frontiers | Frontiers in Agronomy

TYPE Original Research
PUBLISHED 12 January 2026
DOI 10.3389/fagro.2025.1695515

OPEN ACCESS

EDITED BY
Murray B. Isman,
University of British Columbia, Canada

REVIEWED BY
Elisabetta Gargani,
Council for Agricultural Research and Agricultural Economy Analysis | CREA, Italy
Yu-Chen Pu,
Fujian Agriculture and Forestry University, China

*CORRESPONDENCE
Santos Triunfo Leiva-Espinoza
✉ santos.leiva@untrm.edu.pe

RECEIVED 29 August 2025
REVISED 05 December 2025
ACCEPTED 12 December 2025
PUBLISHED 12 January 2026

CITATION
Leiva-Espinoza ST, Román A, Aguilar V, Requejo E, Meza-Maicelo W, Banda-Martinez D, Huaman-Pilco ÁF, Loyola F, Loja M, Oliva-Cruz M and Maicelo-Quintana J (2026) Identification of *Metamasius hemipterus* (Coleoptera: Dryophthoridae) and its biological control through the use of species of the genus *Beauveria* and *Metarhizium*. Amazonas, Peru. *Front. Agron.* 7:1695515. doi: 10.3389/fagro.2025.1695515

Identification of *Metamasius hemipterus* (Coleoptera: Dryophthoridae) and its biological control through the use of species of the genus *Beauveria* and *Metarhizium*. Amazonas, Peru

Santos Triunfo Leiva-Espinoza[1,2*], Alcides Román[3], Vilma Aguilar[2], Elizabeth Requejo[1], Wagner Meza-Maicelo[2], Duber Banda-Martinez[4], Ángel Fernando Huaman-Pilco[2], Fredy Loyola[4], Melissa Loja[2], Manuel Oliva-Cruz[2] and Jorge Maicelo-Quintana[5]

[1]Facultad de Ingeniería y Ciencias Agrarias, Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Chachapoyas, Amazonas, Peru, [2]Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva, Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Chachapoyas, Amazonas, Peru, [3]Estación Experimental Agraria Amazonas, Dirección de Desarrollo Tecnológico Agrario, Instituto Nacional de Innovación Agraria, Chachapoyas, Peru, [4]Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas – Filial Rodríguez de Mendoza, Rodríguez de Mendoza, Amazonas, Peru, [5]Facultad de Ingeniería Zootecnista, Agronegocios y Biotecnología, Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Chachapoyas, Amazonas, Peru

Biological control of insect pests represents a key strategy toward sustainable agriculture. This study, focused on the morphological and molecular identification of the sugarcane weevil, *Metamasius hemipterus*, (Coleoptera: Dryophthoridae), and on the evaluation of the biocontrol potential of four entomopathogenic fungi: *Beauveria bassiana*, *Metarhizium anisopliae*, *Beauveria peruviensis*, and *Metarhizium* sp. Molecular identification was conducted through phylogenetic analysis, while morphological identification was based on the description of characteristics following established protocols. Both approaches confirmed the identity of the pest as *Metamasius hemipterus* L. Bioassays were carried out under a completely randomized design, testing four conidial concentrations, plus a control. *Metarhizium anisopliae* at 1×10^{10} conidia/mL was the most virulent, producing the highest mortality and exhibiting the lowest lethal concentration ($LC_{50} = 2.68 \times 10^9$ conidia/mL) against *Metamasius hemipterus* adults. Conversely, *Beauveria peruviensis* at 1×10^{14} conidia/mL achieved the highest colonization efficiency in insect cadavers, with a mycosis rate of 99.4%. These findings indicate that *Beauveria peruviensis* and *Metarhizium anisopliae* are promising biological control agents against the sugarcane weevil, the major pest of sugarcane in the Amazonas region of Peru.

KEYWORDS

entomopathogen, laboratory bioassays, pests, sugarcane, weevil

1 Introduction

Sugarcane (*Saccharum officinarum*) is one of the most important crops worldwide in terms of its extension and economic contribution. This crop supplies about 80% of sugar and about 40% of the world's energy production (Wang et al., 2025). In Peru, sugarcane is prominent among industrial crops for human consumption, representing 3.4% of national production and covering approximately 141.3 thousand hectares. However, it faces multiple limitations, among which pests and diseases are one of the main threats. Among them is the striped sugarcane weevil, an insect of the genus *Metamasius*, a large group of dryophthorine weevils (Palmieri et al., 2022), which is characterized by the severe damage it causes, especially in mountains and forests (Figure 1), where the most significant losses are attributed to it. This pest is considered of high economic importance in the sugarcane-producing areas of the Amazon region, Peru, where it has not yet been identified and can cause losses of up to 20% of harvestable sugarcane and a 30% reduction in extractable sucrose (Alpizar et al., 2012). However, when adequate control campaigns have been implemented, infestation levels have been reduced to less than 2%, with sucrose yields ranging from 17.11% to 34.55% in intervened areas (Salazar and Chávez, 2013).

Currently, in the Amazonas region of Peru, sugarcane cultivation is undergoing a crisis that is severely impacting the farming communities engaged in panela production (López, 2015). Primarily due to the lack of resources to improve their plantations, a situation exacerbated by damage caused by phytosanitary issues, as well as the effects of climate change, the presence of pests and diseases, and water scarcity—factors that together limit growth and reduce yield (Kumar et al., 2024; Milindi et al., 2024). In addition, old varieties are cultivated that have lost their productive capacity, becoming increasingly susceptible to pests and diseases such as the sugarcane weevil, making them less efficient in extracting juice, which is later processed into sugar or panela (Msomba et al., 2024; Zhao et al., 2025).

Weevil adults are particularly attracted to crop residues after cutting the cane, which allows them to oviposit in conditions conducive to the development of their offspring (Weissling et al., 2003; Casteliani et al., 2020), this situation also favors the entry of pathogens that generate rotting and fermentation processes (Weissling et al., 2003). The larvae feed on both damaged and healthy tissues, intensifying the impact of the pest. Symptoms of infestation include the accumulation of sawdust in the galleries

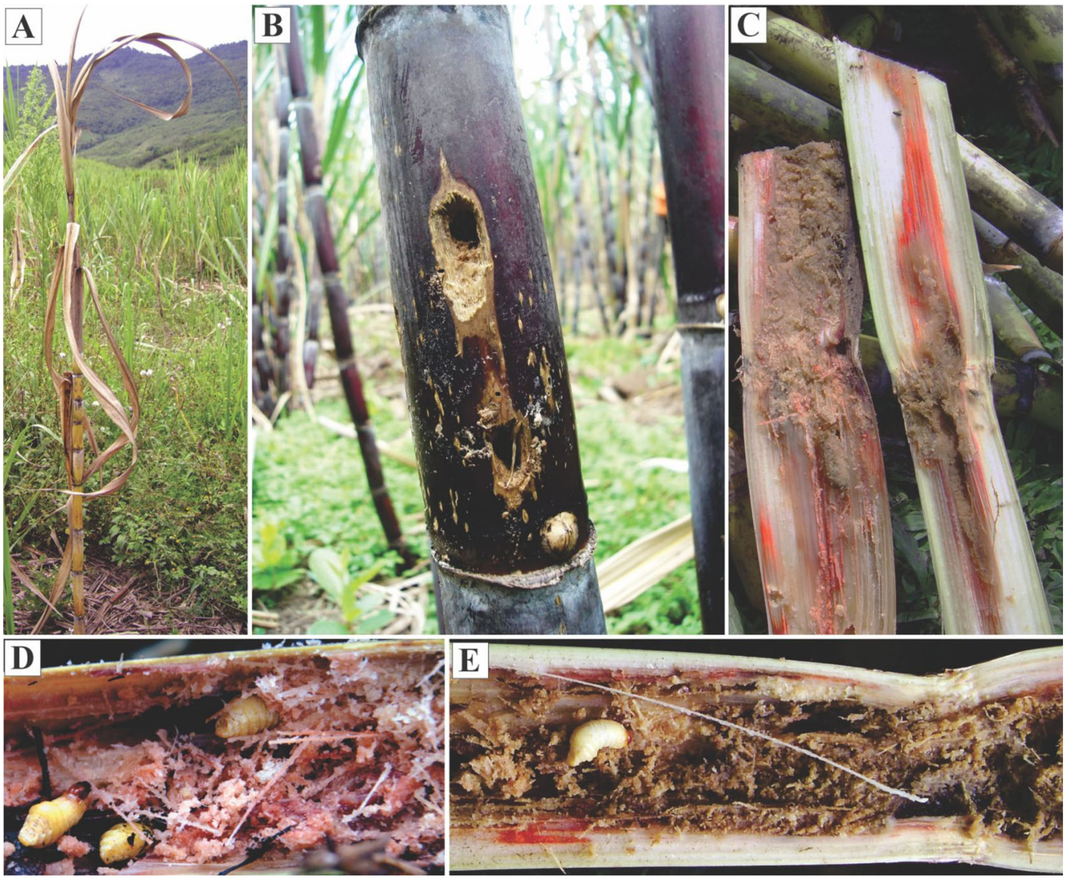

FIGURE 1
Damage caused by the sugarcane weevil. Presence of dry leaves **(A)**; stem perforations **(B)**; initial direct and indirect damage to the stems **(C)**; destruction caused by the larvae of the sugarcane weevil **(D, E)**.

perforated in the stems, which are usually larger than those caused by *Diatraea* (Goyes, 2020; Mendoza et al., 2004) (Figure 1). These effects compromise tissue integrity, decrease juice content, and reduce sugar production (Insuasty, 1997; Risco, 1967).

