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Research Article

Three new records of entomopathogenic fungi infecting the invasive Sineguelas leaf beetle, *Podontia quatuordecimpunctata* (L.) (Chrysomelidae: Galerucinae: Alticini), in the Philippines

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ABSTRACT: The Sineguelas Leaf Beetle (SLB), *Podontia quatuordecimpunctata* (L., 1767*)*, (Chrysomelidae: Galerucinae: Alticini), is a highly destructive invasive and pest of *Spondias purpurea* L., 1762 (Anacardiaceae) in the Philippines. This study reports the first identification of three indigenous entomopathogenic fungi, *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912), *Metarhizium anisopliae* (Metschn.) Sorokin, 1883, and *Aspergillus pseudonomius* Varga, Samson and Frisvad (2011), infecting different life stages of SLB. DNA barcoding confirmed the fungal identities. These pathogens were isolated from SLB populations in Batangas City and Puerto Princesa City, Philippines. The identified fungi show promise for sustainable SLB management. *Beauveria bassiana* exhibited broad-spectrum efficacy against SLB adults and pupae, while *M. anisopliae* caused distinctive necrotic lesions. *Aspergillus pseudonomius* was found infecting SLB eggs. Further research is needed to evaluate the biocontrol potential of these fungi, optimise their application, and address potential contamination risks. Collaborative efforts are essential for developing and implementing integrated pest management strategies incorporating these biological control agents.

KEYWORDS: *Aspergillus pseudonomius*, *Beauveria bassiana*, biological control, invasive species, *Metarhizium anisopliae*

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INTRODUCTION

Podontia quatuordecimpunctata (L., 1767) (Chrysomelidae: Galerucinae: Alticini), commonly known as the sineguelas leaf beetle (SLB), is an invasive flea beetle that poses a significant threat to *S. purpurea* L., 1762 (Anacardiaceae) in the Philippines (Adorada *et al.*, 2023; Calcetas *et al.*, 2023). This pest extensively damages and defoliates its host plant, resulting in substantial economic losses for fruit farmers. In addition to *S. purpurea* (red sineguelas), SLB also infests *S. dulcis* Parkinson, 1773 (yellow sineguelas) and *S. pinnata* (L. f.) Kurz, 1875 in the country (Adorada *et al.*, 2023; Calcetas *et al.*, 2023).

Since its introduction in the Philippines in 2008-2009 and its emergence in 2016, SLB populations have rapidly expanded, causing up to 70% yield reduction in affected areas such as Batangas City (Adorada *et al.,* 2023). Current

management practices heavily rely on applying costly chemical insecticides, primarily Cypermethrin, to control the pest (Adorada *et al.*, 2023). To develop more sustainable and economical pest management strategies, we conducted ongoing monitoring of SLB populations and identified individuals infected with various fungal entomopathogens.

Entomopathogenic Fungi (EPF) have emerged as environmentally benign alternatives to synthetic pesticides for insect control (Burges, 1981). Their potential is underscored by the substantial crop losses attributed to plant pathogens and pests globally, with estimates reaching 21.5% for wheat, 30.0% for rice, 22.5% for maize, 17.2% for potato, and 21.4% for soybean (Savary *et al.*, 2019). Among the most promising EPF genera are *Beauveria*, *Metarhizium*, and *Isaria*, with *B. bassiana* and *M. anisopliae* demonstrating efficacy against over 200 insect species worldwide (Li, 1988; Shah *et al.*, 2022). Under favourable conditions of moderate temperature and high humidity, EPF germinates, penetrates the insect cuticle through enzymatic and mechanical means, and invades the host. Within the insect, the fungus proliferates, forming hyphal bodies, and ultimately emerges to produce conidia for subsequent infections (Sabbahi *et al.*, 2022).

In the Philippines, *M. anisopliae* and *B. bassiana* are the predominant EPFs employed. *M. robertsii* (synonymous with *M. anisopliae*) (Sorokin, 1883) (Hypocreales: Clavicipitaceae) has been successfully used against various insect pests, including the rice brown plant hopper, coconut hispine beetles, corn borer, locust, papaya red spider mite, corn and onion armyworms, rice black bug, and coconut rhinoceros beetle (Table 1). Conversely, *B. bassiana* (Bals. -Criv.) Vuill. (1912) (Hypocreales: Cordycipitaceae) has shown efficacy against rice hoppers, corn borer, fall armyworm, rice oriental armyworm, and rice bug (Table 2).

