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Issue Date	2026-10-27
Doc URL	https://hdl.handle.net/2115/100601
Type	dataset
File Information	Kakui_Beauveria.pdf

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White fungal growth on a captive individual of *Ognevia longipennis* (Shiraki, 1910) (Insecta: Orthoptera) from Sapporo, Hokkaido, Japan

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Abstract

I report a case of conspicuous white fungal growth on a captive individual of *Ognevia longipennis* (Shiraki, 1910), collected in Kiyota-ku, Sapporo, Hokkaido, Japan on 6 October 2026. The individual was last observed alive on 15 October and was found dead on 16 October, with white fungal growth emerging from the intersegmental regions of its body and from between the articles of its appendages. The 18S rRNA gene and the region spanning ITS1, 5.8S rRNA, ITS2, and part of the 28S rRNA gene were amplified and sequenced separately, yielding a contiguous sequence of 2294 bp. Sequence analysis indicated that the fungus belongs to the *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin species complex, a group of entomopathogenic fungi reported from a wide range of insect hosts.

Key words: DNA barcoding, entomopathogens, entomopathogenic fungi, grasshopper, insects

Introduction

Entomopathogenic fungi are fungi and allied organisms that infect insects, cause disease, and may kill their hosts (Araujo & Hughes, 2016). Infections have been documented in insects belonging to 18 of the 28 extant insect orders (cf. Gullan & Cranston, 2014; Araujo & Hughes, 2016). Orthoptera is one of these orders, and entomopathogenic fungi belonging to three groups—Microsporidia, Entomophthoromycota, and Ascomycota—have been reported from orthopterans (Araujo & Hughes, 2016). Here, I report conspicuous white fungal growth on a captive individual of *Ognevia longipennis* (Shiraki, 1910) (Orthoptera), which had been collected and reared in Hokkaido, Japan.

Material and Methods

An individual of *O. longipennis* was collected from a grassland beside the Yamabe River in Kiyota-ku, Sapporo, Hokkaido, Japan, on 6 October 2026. The individual was kept in an insect cage lined with slightly moistened dried sphagnum moss and fed *Petasites* leaves. The individual was alive when checked during food replacement on the evening of 15 October but was found dead during

food replacement on the evening of 16 October. At that time, white fungal growth was observed in the intersegmental regions of the body and appendages. Photographs were taken on 17 October. After photography, a portion of the fungal growth was collected into an Eppendorf tube using a flame-sterilized needle for DNA extraction. The host specimen and the remaining fungal material were stored in a tube with silica gel.

DNA was extracted using the NucleoSpin Tissue XS Kit (Macherey–Nagel, Germany). During extraction, the sample was homogenized using a homogenizer after addition of T1 buffer and Proteinase K. The 18S rRNA gene was amplified by PCR using the primer pair SR1 and SR12 (Nakayama et al., 1996). The primers 18S-b3F, 18S-b4F, 18S-b5F, and 18S-b8F (Kakui et al., 2011, 2021; Kakui & Shimada, 2017) were used for cycle sequencing. The region spanning the 3′ end of the 18S rRNA gene, ITS1, the 5.8S rRNA gene, ITS2, and the 5′ end of the 28S rRNA gene (hereafter referred to as the "ITS-containing region") was amplified and sequenced using the primer pair M1780F and ITS4 (White et al., 1990; Galaktionov et al., 2012). PCR was performed using KOD ONE PCR Master Mix (Toyobo, Japan) for 45 cycles, with denaturation at 98°C for 10 s, annealing at 60°C for the 18S region or 55°C for the ITS-containing region for 5 s, and extension at 68°C for 5 s for the 18S region or 1 s for the ITS cluster. Cycle sequencing was performed using the QuantumDye Terminator Cycle Sequencing Kit ver. 3.1 (Quintara Biosciences, USA) and a SeqStudio Genetic Analyzer (Thermo Fisher Scientific, USA). Sequence fragments were assembled using MEGA7 (Kumar et al., 2016). The separately obtained 18S rRNA gene and ITS-containing region sequences were assembled into a contiguous sequence of 2294 bp spanning the 18S rRNA gene, ITS1, the 5.8S rRNA gene, ITS2, and part of the 28S rRNA gene. The resulting nucleotide sequences were deposited in the International Nucleotide Sequence Database (INSD) under accession number LC959024.