Despite the magnitude of the problem represented by this pest in the region, there is still no evidence of solid efforts to develop control strategies in organic agroecosystems such as sugarcane in Amazonas, Peru. This situation highlights the urgent need to implement sustainable alternatives, among which ethological or biological control has shown effectiveness in other crops, such as coffee (Leiva et al., 2019). In this sense, research must focus on identifying the pest, its biology, the level of damage it produces, and other key aspects that allow closing an evident knowledge gap. In this regard, previous studies have demonstrated the efficacy of entomopathogenic fungi of the genera *Beauveria* and *Metarhizium* against species of the family Dryophthoridae: Coleoptera (Castrillo et al., 2017; Khun et al., 2020; Lopes et al., 2013). These results support the potential use of these fungi as biocontrol agents in integrated pest management programs. Despite the scarcity of specific studies on the sugarcane weevil, *Beauveria peruviensis* has been identified as a possible native biocontrol agent with action on pest coleoptera present in Amazonas, Peru (Bustamante et al., 2019; Chuquibala-Checan et al., 2023).

In this context, the present study aims to identify the striped sugarcane weevil and evaluate the pathogenicity of two native isolates and two commercial strains of entomopathogenic fungi on this pest under laboratory conditions, as a first step towards the formulation of a biological control strategy adapted to local conditions.

Thus, the general objective was to evaluate the efficacy of native and commercial strains of entomopathogenic fungi of the genera *Beauveria* and *Metarhizium* on the mortality of the striped sugarcane weevil under laboratory conditions.

2 Materials and methods

2.1 Sampling of sugarcane weevils

Specimen collection was carried out through direct observation and manipulation of leaves and stems, following the procedures described by Rivera and Pinto (1999). For morphological and molecular identification, a representative sample was selected from each province (Bongara and Rodriguez de Mendoza; Figure 2), consisting of 40 adults, 26 pupae, and 38 larvae at different developmental stages. In this first collection (May–July 2022), 680 additional adults (females and males) with uniform morphological characteristics were also obtained and used in the first bioassay. Subsequently, during the same research period (May 2022 – March 2023), a second bioassay was conducted, for which a new collection was carried out at the same sampling sites, using individuals with the same morphological characteristics as those from the first bioassay. All specimens were transported alive to the Plant Health Research Laboratory (LABISANV) at the National University Toribio Rodríguez de Mendoza of Amazonas (UNTRM). The rearing of insects used in the bioassays was carried out in conditioned plastic containers placed in a controlled environment at 24 °C and an average relative humidity of 80%. Sugarcane pieces were added every 15 days as food.

2.2 Morphological identification

With the aid of a Nikon SMZ1270 stereomicroscope and a Nikon D5 professional camera, each stage of the samples was photographically documented. The morphology of each stage was characterized. Species identification was carried out by identifying key morphological characters with the support of specific keys and previous descriptions, based on observable external characters, following the protocol that includes specific characteristics for the genus *Metamasius*, such as larvae and pupae, and especially the traits of adults (Chamorro, 2019; Palmieri et al., 2022; Sepúlveda-Cano et al., 2009; Vaurie, 1967; Weissling and Giblin-Davis, 2010).

2.3 Molecular identification: extraction, amplification, and alignment of DNA sequences

Total genomic DNA was extracted from individual insect samples (thorax of one adult) collected from the provinces of Bongará and Rodríguez de Mendoza, representative sugarcane-producing areas in the Amazonas region (Figure 2), using the NucleoSpin® DNA Insect kit (Macherey-Nagel, Düren, Germany), following the manufacturer's instructions. DNA samples were stored at –20 °C until further analysis by PCR and quantification using a spectrophotometer (Eppendorf BioSpectrometer® kinetic, Germany).

For the polymerase chain reaction (PCR), a total reaction volume of 25 µL was used, consisting of 12.5 µL of Promega GoTaq PCR Master Mix (2X), 1.25 µL of forward primer (10 µM), 1.25 µL of reverse primer (10 µM), 5 µL of nuclease-free water, and 5 µL of DNA template.

The cytochrome oxidase subunit I (COI) gene was amplified by PCR using the universal primers C1-J-2195 (5'-TTGATTTTTTGGTCATCCAGAAGT-3') and TL2-N-3014 (5'-TCCAATGCACTAATCTGCCATATTA-3'), following the cycling protocol described by (Simon et al., 1994). Amplified products were subsequently sequenced using the Sanger method at MACROGEN (Santiago, Chile).

2.4 Phylogenetic analyses

Sugarcane weevil COI gene sequences obtained by Sanger sequencing were edited with Sequencher v.5.4.6 (Gene Codes, Ann Arbor, Michigan). Subsequently, they were aligned using MEGA X (Molecular Evolutionary Genetic Analysis (Kumar et al., 2018). To identify the sequences, a search was carried out in the NCBI (National Center for Biotechnology Information)

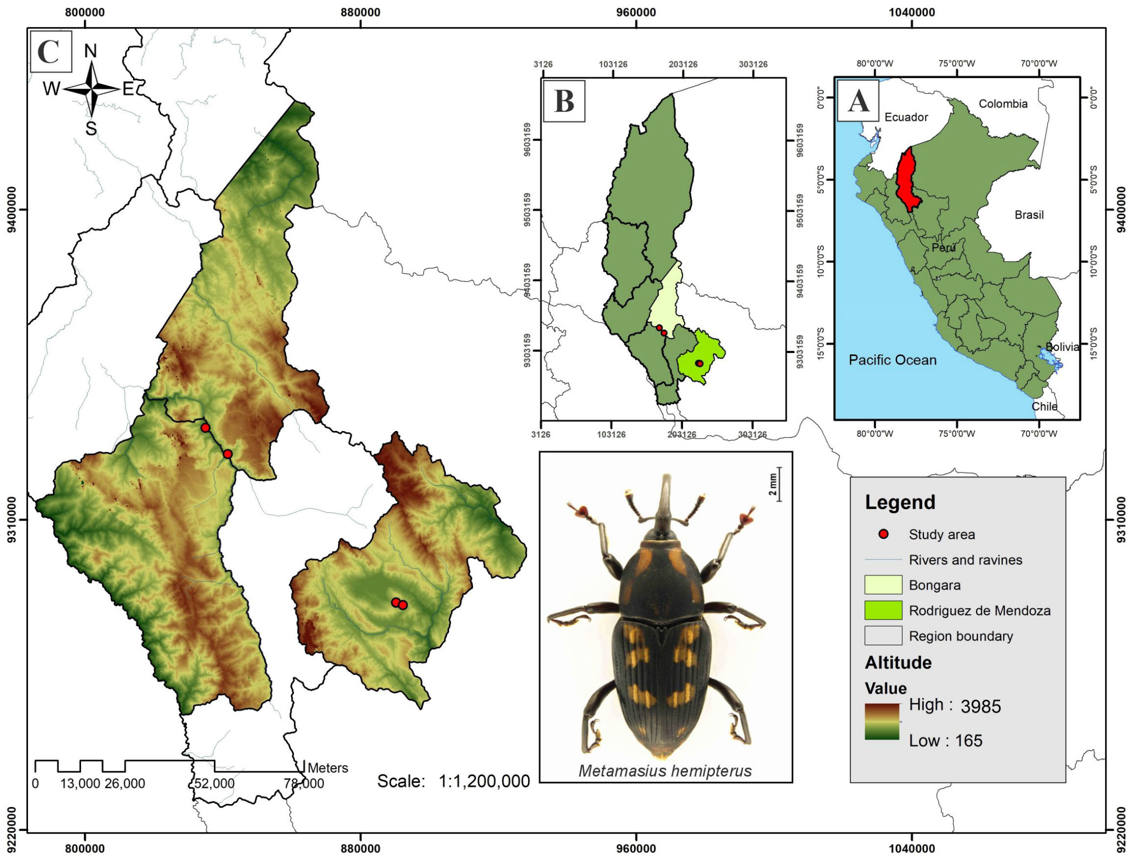

FIGURE 2
Location of sampling areas. Sampling area in Peru **(A)**; sampling area in the Amazonas region **(B)**; sampling points in the provinces of Bongará (left) and Rodríguez de Mendoza (right) **(C)**.

database using the BLASTn tool, with the aim of determining their similarity with previously reported sequences. The phylogenetic analysis was carried out using the maximum likelihood (ML) method, using RAxML-HPC BlackBox, available on the CIPRES Science Gateway platform (https://www.phylo.org/). The GTRGAMMAI model was applied, with 1000 Bootstrap replicates, to evaluate the reliability of the phylogenetic nodes (Díaz-Valderrama et al., 2017; Edler et al., 2021). The sequences obtained were deposited in the NCBI Genbank SLTE06 (PX425762) and STLE07 (PX425761).

2.5 Disinfection of adults for *in vitro* bioassays

In each *in vitro* bioassay, adult insects were disinfected by immersion in 0.5% sodium hypochlorite solution for 3 minutes. To ensure safe handling and prevent the escape of individuals during the procedure, a sterilized fine mesh was employed, allowing efficient manipulation of the specimens without compromising aseptic conditions (Suárez, 2009).

2.6 Reactivation of entomopathogenic fungi

The entomopathogenic fungi species used in this study were obtained from the collection of LABISANV, where they are conserved. Among them, *Beauveria peruviensis* (P19), a new species reported with high potential as a biological control agent of weevils, corresponds to GenBank accession UTRP19, with the holotype deposited as UFV5609, the isotype as ARSEF14196, and registered in MycoBank (No. 829032) (Bustamante et al., 2019; Oliva-Cruz et al., 2024), and *Metarhizium* sp. (MMR-M1), isolated from soil samples from coffee plantations located in the same geographical region as *Beauveria peruviensis*, is still under complete morphological and molecular characterization; therefore, its GenBank accession number is not yet available (Chuquibala-Checan et al., 2023), were used.

In addition, commercial species of entomopathogenic fungi were used, specifically *Beauveria bassiana* (strain CCB LE-265) and *Metarhizium anisopliae* (strain DSM-15168), both registered in the National Agrarian Health Service (SENASA) under registration numbers 300 and 165, respectively. These strains were obtained from authorized distributors in Peru, in wettable powder and liquid formulations, respectively. After acquisition, both strains were isolated and cultured under laboratory conditions to obtain pure cultures. These cultures were placed in 2.5 mL tubes containing PDA medium and stored at –80 °C in the LABISANV strain collection. All material used in the bioassays came exclusively from these preserved strains; therefore, no commercial formulations were used directly, nor was a germination test performed with these products, as the study was based solely on previously preserved, pure, and homogeneous cultures.