This paper reports the identification and characterization of three novel entomopathogenic fungi isolated from different life stages of the invasive Sineguelas Leaf Beetle (SLB), *Podontia quatuordecimpunctata*, in the Philippines using morphological and molecular techniques.

MATERIALS AND METHODS

The SLB is reported from red sineguelas (*Spondias purpurea* L.), golden apple or yellow sineguelas (*S. dulcis* Forst.), and hog plum or "libas" [*S. pinnata* (L.f.) Kurz.] in the Philippines (Adorada *et al.,* 2023). A two-year monitoring program involving weekly checks was conducted from 2021 to 2023 to assess the natural enemies, including predators, parasites, parasitoids, and entomopathogens, associated with the SLB. Concurrently, the biology of both the host plant (Calcetas *et al.*, 2023) and the beetle (Calcetas *et al.*, 2023) were investigated to provide a comprehensive understanding of the pest's ecology and inform subsequent experimental design for identifying potential fungal biocontrol agents.

Discovery and collection of infected specimens

Weekly field surveys were conducted in Batangas City from 2021 to 2023 to collect SLB individuals exhibiting signs of entomopathogens or attacks from parasitoids. Pupal samples were also destructively collected from the base of host trees and examined under a stereomicroscope for the presence of pathogens. Additionally, collected egg masses were regularly inspected.

Infected SLB specimens were aseptically transferred to sterilized glass vials, labelled, and submitted for molecular identification. This sampling methodology was replicated across various Philippine regions, including Southern Luzon (Batangas, Laguna, Cavite, Quezon), Central Luzon (Pampanga, Pangasinan, Nueva Ecija), Northern Luzon (Ilocos Sur, Ilocos Norte), Island Luzon (Occidental Mindoro, Palawan), Central Visayas (Iloilo, Guimaras Island, Negros Oriental), and Mindanao (Cagayan de Oro, Bukidnon). Detailed field observations were conducted at each location, including specimen counts, photography, and data recording.

Identification of entomopathogens of SLB

Fungal specimens were submitted to experts at the University of the Philippines Los Baños for morphological and molecular identification at the species level. Pure cultures of the identified fungi were deposited at the Insect Pathology and Molecular Biology Laboratory, Institute of Weed Science, Ecology and Plant Physiology (IWEP), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños, Laguna; the Center for Natural Sciences and Environmental Research (CENSER), De La Salle University, Sta. Rosa City, Laguna; and the Department of Agriculture, Regional Crop Protection Center-IVA, Marawoy, Lipa City, Batangas.

Insect cadavers or diseased insects exhibiting visible mycelial growth were aseptically sampled. Fungal structures were excised and inoculated onto fresh acidified Potato Dextrose Agar (PDA) plates containing 1-2 drops of 25% lactic acid to suppress bacterial contamination. Plates were incubated at room temperature and monitored daily for fungal growth. Pure cultures were obtained through subculturing and subsequently characterized using Humber's (2012) dichotomous key. Putative entomopathogenic isolates were selected for further identification.

Molecular analysis and sequence analysis

Putative entomopathogenic fungal isolates were cultured in PDB at 28°C and 100 rpm for 48 h to obtain sufficient biomass. Genomic DNA extraction of each isolate

Table 1. Insect pest species hosts *Metarhizium anisopliae* (Metsch.) Sorokin, 1883, in the Philippines