The 18S rRNA gene and ITS-containing region sequences were compared with sequences in the INSD using BLAST (Altschul et al., 1990). For phylogenetic analysis, the ITS-containing region sequence obtained in this study was analyzed together with homologous sequences from closely related taxa identified through the BLAST search. The sequences were aligned using MUSCLE (Edgar, 2004), and a neighbor-joining (NJ) tree was constructed using MEGA7. Node support was assessed using 1000 bootstrap replicates.

Results and Discussion

The white fungal growth observed on *O. longipennis* (Fig. 1) was identified as belonging to the ascomycete genus *Beauveria* based on sequence similarity searches using the 18S rRNA gene and ITS-containing region sequences. Phylogenetic analysis based on the ITS-containing region sequence obtained from the present sample and sequences determined from type strains of 17 *Beauveria* species (accession numbers NR_077147, NR_111594–NR_111602, NR_111678,

NR_136979, NR_151832, NR_158356, NR_200316, NR_202618, and NR_202619) placed the fungus within the *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin species complex (Fig. 2). The *B. bassiana* species complex is widely distributed worldwide and has been detected in various environments, including on plants and in soil. Infections attributed to the *B. bassiana* species complex have been reported in more than 700 insect species belonging to 14 orders (Zimmermann, 2007), indicating that this species complex has a very broad host range. Although two new species have recently been described within this species complex using genomic data (Kobmoo et al., 2021), it remains unclear which species the present sample represents.

To my knowledge, the only published records of *Beauveria* infecting orthopterans in Hokkaido are those reported by Yamagishi et al. (2018), who documented infections in *Podisma sapporensis* (Shiraki, 1910) collected from Mt. Teine and Tenpoku Pass, Hokkaido. The present observation adds a record of *Beauveria* associated with another orthopteran species in Hokkaido. Given the broad host range of the *B. bassiana* species complex, further records from other orthopteran species in Hokkaido may be obtained in future studies.

Acknowledgments

I thank my family for assisting with the collection of the specimen.

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Suggested citation

Keiichi Kakui (2026) White fungal growth on a captive individual of *Ognevia longipennis* (Shiraki, 1910) (Insecta: Orthoptera) from Sapporo, Hokkaido, Japan. figshare.
https://doi.org/10.6084/m9.figshare.34764579

Figures



Fig. 1. White fungal growth on a captive individual of *Ognevia longipennis*, left (A) and dorsal (B) views.

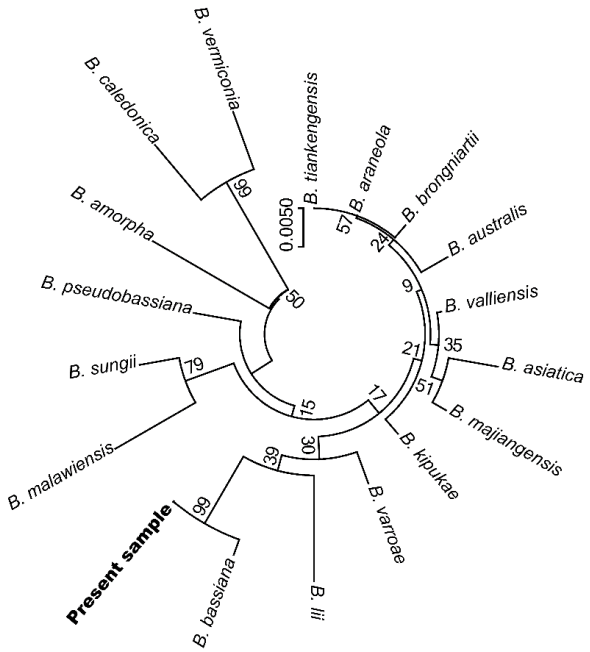


Fig. 2. NJ tree for *Beauveria* from ITS sequences; numbers near nodes are bootstrap values (%).