For the reactivation of the entomopathogenic fungi, the procedure described by Argomedo et al. (2015) for *Trichoderma asperellum* was followed, with adaptations for the species used in this study. In a laminar flow chamber, the tubes stored at –80 °C were allowed to reach room temperature, and a small portion of the mycelium from each strain was taken using a flame-sterilized inoculating loop. The mycelium was transferred to the center of Petri dishes containing potato dextrose agar (PDA). The plates were incubated at 28 ± 2 °C for 10 days until cultures with adequate conidiation were obtained.

2.7 Production of entomopathogenic fungi and preparation of conidia suspension

We weighed 200 g of rice per 500 mL flask, to which boiled water was added and left to stand for two hours. Subsequently, the rice was drained using nets to reduce excess moisture, and, once precooked, it was transferred to polypropylene bags, which were autoclaved at 121 °C for 15 minutes. A portion of the mycelium of each fungus was added from the Petri dishes to the substrate bags and sprayed with sterile distilled water before sealing to avoid contamination. The inoculated bags were incubated at 26 °C and gentle shaking was performed every four days to facilitate aeration and homogenize the growth of the entomopathogen in the substrate. This process lasted for 15 days, until sporulation was completed, evaluated when at least 90% of the grains showed visible coverage of conidia on the surface of the substrate (Gómez et al., 2014).

On the other hand, in the process of preparing the conidia suspension, 1 gram of sporulated rice was weighed for each entomopathogenic fungus, and mixed with 99 mL of distilled water in an Erlenmeyer flask, to which 1 mL of Tween 20 was added as an emulsifier. The mixture was homogenized in a vortexer at 2000 rpm for 45 seconds. For conidia counting, serial dilutions were performed, taking 1 mL of the initial suspension and diluting it in 9 mL of distilled water, obtaining a 10^{-1} dilution, a process that was repeated until a 10^{-3} dilution was obtained.

Finally, the conidia suspension was loaded into a Neubauer chamber using a Pasteur pipette and covered with a coverslip. Conidia counting was performed under the microscope, using 10X magnification for positioning and 40X for quadrant counting. Conidia concentration was determined using the formula proposed by (Reyes-Figueroa et al., 2016):

$$C = (Cc)(4 \text{ x } 10^6)\left(\frac{Fd}{80}\right)$$

Where:

C: Concentration (conidia/mL)
Cc: Mean of conidia counted in the Neubauer chamber
Fd: Dilution factor

2.8 Application of entomopathogenic fungi

A total of 40 previously disinfected adult specimens were immersed for 3 minutes in 100 mL of conidia solution of native (*Beauveria peruviensis*, *Metarhizium* sp.) and commercial (*Beauveria bassiana*, *Metarhizium anisopliae*) entomopathogenic fungi, with concentrations of 1×10^9, 1×10^{10}, 1×10^{12} and 1×10^{14} conidia/mL. After inoculation, the specimens were dried on sterile paper towels and distributed, with tweezers, into four 5 L plastic containers, at a rate of 10 individuals per container, which were considered replicates. Pieces of sugarcane were added to each container as food. During the bioassays, an average temperature of 19°C and a relative humidity of 70% were maintained (Campos and Velásquez, 2016).

Dead individuals were placed in Petri dishes with moistened absorbent paper to determine the cause of mortality. The plates were labeled according to treatment and replicate and incubated at 24°C for 7 days. To maintain humidity, the paper was sprayed daily with distilled water using a spray bottle, avoiding saturation (Vásquez, 2015).

2.9 Determination of the percentage of mortality

The plastic containers were inspected at 5, 10, 15, and 20 days to record the number of dead adults after applying the entomopathogenic fungi. No food was removed during the experiment. The percentage of mortality in each treatment was calculated using the Abbott (1925) formula:

$$\% \text{ mortality} = \frac{PI - PF}{PI} * 100$$

Where:

PI: Initial population
PF: Final population

Cumulative mortality at 20 days was used to estimate the Median Lethal Concentration (LC_{50}) of each fungal species using Probit models (Faria et al., 2015).

2.10 Estimation of the degree of mycosis

Dead adults were incubated at 24 °C for 7 days in Petri dishes containing moistened paper towel circles. The degree of fungal infection was classified using a visual scale, according to the ranges established by Zárate (2016). This scale was defined in four grades: Grade 1, when the fungus covered between 1% and 25% of the insect's body; Grade 2, with coverage between 25% and 50%; Grade 3, between 50% and 75%; and Grade 4, when the fungus covered between 75% and 100% of the insect's body.

2.11 Experimental design and statistical analysis

The study was structured in two independent bioassays, using a Completely Randomized Design (CRD) with two factors: type of entomopathogenic strain and conidia concentration. The "strain" factor included the following levels: *Beauveria bassiana*, *Beauveria peruviensis*, *Metarhizium anisopliae* and *Metarhizium* sp. The "concentration" factor included the levels of 1×10^9, 1×10^{10}, 1×10^{12} and 1×10^{14} conidia/mL. A total of 17 treatments were obtained, each with an assigned control, and four replicates were defined.

Before statistical analysis, the assumptions of normality and homogeneity of variances were verified using the Shapiro-Wilk and Levene tests, respectively. Subsequently, the data were analyzed by analysis of variance (ANOVA) to determine the existence of significant differences between treatments, using R Studio software, version 4.3.1. In case of significant differences, the Tukey test for comparison of means was applied with a significance level of $p \leq 0.05$.

3 Results

3.1 Identification of the sugarcane weevil

3.1.1 Morphological identification

To support molecular identification, morphological identification was performed based on specific morphological characters, based on taxonomic keys and previous descriptions; in this context, the mature larval, pupal, and adult stages were characterized.

The mature larvae of the observed individuals are apodal, with well-defined and easily visible spiracles. The head capsule is leathery and dark brown in color, with no clearly differentiated frontal sutures. On average, the larvae reach a length of 18.5 mm and a width of approximately 0.70 mm. The body consists of twelve segments, with the ninth and tenth being noticeably wider than the others (Figure 3A).

During development, the insect forms a curculioniform pupa, exarata, with dimensions ranging from 16–20 mm long and 8–10 mm wide, housed in a coconid (Figures 3B, C). Adults have a dark brown coloration, with symmetrically arranged orange spots and bands. The head, rostrum, and scutellum are also dark brown, and the antennae are geniculate in shape (Figures 3E, F). The femurs share this dark shade (Figure 3D) (Zorzenon et al., 2000).

Adults presented distinctive characters that are determinant for the species *Metamasisus hemipterus*; namely: the third tarsomere is heart-shaped, antennae truncated at their tip, with a hairy region that occupies less than half of the total length of the club (Rubio-G. and Acuña-Z., 2006; Sepúlveda-Cano et al., 2009) (Figures 3E, F). They have a total length (measured from the tip of the rostrum to the pygidium) that varies between 9 and 16 mm. Specimens with predominantly dark tones (brown) and striated elytra with slight variations in the color pattern were observed, without visible pubescence, dark brown, with a pair of clear mesolateral bands (Figure 3D). The pygidium is exposed, is yellowish brown and is covered with fine golden hairs, more abundant in males than in females. Furthermore, the prothorax and mesothorax are black with yellowish stripes, while the metathorax is darker (Figure 3D).

3.1.2 Phylogenetic identification

Phylogenetic analysis based on COI gene sequencing shows the evolutionary relationship between different species of the genus *Metamasius*, using *Rhynchophorus palmarum* as an outgroup. Sequences obtained from specimens STLE06 (Genbank: PX425762) and STLE07 (Genbank: PX425761) clustered with high similarity to the reference sequence MH252485 of *Metamasius hemipterus*, forming a single clade (Figure 4). This clade is closely related to *Metamasius* sp. nr. *hemipterus* (AY131107), with a support value of 51%. In a separate clade, *Metamasius callizona* (AY131114) clusters independently, indicating further divergence from *Metamasius hemipterus*.

3.2 Percentage mortality

Analysis of variance (ANOVA) of the first bioassay showed a significant effect of the interaction between strain and conidia concentration on the mortality of *Metamasius hemipterus* ($F = 9.56$; $df = 12$; p -value $= 5.15 \times 10^{-10}$). Similarly, in the second bioassay, a significant effect of the interaction between strain and conidia concentration on mortality was observed ($F = 8.29$; $df = 12$; $p - \text{value} = 6.19 \times 10^{-9}$). These results justify the multiple comparison of means to identify the most effective treatments.

In the first bioassay, the treatment with *Metarrhizium anisopliae* (commercial strain) at a concentration of $1 \text{ x } 10^{10}$ conidia/mL recorded the highest mortality in adults of *Metamasius hemipterus*, reaching 77.5%. This result positions it as the most effective treatment in this experimental phase. It was followed in effectiveness by *Beauveria peruviensis* at $1 \text{ x } 10^{14}$ conidia/mL and *Metarrhizium anisopliae* at $1 \text{ x } 10^9$ conidia/mL, both with 60% mortality. These three treatments formed a statistically superior group, with significantly higher values than the remaining treatments and the control (Figure 5A).