Insect Host	Common Name	Order	Family	References
Brontispa longgissima (Gestro, 1885)	Coconut Hispine Beetle	Coleoptera	Cassidinae	Jun Concibido PCA 2024, Unpublished Report
Helopeltis bakeri (Poppius, 1915)	Cacao Mirid Bug	Hemiptera	Miridae	Albacete *et al*., 2023
Locusta migratoria (L., 1758)	Oriental Migratory Locust	Orthoptera	Acrididae	Santiago *et al*., 2001
Nilaparvata lugens (Stål, 1854)	Brown Plant Hopper	Hemiptera	Delphacidae	Rombach *et al*., 1986
Oryctes rhinoceros (L., 1758)	Coconut Rhinoceros Beetle	Coleoptera	Scarabaeidae	Subhathma *et al*., 2013; Jun Concibido PCA 2024, Unpublished Report
Ostrinia furnacalis (Guenee, 1854)	Corn Borer	Lepidoptera	Noctuidae	Santiago and Gabriel 2000
Plesispa reichei Chapius 1875	Hispine Beetle of Coconut	Coleoptera	Cassidinae	Gallego and Gallego 1988
Promecotheca cumingii Baly, 1858	Hispine Beetle of Coconut	Coleoptera	Cassidinae	Gallego and Gallego 1988
Scotinophara sorsogonensis Barrion, Joshi, and Sebastian, 2007	Rice Black Bug	Hemiptera	Pentatomidae	Soriano and Adion 2023; DA-RCPC-IVA 2024, Unpublished Report
Spodoptera exigua (Hubner, 1808)	Onion Armyworm	Lepidoptera	Noctuidae	Montecalvo and Navasero 2021b; 2022
Spodoptera frugiperda (J.E. Smith, 1797)	Fall Armyworm	Lepidoptera	Noctuidae	Montecalvo and Navasero 2021a; 2022; 2023
Tetranychus kanzawai (Kishida)	Red Spider Mite	Acarina	Tetranychidae	Sanjaya *et al*., 2013
Tirathaba rufivena (Busck, 1916)	Coconut Spike Moth	Lepidoptera	Pyralidae	Gallego and Gallego 1988

Table 2. Insect pest species hosts of *B. bassiana* (Bals. -Criv.) Vuill., (1912), in the Philippines

Insect Host	Common name	Order	Family	References
Leptocorisa oratorius (F., 1764)	Rice Bug	Hemiptera	Alydidae	DA-RCPC-IVA 2024, unpublished report
Mythimna separata Walker, 1865	Oriental Armyworm	Lepidoptera	Noctuidae	Montecalvo and Navasero, 2023
Nephotettix virescens (Distant, 1908)	Green Leaf Hopper	Hemiptera	Cicadelidae	Aguda *et al.,* 1984
Nilaparvata lugens (Stål, 1854)	Brown Plant Hopper	Hemiptera	Delphacidae	Aguda *et al*., 1984; Aguda *et al*., 1987
Ostrinia furnacalis (Guenee, 1854)	Corn Borer	Lepidoptera	Noctuidae	Nicolas *et al.,* 2013
Sogatella furcifera (Horvath, 1899)	White Backed Plant Hopper	Hemiptera	Delphacidae	Aguda *et al*., 1984
Spodoptera frugiperda (J.E. Smith, 1797)	Fall Armyworm	Lepidoptera	Noctuidae	Montecalvo and Navasero, 2021

was performed using the Jena Bioscience Animal and Fungi DNA extraction kit (Jena Bioscience, Germany) according to the manufacturer's instructions.

To confirm species identity, the Internal Transcribed Spacer (ITS) region, a universal DNA barcode, was amplified and sequenced. PCR amplification utilizes the primer pair ITS 4 (5'- TCC TCC GCT TAT TGA TAT GC-3') and ITS 5 (5'- GGA AGT AAA AGT CGT AAC AAG G- 3') with 2x Taq Mastermix (Vivantis). The cycling conditions included an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation (94°C for 1 min 30 s), annealing

(55°C for 2 min), and extension (72°C for 3 min), with a final extension step at 72°C for 5 min.

Agarose gel electrophoresis verified successful PCR amplification. Amplicons with the expected band size were submitted to Macrogen, Inc. (through Kinovett Scientific Solutions Co.) for the Sanger nucleotide sequencing process. The resulting sequences were individually edited using BioEdit software. BLASTn searches against the NCBI GenBank database were performed to identify the fungal isolates based on sequence similarity.