北海道で採集・飼育したハネナガフキバッタに発生した白色菌類

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摘要

2026 年 10 月に北海道札幌市清田区で採集，飼育していたハネナガフキバッタから白色菌類の発生が確認された．試料から 18S–ITS1–5.8S–ITS2–28S 領域の塩基配列（2294 塩基対）を決定し，相同性検索および系統解析を行ったところ，確認された菌類は，非常に広い宿主範囲を示す昆虫病原糸状菌である，*Beauveria bassiana* (Balsamo-Crivelli) Vuillemin 種複合体であると判断された．筆者の知る限り，本報告は，北海道産バッタ目昆虫からの *Beauveria bassiana* 種複合体の報告として，サッポロフキバッタに続く 2 例目である．

キーワード

DNA バーコーディング，昆虫病原糸状菌，バッタ，昆虫

はじめに

昆虫病原糸状菌 entomopathogenic fungi は，昆虫に感染し宿主を死に至らしめることのある菌類および菌類に近縁な生物群である（Araujo & Hughes, 2016）．現生 28 目（cf. Gullan & Cranston, 2014）のうち 18 目の昆虫への感染が確認されている（Araujo & Hughes, 2016）．その一つであるバッタ目からは微胞子虫類 Microsporidia，ハエカビ類 Entomophthoromycota，子嚢菌類 Ascomycota の 3 群に属する昆虫病原糸状菌類の感染例が報告されている（Araujo & Hughes, 2016）．今回，北海道で採集，飼育していたバッタ目の一種ハネナガフキバッタ *Ognevia longipennis* (Shiraki, 1910) から，白色菌類の発生がみられたので報告する．

材料と方法

ハネナガフキバッタは 2026 年 10 月 6 日に，札幌市清田区山部川脇の草地で採集した．同個体はやや湿らせた乾燥水苔を敷いた虫かごに入れ，フキの葉を餌として与え飼育を行った．10 月 15 日夕方の餌替え時には生体が確認できた．10 月 16 日夕方の餌替え時に死亡しているのを確認した．その時点で体節や付属肢の節間などに白色菌類の発生が確認された．10 月 17 日に写真撮影を行った．写真撮影後，白色菌類の一部を火炎滅菌した針を

用いてエッペンドルフチューブに回収し，DNA 抽出に供した．宿主及び残りの菌類については，シリカゲルとともにチューブに入れて保管した．

DNA 抽出は，NucleoSpin Tissue XS Kit（Macherey–Nagel，ドイツ）を用いて行った．抽出処理に際して，T1 バッファーと Proteinase K を投入後，ホモゲナイザーを用いてホモゲナイズを行った．18S rRNA 遺伝子の PCR には SR1 と SR12 のプライマーペア（Nakayama et al., 1996）を用い，サイクルシーケンシングには 18S-b3F，18S-b4F，18S-b5F，18S-b8F（Kakui et al., 2011, 2021; Kakui & Shimada, 2017）を用いた．18S–ITS1–5.8S–ITS2–28S 領域（以降 ITS と呼ぶ）の PCR とサイクルシーケンシングには M1780F と ITS4 のプライマーペア（White et al., 1990; Galaktionov et al., 2012)を用いた．PCR は KOD ONE PCR Master Mix（東洋紡，日本）を用いて 98℃10 秒，60℃（18S）または 55℃（ITS）5 秒，68℃5 秒（18S）または 1 秒（ITS）を 45 サイクルという条件で実施した．サイクルシーケンシングには QuantumDye Terminator Cycle Sequencing Kit ver. 3.1（Quintara Biosciences，アメリカ）と SeqStudio Genetic Analyzer（Thermo Fisher Scientific，アメリカ）を用いた．得られた塩基配列断片の結合には MEGA7（Kumar et al., 2016）を用いた．決定した 2294 塩基対の塩基配列は国際塩基配列データベース（INSD）に登録した（登録番号 LC959024）．