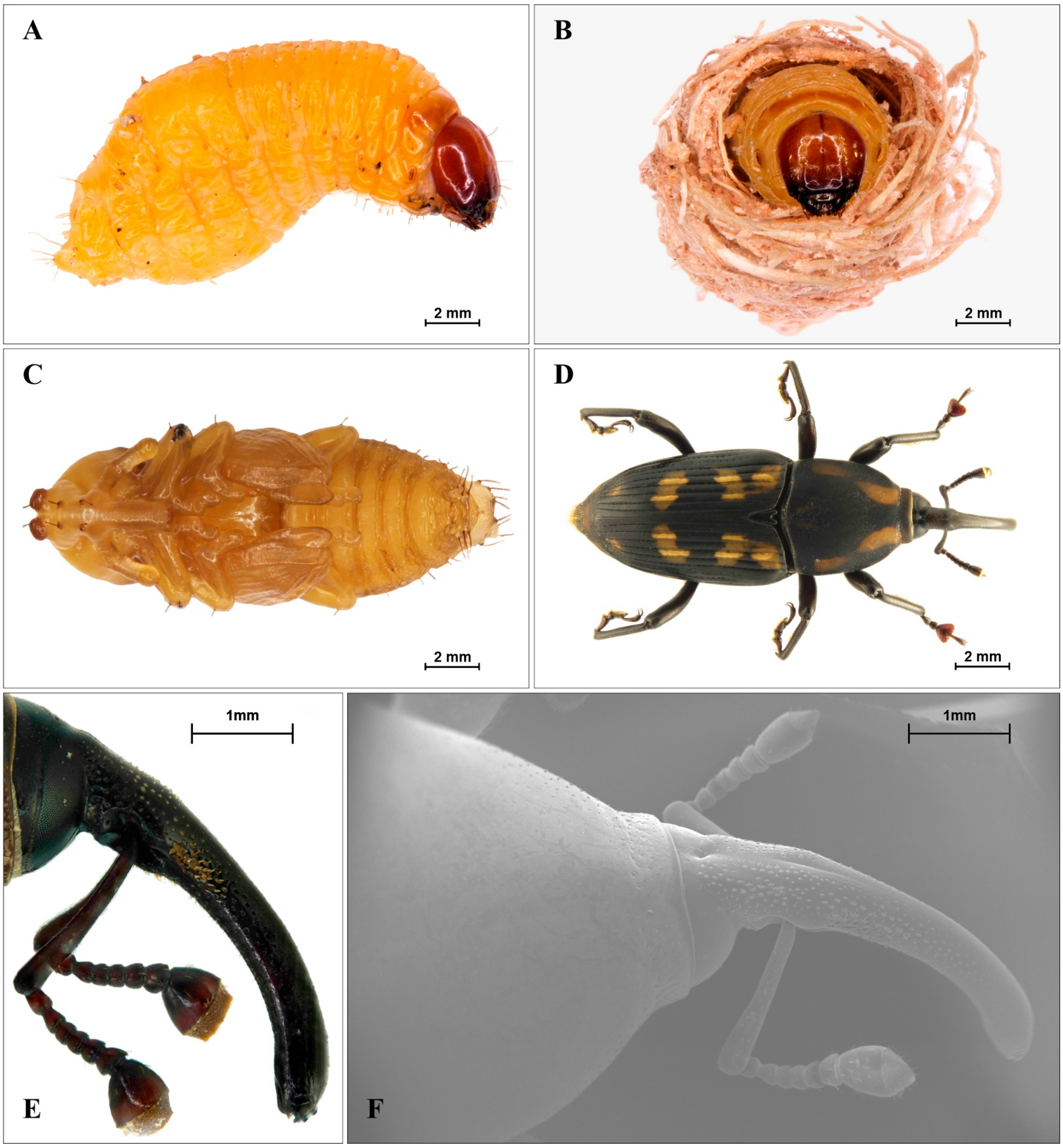

FIGURE 3
Morphological characteristics of *Metamasius hemipterus*. Frontal view of third instar larva **(A)**; Morphological characteristics of the larva in cocoon, beginning of the pre-pupal stage **(B)**; Ventral view of the pupal stage **(C)**; Dorsal view of the adult stage **(D)**; Lateral view of the head and antennae - adult stage **(E)**; Electron microscopic view of the head of the adult stage **(F)**.

During the second bioassay, *Metarhizium anisopliae* (commercial) at 1 x 10^{10} conidia/mL maintained its performance, reaching 75% mortality. *Beauveria peruviensis* at 1 x 10^{14} conidia/.mL and *Metarhizium anisopliae* at 1 x 10^{9} conidia/mL recorded 62.5% and 57.5% mortality, respectively, showing no significant differences concerning the most effective treatment. Likewise, *Beauveria peruviensis* at 1 x 10^{12} conidia/mL and *Metarhizium* sp. at 1 x 10^{10} conidia/mL achieved 52.5% and 55% mortality, respectively. These treatments were also part of the highest efficacy group, differing statistically from the other treatments and the control (Figure 5B). In both bioassays, the individuals in the control treatment showed no mortality and remained alive throughout the experiment.

3.3 Median lethal concentration

The concentration-mortality data fitted the Probit model adequately in the bioassays performed (n = 160; DF = 14), with chi-square (χ^2) values ranging from 6.21 to 10.21 and p-values greater than 0.05, indicating that the null hypothesis of good fit was not rejected (Table 1).

In both bioassays, *Metarhizium anisopliae* required the lowest concentrations to achieve 50% mortality in the *Metamasius hemipterus* population, with a mean of 2.68 × 10^{9} conidia/mL. In contrast, *Beauveria peruviensis* exhibited a mean of 6.31 × 10^{11} conidia/mL, while *Metarhizium* sp. required 4.76 × 10^{11} conidia/mL to reach the same mortality level. Mortality in the control group was

FIGURE 4
Phylogenetic tree of *Metamasius hemipterus* based on the mitochondrial COI gene, inferred by maximum likelihood (RAxML) with 1000 bootstrap replicates. Insect samples STLE06 and STLE07 clustered with the reference sequence MH252485 (*Metamasius hemipterus*). *Rhynchophorus palmarum* was used as the outgroup.

0%. Regarding *Beauveria bassiana*, it was not possible to determine the median lethal concentration (LC_{50}), since none of the concentrations tested—including the highest applied (1×10^{14} conidia/mL)—achieved 50% mortality (Figure 5).

3.4 Percentage of mycosis

The analysis of variance (ANOVA) revealed statistically significant differences among treatments in both bioassays regarding the incidence of mycosis in *Metamasius hemipterus* (first bioassay: F = 1.80; df = 9; p = 0.03; second bioassay: F = 2.15; df = 9; p- value= 0.04). These results justify multiple mean comparisons to identify the treatments with the highest fungal infection capacity against the insects.

In the first bioassay, the native isolate of *Beauveria peruviensis* applied at a concentration of 1×10^{14} conidia/mL reached 100% mycosis, standing out as the most effective treatment in colonizing the insect. Other treatments showed slightly lower percentages, although without significant differences with respect to the most effective treatment. These included *Beauveria peruviensis* at 1×10^{12} and 1×10^{10} conidia/mL, with mycosis values of 99.4% and 96.5%, respectively. Likewise, *Beauveria bassiana* presented high levels of mycosis at all concentrations evaluated: 95% (1×10^{12} and 1×10^{10} conidia/mL), 94% (1×10^{14} conidia/mL), and 93.6% (1×10^{9} conidia/mL). These treatments evidenced significantly higher infection capacity than the other treatments and the control group (Figure 6A).

During the second bioassay, *Beauveria peruviensis* at 1×10^{14} conidia/mL showed high effectiveness, with a mycosis percentage of 98.7%, remaining the most efficient treatment. The other concentrations of *Beauveria peruviensis* and *Beauveria bassiana*, which had previously shown good results, also retained their effectiveness without statistical differences concerning the most effective treatment. However, they differed significantly from the treatments evaluated (Figure 6B).

4 Discussion

The sugarcane weevil is the main pest affecting sugarcane crops in the Amazon region of Peru. In this study, we fully identified the species using molecular and morphological tools based on specific keys and previous descriptions. Subsequently, we conducted *in vitro* biological control trials using two native and two commercial entomopathogens, both belonging to the genera *Beauveria* and *Metarhizium*. This study allows us to identify the harmful agent and the entomopathogens with the greatest biocontrol potential for future fieldwork, thereby contributing to the management of this important sugarcane pest in this region of the world.

The results obtained in this study agree with previous morphological descriptions of *Metamasius hemipterus* in its different developmental stages, as documented by por Weissling and Giblin-Davis (2003); Sepúlveda-Cano et al. (2009) and Zorzenon et al. (2000). In the larval stage, the observed specimens presented general characteristics with those reported by Weissling and Giblin-Davis (2010), such as the absence of locomotor appendages, easily distinguishable spiracles, and a dark brown leathery cephalic capsule. The larvae reached an average length of 18.5 mm and a width of approximately 0.70 mm, with coloration