Image acquisition

Colour images capturing the fungal entomopathogens were obtained using a Nikon®-D7100 DSLR camera (24.3 megapixels) equipped with a Nikon® Micro 105 mm 1:2.8G lens mounted on a microscope arm track stand. Time-lapse photography was employed, with the camera remotely controlled via a Mac® computer using Helicon Remote® software. Light-Emitting Diode (LED) ring lights and bulbs, diffused by a Pixco® camera flash diffuser, provided illumination. Captured images were tethered to Helicon Remote® (ver. 3.9.12 M) for live view and subsequently processed using Helicon Focus® (ver. 7.7.4) for image stacking. Final image adjustments, including cropping and blemish removal, were performed using Windows-based Adobe Photoshop Elements 2020®, and images were saved in TIFF format.

RESULTS AND DISCUSSIONS

This study discovered three fungal entomopathogens infecting SLB in the Philippines: *B. bassiana* strain Pq, *M. anisopliae*, and *Aspergillus pseudonomius*. Below, we present findings on these pathogens, including their biological characteristics and potential as biocontrol agents for SLB.

White muscardine fungus, *B. bassiana* strain Pq (Batangas City, Puerto Princesa City)

Beauveria bassiana (Bals.) Vuill. (Hypocreales: anamorphic Clavicipitaceae) is a soilborne, facultative necrotrophic fungus renowned for causing the "white muscardine" disease in insects (Rehner *et al*., 2011). Historically significant as the first documented fungal pathogen of animals (silkworm, 1835), *B. bassiana* has been utilized as a medicine in Japan for over a millennium (Ignoffo, 1973). This fungus produces bioactive compounds, including cyclodepsipeptides and beauvericin, which contribute to its insecticidal properties (Miller *et al.*, 1983). While the teleomorph stage, *Cordyceps bassiana*, has been reported, its distribution is primarily restricted to Eastern Asia (Li *et al.*, 2001).

For biological control, *B. bassiana* is typically applied as an inundative spray, delivering high concentrations of aerial conidia in dry or liquid formulations (Mascarin & Jaronski, 2016). Notably, the fungus poses minimal risk to non-target insects and mammals, including humans (Zimmermann, 2007). A previous study in Bangladesh demonstrated the efficacy of a microbial pesticide formulation containing *M. anisopliae*, *Trichoderma harzianum* Rifai, *B. bassiana*, and *T. viride* Pers. (Lycomax, Russell IPM) against SLB. When applied as a drench to hog plum, this formulation resulted in 75.00% larval, 72.22% adult, and 51.85% pupal mortality (Rahman *et al.*, 2022).

Two strains of the white muscardine fungus (*B. bassiana* strain Pq) were isolated from adult SLBs collected in San Miguel, Batangas City (isolate 9.1) (Figures 1A-1C), and Brookes Point, Palawan (isolate 7.2.2) Isolate 9.1, obtained from a pupa collected in Batangas, exhibited a cottony colony morphology, while isolate 7.2.2, isolated from adults and pupae in Puerto Princesa City, Palawan, displayed a powdery appearance (Figures 2A and 2B). Molecular identification based on ITS sequencing confirmed both isolates as *B. bassiana* with 100% nucleotide identity to GenBank accession number MT586632.1 (Table 3). The nucleotide sequences of *B. bassiana* isolates 7.2.2 and 9.1 were uploaded in the GenBank under accession numbers OR426353 and OR426354, respectively (Table 3).

Larval symptoms of fungal infection

Earlier observations during SLB life history studies (Calcetas *et al.*, 2023) revealed an atypical larval individual that showed prolonged development (16-19 days), followed by death and later fungal growth on the mouthparts. While this case was excluded from the original life table analysis due to its outlier status, it provided preliminary evidence of potential fungal infection. Healthy, voracious SLB larvae typically display rapid growth and produce substantial fecal matter. Conversely, larvae exhibiting signs of ill health or reduced feeding activity tend to have thinner, less voluminous fecal pellets.

