感染率に大きな差（0.1～66.4%）があること、そして体表の全部位に広く存在していることを示した。これらの菌を用いたシロアリ防除システムを構築する上で非常に貴重なデータである。

３．数百頭のシロアリの体表を顕微鏡で詳細に観察することは困難であることから、分子生物学的な検出法の開発に取り組み、種特異的なプライマーを開発することにより、これら２種を同時に検出することのできる新たな方法を提案した。感染の迅速な判定など、今後の応用面への大きな貢献が期待される成果である。

以上のように、本論文は２種のシロアリ外部寄生菌を日本で初めて記載するとともに、北海道から沖縄までの17地点から採集したヤマトシロアリ属シロアリにおける感染の有無および感染率を詳細に調査し、さらに、これら２種の検出法として種特異的なプライマーを用いた分子生物学的な方法を開発したものであり、菌類生態学、昆虫生理学、および木材保存学の発展に寄与するところが大きい。

よって、本論文は博士（農学）の学位論文として価値あるものと認める。

なお、平成３０年２月１４日、論文並びにそれに関連した分野にわたり試問した結果、博士（農学）の学位を授与される学力が十分あるものと認めた。

注）論文内容の要旨、審査の結果の要旨及び学位論文は、本学学術情報リポジトリに掲載し、公表とする。

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要旨公開可能日：　　年　　月　　日以降（学位授与日から３ヶ月以内）