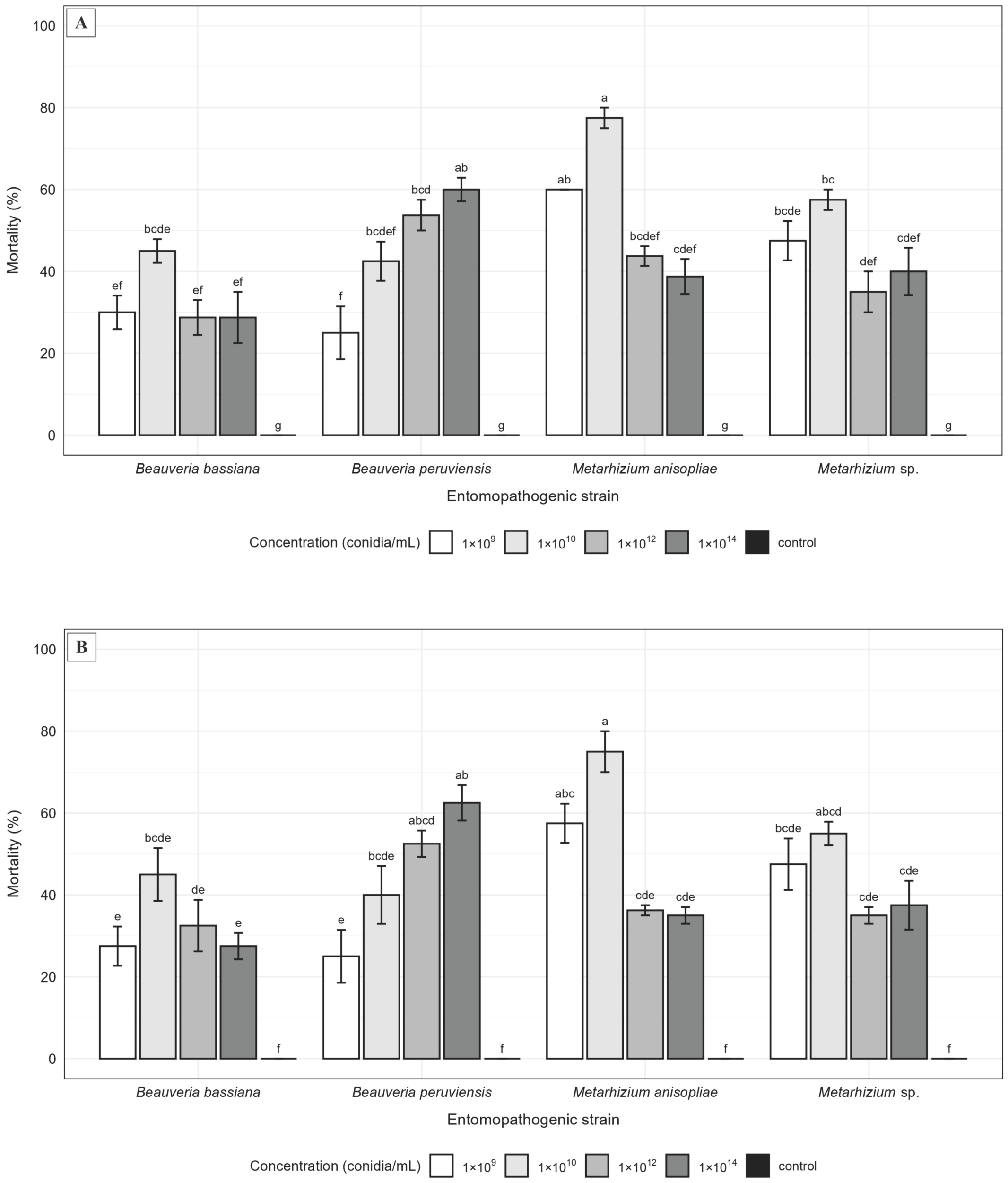

FIGURE 5
Mean mortality (%) of two commercial strains, *Beauveria bassiana* and *Metarhizium anisopliae*, and two native strains, *Beauveria peruviensis* and *Metarhizium* sp., against *Metamasius hemipterus* after 20 days of incubation. **(A)** trial 1; **(B)** trial 2. Means followed by the same letter in each column are non-significant; Tukey test ($\leq$ 0.05).

ranging from cream to pale yellow. Likewise, the robust shape of the ninth and tenth segments is a distinctive morphological feature facilitating their identification.

Adult sugarcane weevils are predominantly dark brown with symmetrically distributed orange spots and stripes. They exhibited crucial distinguishing features for the species *Metamasisus hemipterus*: a heart-shaped third tarsomere, antennae truncated at their tips, and a hairy region that occupies less than half of the total length of the club (Rubio-G. and Acuña-Z., 2006; Sepúlveda-Cano et al., 2009) (Figures 3D-F).

The striated, non-pubescent elytra exhibited mesolateral and median bands with slight variations in color pattern, consistent with the findings of Palmieri et al. (2022). These features were common in the present study. Furthermore, the specimens found had an exposed pygidium, with brown coloration and the presence of golden setae, consistent with the observations of Zorzenon et al. (2000), who also observed differences in the density of these setae between males and females.

Another relevant characteristic was the coloration of the femora, which ranged from reddish to dark brown, suggesting a

TABLE 1 Lethal concentrations (LC 50) of two species of commercial entomopathogens (*Metarhizium anisopliae* and *Beauveria bassiana*) and two native entomopathogens (*Beauveria peruviensis* and *Metarhizium* sp.) in adults of *Metamasius hemipterus*.

Species of entomopathogens	Bioassay	LC50 (conidia/mL)	Lower 95% CI	Upper 95% CI	$\chi 2$	DF	n	p-value
Beauveria peruviensis	1	6.31×10^{11}	3.98×10^{10}	1.58×10^{13}	6.21	14	16	0.96
Metarhizium sp.		7.94×10^{11}	3.98×10^{10}	2.51×10^{13}	7.90	14	16	0.89
Metarhizium anisopliae		3.31×10^{9}	9.12×10^{6}	1.00×10^{13}	7.04	14	16	0.93
Beauveria bassiana		ND	ND	ND	–	–	–	–
Beauveria peruviensis	2	6.31×10^{11}	5.01×10^{10}	1.00×10^{13}	7.42	14	16	0.92
Metarhizium sp.		1.58×10^{11}	1.26×10^{10}	2.51×10^{12}	10.21	14	16	0.75
Metarhizium anisopliae		2.04×10^{9}	7.08×10^{6}	5.01×10^{12}	6.30	14	16	0.96
Beauveria bassiana		ND	ND	ND	–	–	–	–

χ^2, chi-square statistic; DF, degrees of freedom; n, sample size; ND, not determined.

certain degree of polymorphism in the species. Finally, orange spots were identified in the prosternum, mesosternum, and metasternum regions, as well as a central spot in the shape of an inverted "T" on the abdominal sternites, elements that are consistent with the descriptions made by Zorzenon et al. (2000).

Amplification of the COI gene in *Metamasius* sp. using primers C1-J-2195 and TL2-N-3014 was successful, obtaining a fragment of approximately 800 bp, consistent with previous studies in Coleoptera (Duque-Gamboa et al., 2012; Folmer et al., 1994; Simon et al., 1994). This marker has been consolidated as a fundamental tool in DNA barcoding, as it presents conserved regions that allow efficient amplification in a wide variety of insects, together with hypervariable regions that facilitate species differentiation (Al-Sarar et al., 2024; Ma et al., 2022; Takiya et al., 2006; Xu et al., 2021).

In the case of *Metamasius hemipterus*., comparing sequences obtained with databases such as GenBank aided taxonomic identification and species delimitation within the genus. The specimens coded as STLE06 and STLE07 were grouped in the same clade with *Metamasius hemipterus*, which supports their assignment to this species and evidences a close phylogenetic relationship with this taxon (Coleoptera: Dryophthoridae)).

Entomopathogenic fungi have been consolidated in recent decades as effective tools for biological pest control, due to their ability to infect insects through the cuticle, without the need to be ingested. This particularity and the production of secondary metabolites and proteins with toxic effects contribute to their lethal action on diverse hosts (Rustiguel et al., 2018). In this study, two independent assays were conducted to evaluate the potential of two commercial strains, *Beauveria bassiana* and *Metarhizium anisopliae*, as well as two native strains, *Beauveria peruviensis* and *Metarhizium* sp, at doses of 1×10^9, 1×10^{10}, 1×10^{12} and 1×10^{14} conidia/mL. The trials measured the mortality rate, the median lethal concentration (LC_{50}), and the percentage of fungal infections induced by each treatment.

The insect cuticle constitutes the first physical barrier, and the interactions occurring on this surface determine whether the entomopathogen progresses or whether the host activates its defense mechanisms (Ortiz-Urquiza and Keyhani, 2013). From the exterior to the interior, the cuticle is organized into the epicuticle, procuticle, and epidermis. The epicuticle features a hydrophobic lipid layer and various antimicrobial compounds capable of inhibiting conidial adhesion and germination (Pedrini et al., 2007; Qu and Wang, 2018). The epidermis, in turn, contributes to defense through the secretion of inhibitors of fungal proteases and the activation of melanization pathways that restrict mycelial growth (Qazi and Khachatourians, 2007). Additionally, insects adopt behaviors that reduce the likelihood of infection, such as behavioral fever, through which they raise their body temperature to prevent the development of species sensitive to thermal fluctuations (Shang et al., 2015; Zhang et al., 2025).

For the entomopathogenic fungus to penetrate the insect cuticle, its conidia must adhere to the surface, germinate, form penetration structures (appressoria), penetrate the cuticle (combined action of enzymes and mechanical pressure), cause the insect's death, colonize its hemocoel, sporulate, and finally invade the entire insect body, leading to mycosis (Lu and St. Leger, 2016; Mariappan et al., 2025). In this study, the pathogenicity tests identified the commercial strain of *Metarhizium anisopliae*, at a concentration of 1×10^{10} conidia/mL, as the most effective treatment against *Metamasius hemipterus*, reaching an average cumulative mortality rate of 76.3% at the end of the 20-day evaluation. This result is consistent with previous studies demonstrating the efficacy of this fungus against other pest beetles. For example, Saleem and Ibrahim (2019) reported that an isolate of *Metarhizium anisopliae* applied at a dose of 1×10^8 conidia/mL achieved 60.8% mortality on third instar larvae of *Oryctes agamemnon*, a pest of *Phoenix dactylifera* L., at 28 days of evaluation. Similarly, Yasin et al. (2019) evaluated 19 entomopathogenic fungi against adults of *Rhynchophorus ferrugineus*, highlighting *Metarhizium anisopliae* isolate WG-44, which at a dose of 1×10^8 conidia/mL achieved 59.7% mortality at 12 days.