Pupal stage and fungal infection

The pupal stage of SLB was frequently observed to be infected by *B. bassiana* (Figure 2B). Random sampling of pupae revealed a mortality rate of 60% (n=12), likely attributed to an unidentified parasitic fungus. An additional 20% (n=4) of pupae were infected with *B. bassiana*, while only 20% (n=4) successfully emerged as adults. The earthen pupal chamber offers protection against various threats, including predators, parasitoids, adverse weather conditions, and microbial pathogens. This protective structure likely contributes to the overall survival of SLB and is considered an adaptive trait within the flea beetle lineage (Prathapan & Chaboo, 2011).

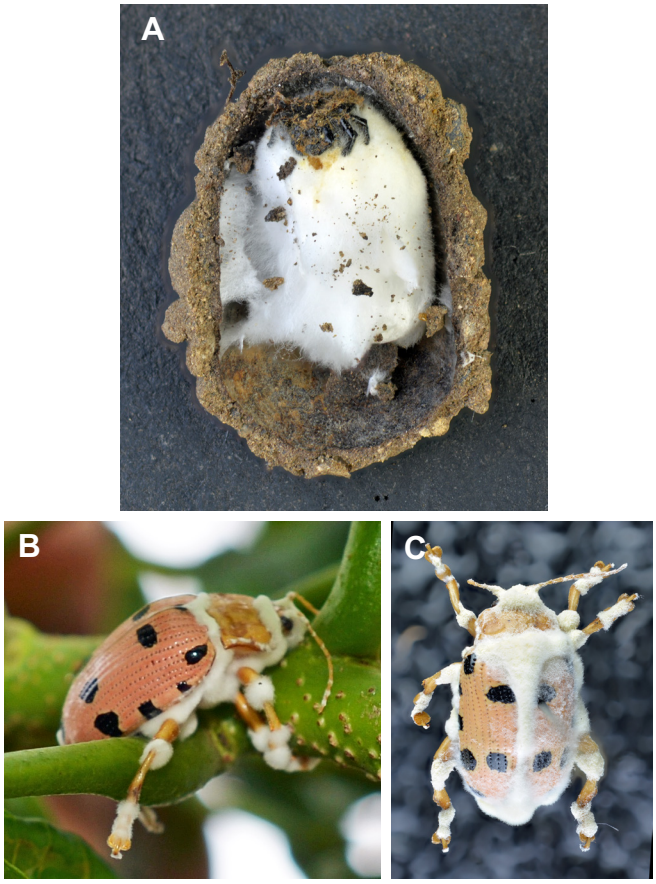


Figure 1. *Podontia quatuordecimpunctata* (SLB) infected with *B. bassiana*. (A) SLB pupa infected with *Beauveria bassiana*. (B) Adult SLB infected with *B. bassiana* hanging on a tree. (C) Dorsal view of an adult SLB infected with *B. bassiana*. (Digital photos courtesy of Orlando A. Calcetas).