決定した 18S + ITS 配列は，ITS 配列の BLAST（Altschul et al., 1990）での相同性検索により近縁だと判断された種の ITS 配列とともに MUSCLE（Edgar, 2004）で整列，MEGA7 を用いて近隣結合法による統樹（NJ 系統樹）を作成した．1000 回反復のブートストラップ法を用いて樹形の信頼性を評価した．

結果と考察

ハネナガフキバッタに発生した白色菌類（図 1）は，18S，ITS の塩基配列の相同性検索の結果，子嚢菌類のボーベリア属 *Beauveria* に属する昆虫病原糸状菌であると判断された．また今回の試料から決定した ITS 配列と，*Beauveria* の 17 種のタイプ株から決定された ITS 配列（アクセション番号 NR_077147, NR_111594–NR_111602, NR_111678, NR_136979, NR_151832, NR_158356, NR_200316, NR_202618, NR_202619）に基づく系統解析の結果，今回の試料は *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin, 1912 種複合体であると判断された（図 2）．*Beauveria bassiana* 種複合体は世界中の植物体上や土壌中を含めた様々な環境から検出される普遍的な菌類で，14 目 700 種を超える昆虫への感染が報告されている（Zimmermann, 2007），非常に広い宿主範囲を示す菌類である．近年ゲノムデータを活用して本種複合体から 2 新種が記載されているが（Kobmoo et al., 2021），今回の試料がいずれの種に該当するかは定かでない．

北海道におけるバッタ目からの *Beauveria* の公開された記録報告は，筆者の知る限り，山岸ら (2018) による北海道手稲山・天北峠にて採集されたサッポロフキバッタ *Podisma sapporensis* (Shiraki, 1910) への感染報告に限られる．本種複合体が広い宿主範囲を示すことからは，今後，他のバッタ目昆虫からの感染報告も予想される．

謝辞

ハネナガフキバッタの採集に協力いただいた家族に感謝申し上げる．

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引用例

角井敬知 (2026) 北海道で採集・飼育したハネナガフキバッタに発生した白色菌類. figshare. https://doi.org/10.6084/m9.figshare.34764579

図版



図 1. 白色菌類が発生したハネナガフキバッタ．A, 左側面図．B, 背面図.

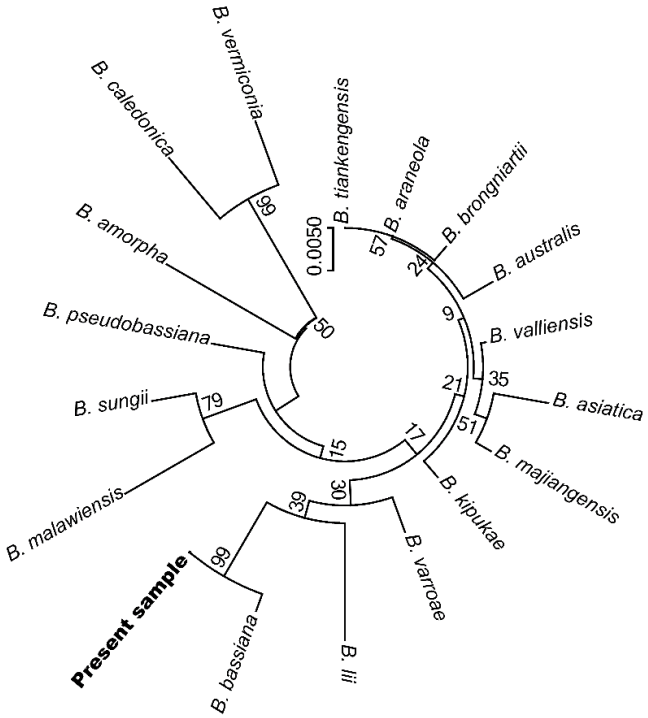


図 2. ITS 配列に基づく *Beauveria* の NJ 系統樹．節近傍の値はブートストラップ値（%）.