Likewise, Khun et al. (2020) evaluated the pathogenicity of six isolates of *Metarhizium anisopliae*, six of *Beauveria bassiana*, and one commercial species of *Beauveria bassiana* against adults of the

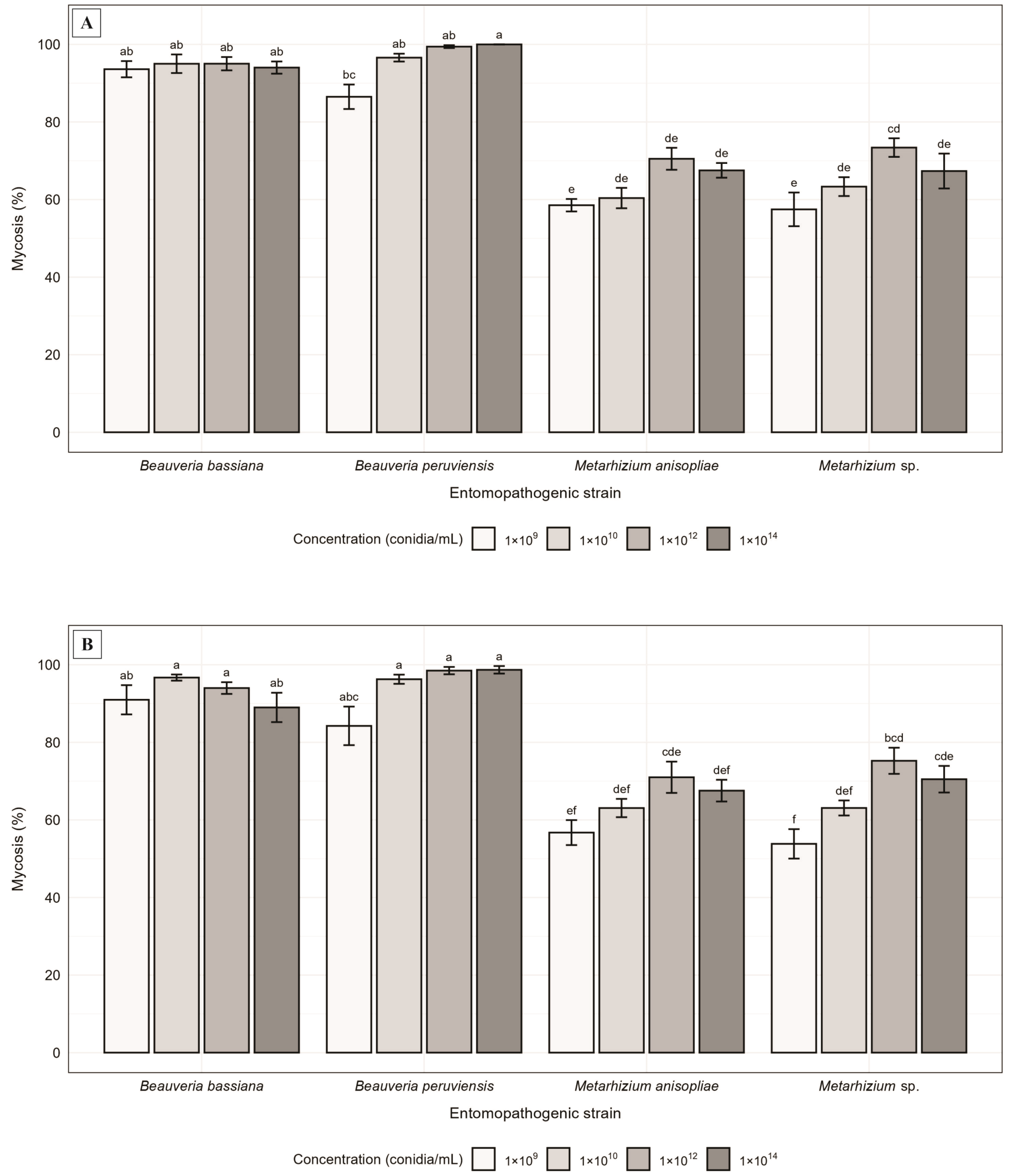

FIGURE 6
Average percentage of mycosis caused by two commercial strains of *Beauveria bassiana* and *Metarhizium anisopliae*, and two native isolates of *Beauveria peruviensis* and *Metarhizium* sp. on *Metamasius hemipterus* after seven days of incubation. **(A)** trial 1; **(B)** trial 2. Means followed by the same letter in each column are not significant; Tukey test (≤ 0.05).

macadamia seed weevil (*Kuschelorhynchus macadamiae*), highlighting the isolate ECS1/BRIP 70272 of *Metarhizium anisopliae* at a dose of 1 x 10^7 conidia/mL that achieved 97.5% mortality at the end of the 12 days of evaluation. In another similar study, Negrete-González et al. (2018) analyzed the virulence of twelve native isolates of *Cordyceps bassiana* and nine of *Metarhizium anisopliae* against adults of *Cosmopolites sordidus*, applying doses of 1 x 10^4, 1 x 10^5, 1 x 10^6, 1 x 10^7, and 1 x 10^8 conidia/mL in the laboratory and 2 x 10^{13} conidia/ha under field conditions. *Metarhizium anisopliae* isolate Ma148 was the most effective in the laboratory, with a mortality of 76.9%, and reduced *C. sordidus* populations in the field by 48.5%. In addition, co-application with the *C. bassiana* isolate Cb174, which had the highest laboratory mortality within its species, resulted in an additional 38.1% reduction in the field.

In *in vitro* assays, a commercial strain is expected to exhibit higher virulence than native isolates, although this does not always represent the decisive factor for achieving effective pest control (Benito-Delgado et al., 2025). In this study, the higher virulence observed in *Metarhizium anisopliae* could be associated with its ability to adhere firmly to the cuticle of *Metamasius hemipterus* through conidial surface hydrophobins, the MAD1 adhesin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), all of which are involved in conidial attachment (Broetto et al., 2010; C. Wang and St Leger, 2007; Zhang et al., 2025). Additionally, *Metarhizium anisopliae* is capable of penetrating the cuticle directly, without forming appressoria (Butt et al., 1995). Following adhesion, the fungus would have initiated cuticle degradation through the action of proteases such as the subtilisin-like serine protease (Pr1) and the trypsin-like protease (Pr2), whose higher activity is commonly associated with increased virulence (Liu et al., 2007; Mustafa and Kaur, 2009; Zare et al., 2014). This activity is complemented by chitinases and lipases, whose elevated expression accelerates penetration in insects with heavily sclerotized cuticles, such as *Metamasius hemipterus* (Gebremariam et al., 2022; Xiao et al., 2012).

Contrary to expectations, the native isolate of *Beauveria peruviensis* at doses of 1 x 10^{14} and 1 x 10^{12} conidia/mL showed slightly lower mortality percentages, with cumulative mortality averages of 61.3% and 52.5%, respectively, differences that were not statistically significant compared to the most effective treatment, which positions *Beauveria peruviensis* as an alternative as effective as the commercial strain. In this regard, Chuquibala-Checan et al. (2023), in an investigation that evaluated the pathogenicity of three entomopathogenic fungi against the coffee berry borer (*Hypothenemus. hampei*) at different doses Chuquibala-Checan et al. (2023) reported that *Beauveria peruviensis*, applied at a dose of 1 <x 10^9 conidia/mL against *Hypothenemus hampei*, achieved over 80% mortality just four days after inoculation, considering two independent trials. These results exceeded those obtained in this study, despite the use of higher doses, which reinforces the hypothesis that the specificity between the fungus and the host insect and the experimental conditions are important factors for the efficacy of biological control (Delgado and Murcia-Ordoñez, 2011).

Native entomopathogenic fungi can trigger lethal epizootics in insect pest populations within agroecosystems (Dwi Sutanto et al., 2024; Mathulwe et al., 2021). *Beauveria peruviensis* was initially found causing a natural epizootic in *Hypothenemus hampei* on coffee plantations (Bustamante et al., 2019). As an isolate native to the Amazon region, it is adapted to the climate and environmental conditions in which coffee and sugarcane crops often coexist, positioning it as a potential alternative to the use of commercial strains. This local adaptation reflects the fact that native species tend to be better adapted to the conditions of the area in which they were isolated, a crucial aspect in agroecosystems subject to environmental fluctuations (Benito-Delgado et al., 2025). This is complemented by their sporulation habits, which allow them to remain active for longer periods in the environment, giving them greater persistence and high epizootic potential (Castrillo et al., 2020).

Another native isolate evaluated in the study, *Metarhizium* sp. at 1 x 10^{10} conidia/mL, displayed a level of virulence comparable to that of *M. anisopliae* at 1 x 10^9 conidia/mL, yielding cumulative mortalities of 58.8% and 56.3%, respectively, by day 20. Similarly, Chuquibala-Checan et al. (2023) reported that a native isolate of *Metarhizium* sp. applied at 1 x 10^9 conidia/mL exceeded 80% mortality by the fourth evaluation day in both trials. Similarly, Valle and Caicedo (2019) evaluated four native isolates of *Metarhizium* sp. at a dose of 1 x 10^8 conidia/mL, and reported that isolate T16301 achieved 76% cumulative mortality at day 15 of evaluation.

The mortality values obtained for each entomopathogenic strain were used to calculate the LC_{50}, which enables a quantitative comparison of virulence among fungi, as it indicates the concentration required to cause 50% mortality in *Metamasius hemipterus* adults (Stephenson et al., 2006). In this study, it was observed that, after 20 days post-inoculation, *Metarhizium anisopliae* was the most virulent fungus, requiring the lowest mean concentration (2.68 × 10^9 conidia/mL) to reach the LC_{50}. However, when contrasted with other studies, evidence shows that in different pest hosts, *Metarhizium anisopliae* has required even lower concentrations to achieve 50% mortality. Yasin et al. (2019) reported that, at 21 days, two strains of the fungus caused 50% mortality of *Rhynchophorus ferrugineus* adults with a mean of 5.71 × 10^6 conidia/mL. More notably, Khun et al. (2020) found that, after 12 days of exposure, the LC_{50} against adults of *Kuschelorhynchus macadamiae* was reached with only 1.48 × 10^5 conidia/mL.

For their part, *Beauveria peruviensis* and *Metarhizium* sp. required concentrations of 6.31 × 10^{11} and 4.76 × 10^{11} conidia/mL, respectively. Chuquibala-Checan et al. (2023), however, reported that, against *Hypothenemus hampei*, the LC_{50} for *Beauveria peruviensis* and *Metarhizium* sp. was only 6.1 × 10^2 and 1.1 × 10^4 conidia/mL, respectively. In the case of B. bassiana, none of the concentrations evaluated in this study reached 50% mortality, which prevented the calculation of its LC_{50} using reliable models (Pillai et al., 2021). Nonetheless, Chuquibala-Checan et al. (2023) reported that the same strain of *Beauveria bassiana* used in the present study (CCB-LE265) exhibited one of the lowest concentrations to achieve 50% mortality in *Hypothenemus hampei* adults, with only 9.6 × 10^2 conidia/mL.