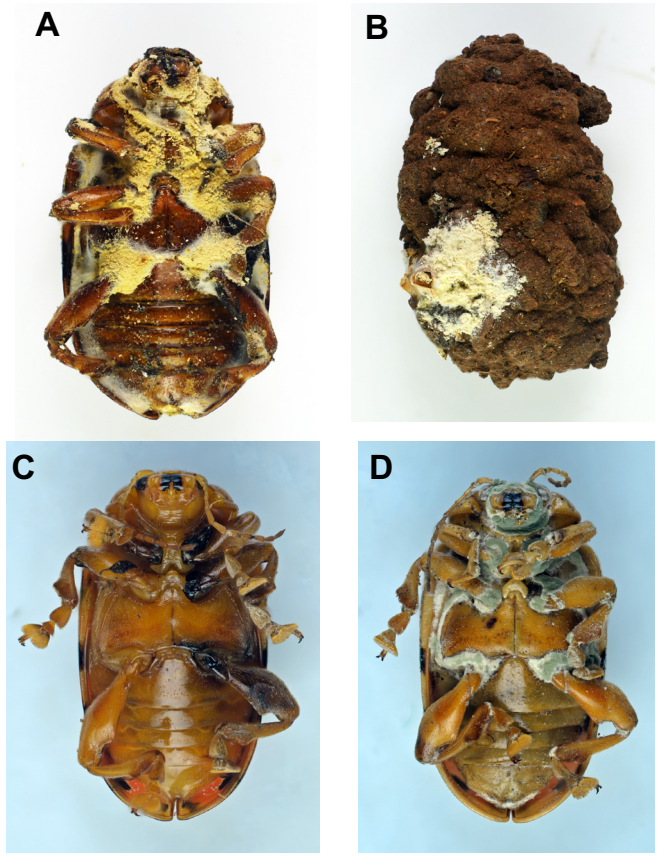


Figure 2. *Podontia quatuordecimpunctata* (SLB) infected by entomopathogenic fungi. (A) Adult SLB infected with *Beauveria bassiana* collected in Brookes Point, Palawan. (B) SLB pupa infected with *Beauveria bassiana* collected in Brookes Point, Palawan. (C) Necrotic lesion symptoms on an adult SLB infected with *Metarhizium anisopliae*. (D) Adult SLB with green muscardine fungal growth (*M. anisopliae*) on its body. (Digital photos courtesy of Orlando A. Calcetas).

Table 3. Molecular identification of three fungal entomopathogens infecting the SLB, *P. quatuordecimpunctata* (L.) in the Philippines

Isolate Code	Species Identification	Assigned Accession Number	BLAST HIT	
			GenBank Accession Number	Percentage Nucleotide Identity
2.1	*Aspergillus psuedonomius*	OR426351	MH279418.1	100
7.2.2	*Beauveria bassiana*	OR426353	MT586632.1	100
9.1	*Beauveria bassiana*	OR426354	MT586632.1	100

Pupal site and infection dynamics

The earthen pupal chamber often served as a substrate for subsequent generations of SLB, indicating a potential role in pathogen transmission (Calcetas *et al.*, 2023). Applying fungal spore preparations to the tree base could enhance pathogen dissemination and reduce reliance on chemical control measures, thereby increasing the likelihood of fungal infections within the pest population over time. While our observations revealed that both larval and pupal stages were susceptible to fungal infection, the absence of a protective earthen covering rendered pupae more vulnerable to desiccation or pathogen attack (Calcetas *et al.*, 2023). For the bioassay on pupae, the mortality time for infected pupae ranged from 2–13 days after exposure, with infection rates between 10-40%.

Adult susceptibility to infection

For the bioassay on adults, the infection rates for *B. bassiana* isolated from SLB were found to be 100% (n=10) of individuals exhibiting fungal growth beneath the

semi-transparent abdominal venter within seven days post-exposure (Figures 1B and IC). Subsequently, characteristic white, powdery conidia were observed erupting from the insect's integument. Field surveys conducted during the wet season in Batangas City revealed a significant increase in *B. bassiana* infections among SLB populations in October (n=30 adults). The concurrent decline in SLB population density following the surge in *B. bassiana* infections supports the pathogen's role as a potential biological control agent. Detailed findings on pupal infections are presented in a separate study focusing on the population dynamics of SLB and its natural enemies.

*Mass Production and Application of B. bassiana***:**

Large-scale production and formulation of *B. bassiana* have been established in various countries (Feng *et al.*, 1994). Early methods relied on diphasic fermentation, combining solid and liquid Subsequent advancements led to solid-state fermentation techniques, such as the use of wheat bran as a substrate (Rombach *et al.*, 1988), and commercial-scale production methods developed by Mycotech Corporation, USA (Bradley *et al.* 1992). In the Philippines, the Bureau of Plant Industry-Crop Pest Management Division (BPI-CPMD) and the Department of Agriculture-Regional Crop Protection Center-IVA-CALABARAZON have successfully employed corn or rice as substrates for *B. bassiana* production (BPI-CPMD, 2021).

To effectively manage SLB populations, we propose the mass production of *B. bassiana* strain Pq by the DA-RCPC-IVA for distribution to Local Government Units (LGUs) in Batangas. Application of the fungal formulation should coincide with the post-harvest period (June) when new shoot growth stimulates SLB population increase. Timely intervention can significantly reduce pest numbers during the critical fruiting season (January-February). The entomopathogenic fungi *B. bassiana* is recommended to be applied in the soil since they will persist in this environment and have a higher probability of efficacy for the developing pupae due to the same resource (soil around the base of the plant) reusability (Calcetas *et al.*, 2023).