Variations in LC_{50} values can be attributed, first, to the differential susceptibility of hosts, as the structural and chemical characteristics of the cuticle condition fungal penetration and, consequently, the mortality achieved (Ortiz-Urquiza and Keyhani, 2013). Likewise, the same fungal strain may display contrasting levels of virulence depending on the host species evaluated, which limits direct comparisons across different pests (Zamora-Avilés et al., 2024). Additionally, methodological differences in suspension preparation may significantly influence LC_{50} estimates, since conidial viability, vigor, and homogeneity, together with the mode of application, can substantially affect outcomes (Faria et al., 2015). Finally, the evaluation period constitutes a critical factor, as the post-inoculation days considered for mortality assessment directly impact the estimation of the median lethal dose, which explains discrepancies among studies employing different observation intervals (Khun et al., 2020; Yasin et al., 2019).

Regarding the mycosis assays, *Beauveria peruviensis* at a dose of 1 x 10^{14} conidia/mL was the most effective treatment, registering a cumulative mycosis of 99.4% on the seventh day. Lower doses, such as 1 x 10^{12} and 1 x 10^{10} conidia/mL, also showed high levels of mycosis (98.9% and 96.5%, respectively), with no significant statistical differences. These results are consistent with those of Oliva-Cruz et al. (2024), who reported 84.1% mycosis in *Hypothenemus hampei* using a combination of *Beauveria* *peruviensis* (P19) and *Metarhizium* sp. (PMR-M12) strains, both at 1 x 10^7 conidia/mL.

Likewise, *Beauveria bassiana* showed high levels of mycosis at all concentrations evaluated (1 x 10^{10}, 1 x 10^{12}, 1 x 10^9, and 1 x 10^{14} conidia/mL), reaching 95.9%, 94.5%, 92.3%, and 91.5% of accumulated mycosis, respectively. These values were higher than those obtained by Yasin et al. (2019), who evaluated ten isolates of *Beauveria bassiana* and reported mycosis between 0% and 78% with 1 x 10^7 conidia/mL, and between 16.2% and 95.3% with 1 x 10^8 conidia/mL, with isolate WG-41 being the most effective.

Our results showed that there is no proportional relationship between mortality and mycosis rates, which is consistent with observations reported for other insect pests (Merino et al., 2007; Rodríguez et al., 2006). The high mortality caused by *Metarhizium anisopliae* could be attributed to the elevated concentration used and to the activity of fungal enzymes associated with its infective process, whereas the lower and more variable mortality recorded for the remaining strains suggests the need to evaluate higher concentrations and to consider the influence of genetic differences linked to host specialization and the geographic origin of the isolates. Nevertheless, the low percentage of mycosis observed in *Metarhizium anisopliae* indicates a limited ability to emerge and sporulate on the host surface (Coates et al., 2002; Falconi et al., 2010; Jaronski, 1997; Rodríguez et al., 2006). In contrast, *Beauveria* strains exhibited greater sporulation than *Metarhizium*, a trait that, from an applied perspective, could favor horizontal transmission of the fungus, as susceptible individuals would come into contact with the new generation of conidia produced on mycosed cadavers (Benito-Delgado et al., 2025; Falconi et al., 2010). Considering that autodissemination is key in biological control and that commercial products often show limited adaptation to different agroecological zones, *Beauveria peruviensis*, being native and having shown high levels of mortality and sporulation in this study, emerges as a promising candidate for formulations incorporating vegetable oils that enhance conidial adhesion and germination, thereby contributing to a reduction in agrochemical use (Benito-Delgado et al., 2025; Sujithra et al., 2025).

The results of this study not only provide evidence of the potential of entomopathogenic fungi for controlling *Metamasius hemipterus*, but also open up the possibility of advancing toward the development of biological formulations based on native fungi and evaluating their performance under field conditions.

5 Conclusions

Through the use of molecular and morphological tools, the full identity of *Metamasius hemipterus* L. is confirmed for the first time as the primary pest of sugarcane plantations in the Amazonas region of Peru.

This results provide clear evidence that *Metarhizium anisopliae* and *Beauveria peruviensis* exhibit highest biological control potential against *Metamasius hemipterus* (Coleoptera: Dryophthoridae). This information will allow to address the practical challenges of sugarcane production and the development of environmentally friendly strategies for controlling the main sugarcane pest in this part of the world.

Data availability statement

The data are publicly available in the following GitHub repository: https://github.com/WAHGNERM2002/Sugarcane-weevil-Traits/tree/main?tab=readme-ov-file.

Author contributions

SL-E: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AR: Investigation, Methodology, Writing – original draft, Writing – review & editing. VA: Writing – original draft, Formal Analysis, Investigation, Methodology, Writing – review & editing. ER: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. WM-M: Writing – review & editing, Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft. DB-M: Writing – original draft, Data curation, Formal Analysis, Investigation, Methodology, Writing – review & editing. AH-P: Writing – review & editing, Conceptualization, Investigation, Methodology, Visualization, Writing – original draft. FL: Writing – review & editing, Conceptualization, Investigation. ML: Writing – review & editing, Conceptualization, Investigation, Methodology, Writing – original draft. MO-C: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. JM-Q: Writing – review & editing, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This study was developed within the framework of the project “Creación del servicio de promoción de la ciencia, tecnología e innovación tecnológica en centro de investigación para el manejo integrado de plagas y enfermedades ‘CEMIPE’ of the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas” (CUI 2671977). The authors would also like to thank the Vice-rectorate of Research of the Universidad Nacional Toribio Rodríguez de Mendoza for the financial support granted for the payment of the APC of the article.

Acknowledgments

The authors are grateful to the Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES).

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

References

Abbott, W. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18, 265–267. doi: 10.1093/jee/18.2.265a

Alpizar, D., Fallas, M., Allan, O., Gonzales, L., Chinchilla, C., and Bulgarelli, J. (2012). Trampeo masivo con feromonas del gorgojo de la caña de azúcar de las Indias Occidentales y el gorgojo americano (Coleoptera: Curculionidae) en palmito. *Florida Entomologist*. 38, 245–252. doi: 10.1653/0015-4040(2002)085[0426:PMTOTW]2.0.CO;2

Al-Sarar, A. S., Abobakr, Y., Alzabib, A. A., and Saleh, A. A. (2024). First report on banana weevil, *Cosmopolites sordidus* (Germar 1823) (Coleoptera: Curculionidae), an exotic economically important pest from Saudi Arabia. *Neotropical Entomol.* 53, 461–468. doi: 10.1007/s13744-024-01141-w

Argomedo, A., Rodríguez-Reyes, S., Rodríguez-Torres, C., Saldaña, M., Santos, W., and Krugg, J. W. (2015). Efecto del fungicida Metalaxyl sobre la germinación, crecimiento y capacidad antagónica de Trichoderma asperellum en condiciones de laboratorio. *Rev. REBIOLEST* 3, 37–42. Available online at: https://revistas.unitru.edu.pe/index.php/ECCBB/article/view/1709. (Accessed June 23, 2025).

Benito-Delgado, Á., Diez-Hermano, S., and Diez, J. J. (2025). Possibilities of native endophytic fungi as entomopathogenic biocontrol agents at a local scale: The case of deciduous and non-deciduous Mediterranean forest trees. *J. Forest. Res.* 36, 26. doi: 10.1007/s11676-024-01815-6

Broetto, L., Da Silva, W. O. B., Bailão, A. M., De Almeida Soares, C., Vainstein, M. H., and Schrank, A. (2010). Glyceraldehyde-3-phosphate dehydrogenase of the entomopathogenic fungus *Metarhizium anisopliae*: Cell-surface localization and role in host adhesion: *Metarhizium* GAPDH: cell surface and role in host adhesion. *FEMS Microbiol. Lett.* 312, 101–109. doi: 10.1111/j.1574-6968.2010.02103.x

Bustamante, D. E., Oliva, M., Leiva, S., Mendoza, J. E., Bobadilla, L., Angulo, G., et al. (2019). Phylogeny and species delimitations in the entomopathogenic genus Beauveria (Hypocreales, Ascomycota), including the description of B. Peruviensis sp. Nov. *MycoKeys* 58, 47–68. doi: 10.3897/mycokeys.58.35764

Butt, T. M., Ibrahim, L., Clark, S. J., and Beckett, A. (1995). The germination behaviour of *Metarhizium anisopliae* on the surface of aphid and flea beetle cuticles. *Mycol. Res.* 99, 945–950. doi: 10.1016/S0953-7562(09)80754-5

Campos, J., and Velásquez, H. (2016). Actividad biológica de dos cepas de *Metarhizium anisopliae* (Deuteromycota: Hyphomycetes) sobre *Rhynchophorus palmarum L. (Coleóptera: Curculionidae) en condiciones de laboratorio* (Villavicencio, Meta, Colombia: Tesis de grado, Bongara).