The potential of microbial biopesticides to manage insect pests while safeguarding human health and the environment is evident. The symbiotic bacterium *Photorhabdus temperata*, associated with entomopathogenic nematodes belonging to *Heterorhabditis* (Rhabditida: Heterorhabditidae) (Fischer-Le Saux *et al.*, 1999), has demonstrated efficacy against over 200 soil-borne insect species. In Bangladesh, *P. temperata* was tested against the hog plum beetle, *P. quatuordecimpunctata*, on *Spondias cythera*. Treatment with different concentrations (0%, 1%, and 10%) of the bacterial culture medium resulted in 10%, 100%, and 85% mortality rates, respectively, among first and second instar larvae after 96 h (Asaduzzaman *et al.*, 2018).

The development of microbial biopesticides with broad-spectrum activity is essential for achieving sustainable agriculture. Such products can effectively manage insect pests while safeguarding human health and the environment (Sabbahi *et al.*, 2022).

Green muscardine fungus, *Metarhizium anisopliae* (Metschn.) Sorokin, 1883

The *Metarhizium anisopliae* has been widely employed as a biological control agent against various agricultural pests and human disease vectors (Peng *et al.*, 2022). Its ubiquitous distribution encompasses soil, plant rhizosphere, and arthropod carcasses (Hu & St. Leger, 2002; Rehner & Kepler, 2017). Unlike *B. bassiana*, *M. anisopliae* lacks a teleomorph stage (Holder & Keyhani, 2005; Sung *et al.*, 2006). The fungus primarily infects insects through direct penetration of the cuticle using conidia, facilitating its application in liquid and dry biopesticide formulations. The infection process involves adhesion, germination, appressorium formation, host penetration, fungal growth, and eventual host death (Gao *et al.*, 2021). However, compared to chemical insecticides, *M. anisopliae* exhibits a slower killing rate, typically requiring approximately five days under optimal conditions (Fang *et al.*, 2007). Extended infection periods have been observed in other insect hosts, such as the rice black bug, *Scotinophara sorsogonensis* Barrion, Joshi, and Sebastian, 2007 (Hemiptera: Pentatomidae), where fungal growth was evident approximately 14 days post-infection (Calcetas and Adorada 2024, unpublished report).

Fungal infection in adult SLB

Laboratory experiments demonstrated that adult SLB exposed to *M. anisopliae* developed characteristic blackish, necrotic lesions primarily on the ventral surface (legs, metasternum, abdomen), with occasional involvement of the head, pronotum, and antennae (Figures 2C and 2D). Infected individuals exhibited reduced mobility and succumbed to the infection within approximately two weeks. A 66.67% infection rate (n=15) was observed within this timeframe. Interestingly, this distinctive necrotic lesion, commonly associated with *M. anisopliae* infections in SLB, has not been reported in the rice black bug (*S. sorsogonensis*) documented infections with this fungus (Calcetas, 2024 unpublished report). This discrepancy may be attributed to the contrasting integument colouration between the two insect species. While SLB possesses an orange or yellow exoskeleton that readily reveals necrotic lesions, the black integument of the rice black bug may obscure such symptoms. Consistent with previous research (Navarrette & Barrion, 2007), infected SLB individuals developed a characteristic fungal growth pattern,

initiating with white hyphae and subsequently producing green conidia. The mummified insect cadavers were readily observed in the field. Additional field observations in Brookes Point confirmed the presence of *M. anisopliae* infections in natural SLB populations (Mercene, 2023 unpublished report).

Laboratory trial of *Metarhizium robertsii* on SLB

The green muscardine fungus (Clavicipitaceae: *Metarhizium robertsii* (=*M. anisopliae*) (Sorokin, 1883) rendered the insect lame or slow to move and eventually, they died after two weeks of exposure.

***Aspergillus pseudonomius* Varga, Samson and Frisvad (2011)**

Aspergillus Micheli, 1729, a genus within the Trichococomaceae family, encompasses several hundred ubiquitous fungal species capable of dispersing globally via air currents, soil, water, and decaying vegetation (Bennett & Klich, 2003; Wang, 2012).

Aspergillus pseudonomius Varga, Samson and Frisvad (2011), is closely related to *Aspergillus nomius* Kurtzman, B.W. Horn and Hesseltine, 1987. This fungus produces aflatoxin B1 but not the G-type aflatoxins (Massi *et al.*, 2014). Initially misidentified as *A. nomius*, *A. pseudonomius* was first isolated from bees (Peterson *et al.*, 2001) and subsequently characterized as a distinct species (Varga *et al.*, 2011). It forms white to yellowish-green colonies with septate hyphae and smooth-walled, round to oval conidia. Beyond its potential mycotoxigenic properties, *A. pseudonomius* produces secondary metabolites known as pseudonomilides with cytotoxic, antifungal, and antiviral activities. This species has a broad ecological distribution, including its association with the economically important Brazil nut tree (*Bertholletia excelsa* Bonpl.) in the Amazon rainforest.

Aspergillus pseudonomius infection was observed in SLB eggs that failed to hatch during fecundity and hatchability studies. Infected eggs exhibited a white, mycelial growth. Subsequent isolation and identification confirmed the presence of the fungus (Figure 3). The obtained nucleotide sequence was uploaded in the GenBank-NCBI database under accession number OR426351 (Table 3). Early symptoms of *A. pseudonomius* infection in SLB eggs included a wet and slimy appearance, followed by the development of visible, white, powdery spores upon desiccation of the egg mass. While previously classified within the *A. nomius* complex, *A. pseudonomius* has been recognized as a distinct species capable of producing aflatoxin B1 (but not G-type aflatoxins) (Varga *et al.*, 2011). Phylogenetic analyses based on ITS, β-tubulin, and calmodulin gene sequences have revealed additional *A. pseudonomius* isolates from insects and soil samples collected in various North American States (Peterson *et al.*, 2001).



Figure 3. Egg cluster of the Sineguelas leaf beetle, *Podontia quatuordecimpunctata* (L., 1767), infected with *Aspergillus pseudonomius* Varga, Samson and Frisvad (2011). (Digital photo courtesy of Orlando A. Calcetas).

CONCLUSION

This study identifies *Beauveria bassiana*, *Metarhizium anisopliae*, and *Aspergillus pseudonomius* as novel entomopathogens infecting the sineguelas leaf beetle (SLB), *Podontia quatuordecimpunctata*, in the Philippines using molecular techniques. These findings underscore the potential of indigenous fungal pathogens for the sustainable management of this economically significant agricultural pest.

Beauveria bassiana exhibited broad-spectrum efficacy, infecting both adult and pupal stages of SLB. The fungus' polycyclic nature, characterized by its ability to rapidly produce multiple generations, underscores its potential as a promising biocontrol agent. Mass production and subsequent foliar application during critical crop phenological stages warrant further investigation to optimize efficacy. The characterized *B. bassiana* strain Pq demonstrates similar potential and warrants field testing to mitigate SLB populations and reduce reliance on chemical insecticides.

Metarhizium anisopliae induced distinct necrotic lesions in adult SLB, a potential diagnostic marker for infection. Elucidating the underlying mechanisms of this phenomenon is crucial for developing effective control strategies. The entomopathogen *B. bassiana* is more effective at 100% mortality after seven days compared to *M. anisopliae* at 66% mortality after approximately two weeks against SLB.

The discovery of *Aspergillus pseudonomius* infecting SLB eggs highlights the complex interactions within the pest-pathogen system and necessitates further research to

assess its biocontrol potential while mitigating potential mycotoxin risks.

These entomopathogens will be provided to Department of Agriculture- Regional Crop Protection Centers (DA-RCPCs) across the country with SLB pest problems. Given the potential presence of these pathogens in different DA-RCPC laboratories nationwide, we strongly recommend implementing regular quality assurance or monitoring procedures using molecular techniques to prevent contamination. To fully realize the potential of fungal biocontrol, rigorous research, development, and quality control are essential. Collaborative efforts among stakeholders are necessary to facilitate the widespread adoption and implementation of integrated pest management strategies incorporating these biological control agents.

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