Casteliani, A., de Fatima Martins, L., Maringoli Cardoso, J. F., Oliveira Silva, M. S., Abe da Silva, R. S., Chacon-Orozco, J. G., et al. (2020). Aspectos del comportamiento de *Sphenophorus levis* (Coleoptera: Curculionidae), daño a la caña de azúcar y su infección natural por *Steinernema carpocapsae* (Nematoda: Rhabditidae). *Crop Prot.* 137, 105262. doi: 10.1016/j.cropro.2020.105262

Castrillo, L. A., Mayfield, A. E., Griggs, M. H., Camp, R., Mudder, B., Taylor, A., et al. (2017). Mortality and reduced brood production in walnut twig beetles, *Pityophthorus juglandis* (Coleoptera: Curculionidae), following exposure to commercial strains of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium brunneum*. *Biol. Control* 114, 79–86. doi: 10.1016/j.biocontrol.2017.08.007

Castrillo, L. A., Wraight, S. P., Galaini-Wraight, S., Matsumoto, T. K., Howes, R. L., and Keith, L. M. (2020). Genetic diversity among naturally-occurring strains of Beauveria bassiana associated with the introduced coffee berry borer, Hypothenemus hampei, (Coleoptera: Curculionidae) on Hawai'i Island. *J. Invertebrate Pathol.* 175, 107456. doi: 10.1016/j.jip.2020.107456

Chamorro, M. (2019). An illustrated synoptic key and comparative morphology of the larvae of dryophthorinae (Coleoptera, curculionidae) genera with emphasis on the mouthparts. *Diversity* 11, 4. doi: 10.3390/d11010004

Chuquibala-Checan, B., Torres-de La Cruz, M., Leiva, S., Hernandez-Diaz, E., Rubio, K., Goñas, M., et al. (2023). *In Vitro* Biological Activity of Beauveria bassiana, Beauveria Peruviensis, and Metarhizium sp. against Hypothenemus hampei (Coleoptera: Curculionidae). *Int. J. Agron.* 2023, 1–9. doi: 10.1155/2023/4982399

Coates, B. S., Hellmich, R. L., and Lewis, L. C. (2002). Allelic variation of a Beauveria bassiana (Ascomycota: Hypocreales) minisatellite is independent of host range and geographic origin. *Genome* 45, 125–132. doi: 10.1139/g01-132

Delgado, P. A. M., and Murcia-Ordoñez, B. (2011). Hongos entomopatógenos como alternativa para el control biológico de plagas. *Ambiente e Agua - Interdiscip. J. Appl. Sci.* 6, 77–90. doi: 10.4136/ambi-agua.187

Díaz-Valderrama, J. R., Nguyen, H. D. T., and Aime, M. C. (2017). *Wallemia Peruviensis* sp. Nov., a new xerophilic fungus from an agricultural setting in South America. *Extremophiles* 21, 1017–1025. doi: 10.1007/s00792-017-0960-0

Duque-Gamboa, D., Caicedo-Vallejo, A., Montoya-Lerma, J., Vallejo-Espinosa, L., and Muñoz-Flórez, J. (2012). Caracterización molecular del complejo picudo de plátano (coleóptera: Curculionidae), géneros Cosmopolites y Metamasius, en tres localidades de Colombia. *Acta Agronómica* 61, 26–27. Available online at: https://www.redalyc.org/articulo.oa?id=169959497013 (Accessed September 11, 2025).

Dwi Sutanto, K., Husain, M., Rasool, K. G., Elbashir, M. I., Mankin, R. W., Omar, A. O., et al. (2024). Isolation, molecular identification, and efficacy of indigenous entomopathogenic fungus isolates against Tribolium confusum (Coleoptera: Tenebrionidae) larvae and adults. *Plant Prot. Sci.* 60, 258–266. doi: 10.17221/131/2023-PPS

Edler, D., Klein, J., Antonelli, A., and Silvestro, D. (2021). raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods Ecol. Evol.* 12, 373–377. doi: 10.1111/2041-210X.13512

Falconi, F., Flores, A., and Castellanos, P. (2010). Letalidad de hongos entomopatogenos sobre Dysdercus Peruvianus (Hemiptera: Pirrhocoridae). *Rev. Peruana Biol.* 17, 225–229. doi: 10.15381/rpb.v17i2.32

Faria, M., Lopes, R. B., Souza, D. A., and Wraight, S. P. (2015). Conidial vigor vs. Viability as predictors of virulence of entomopathogenic fungi. *J. Invertebrate Pathol.* 125, 68–72. doi: 10.1016/j.jip.2014.12.012

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299. Available online at: https://pubmed.ncbi.nlm.nih.gov/7881515/ (Accessed August 19, 2025).

Gebremariam, A., Chekol, Y., and Assefa, F. (2022). Extracellular enzyme activity of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* and their pathogenicity potential as a bio-control agent against whitefly pests, *Bemisia tabaci* and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). *BMC Res. Notes* 15, 117. doi: 10.1186/s13104-022-06004-4

Gómez, H., Zapata, A., Torres, E., and Tenorio, M. (2014). *Manual de producción y uso de hongos entomopatógenos* (Lima, Peru: Servcio Nacional de Sanidad Agraria (SENASA).

Goyes, J. J. (2020). *Descripción del picudo rayado (*Metamasius hemipterus *L) en el cultivo de la caña de azúcar (Saccharum officinarum.* UTB, BABAHOYO. Available online at: http://dspace.utb.edu.ec/handle/49000/8210 (Accessed August 30, 2025).

Insuasty, O. (1997). *Plagas de la caña de azúcar para panela*. Available online at: http://hdl.handle.net/20.500.12324/675 (Accessed August 25, 2025).

Jaronski, S. (1997). *New Paradigms in Formulating Mycoinsecticides. Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328.* Eds. G. R. Goss, M. J. Hopkinson and H. M. Collins (Philadelphia: ASTM), 99–114.

Khun, K. K., Ash, G. J., Stevens, M. M., Huwer, R. K., and Wilson, B. A. L. (2020). Response of the macadamia seed weevil Kuschelorhynchus macadamiae (Coleoptera: Curculionidae) to Metarhizium anisopliae and Beauveria bassiana in laboratory bioassays. *J. Invertebrate Pathol.* 174, 107437. doi: 10.1016/j.jip.2020.107437

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–1549. doi: 10.1093/molbev/msy096

Kumar, T., Wang, J.-G., Xu, C.-H., Lu, X., Mao, J., Lin, X.-Q., et al. (2024). Genetic engineering for enhancing sugarcane tolerance to biotic and abiotic stresses. *Plants* 13, 1739. doi: 10.3390/plants13131739

Leiva, S., Oliva-Cruz, M., Rubio-Rojas, K., Maicelo-Quintana, J., and Milla-Pino, M. (2019). Use of color traps and alcoholic attractants for the capture of the coffee berry borer (Hypothenemus hampei) in highly infested coffee plantations. *Rev. Colombiana Entomol.* 45, Article 2. doi: 10.25100/socolen.v45i2.8537

Liu, S.-Q., Meng, Z.-H., Yang, J.-K., Fu, Y.-X., and Zhang, K.-Q. (2007). Characterizing structural features of cuticle-degrading proteases from fungi by molecular modeling. *BMC Struct. Biol.* 7, 33. doi: 10.1186/1472-6807-7-33

Lopes, R. B., Mesquita, A. L. M., Tigano, M. S., Souza, D. A., Martins, I., and Faria, M. (2013). Diversity of indigenous *Beauveria* and *Metarhizium* spp. in a commercial banana field and their virulence toward *Cosmopolites sordidus* (Coleoptera: Curculionidae). *Fungal Ecol.* 6, 356–364. doi: 10.1016/j.funeco.2013.06.007

López, J. (2015). *La caña de azucar (Saccharum officinarum) para la producción de panela. Caso: Nordeste del departamento de Antioquia* (Antioquia, Colombia: Universidad Nacional Abierta y a Distancia (UNAD).

Lu, H.-L., and St. Leger, R. J. (2016). "Insect immunity to entomopathogenic fungi," in *Advances in Genetics*, vol. 94. (Amsterdam, The Netherlands: Elsevier), 251–285. doi: 10.1016/bs.adgen.2015.11.002

Ma, Z., Ren, J., and Zhang, R. (2022). Identifying the genetic distance threshold for entiminae (Coleoptera: curculionidae) species delimitation via COI barcodes. *Insects* 13, 261. doi: 10.3390/insects13030261

Mariappan, K., Samuel, J. N., Uthandi, S., Sevugapperumal, N., Subbarayalu, M., and Krishnamoorthi, P. (2025). Harnessing the potential of indigenous entomopathogenic fungi isolated from the soils of Tamil Nadu, India, for the management of the cotton aphid, Aphis gossypii Glover (Hemiptera: Aphididae). *J. Cotton Res.* 8, 34. doi: 10.1186/s42397-025-00232-2

Mathulwe, L. L., Jacobs, K., Malan, A. P., Birkhofer, K., Addison, M. F., and Addison, P. (2021). Characterisation of Metarhizium majus (Hypocreales: Clavicipitaceae) isolated from the Western Cape Province, South Africa. *PloS One* 16, e0240955. doi: 10.1371/journal.pone.0240955

Mendoza, J., Ayora, A., and Gualle, D. (2004). Determinación de la importancia del picudo rayado, *Metamasius hemipterus L., en caña de azúcar* (Ecuador: Entomología del CINCAE).

Merino, L., France, A., and Gerding, M. (2007). Selección de Aislamientos Nativos de Hongos Patogénicos a Vespula germanica (Hymenoptera: Vespidae). *Agric. Técnica* 67, 335–342. doi: 10.4067/S0365-28072007000400001

Milindi, P. S., Nsenuka, E. E., and Chopra, S. S. (2024). Driving sustainability in the sugarcane industry: Life Cycle Assessment of conventional and emerging spraying technologies in Tanzania. *Sci. Total Environ.* 955, 176963. doi: 10.1016/j.scitotenv.2024.176963

Msomba, B. H., Ndaki, P. M., and Joseph, C. O. (2024). Sugarcane sustainability in a changing climate: A systematic review on pests, diseases, and adaptive strategies. *Front. Agron.* 6. doi: 10.3389/fagro.2024.1423233

Mustafa, U., and Kaur, G. (2009). Extracellular enzyme production in *Metarhizium anisopliae* isolates. *Folia Microbiol.* 54, 499–504. doi: 10.1007/s12223-009-0071-0

Negrete-González, D., Ávalos-Chávez, M. A., Lezama-Gutiérrez, R., Chan-Cupul, W., Molina-Ochoa, J., and Galindo-Velasco, E. (2018). Suitability of Cordyceps bassiana and *Metarhizium anisopliae* for biological control of *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) in an organic Mexican banana plantation: Laboratory and field trials. *J. Plant Dis. Prot.* 125, 73–81. doi: 10.1007/s41348-017-0126-4

Oliva-Cruz, M., Servan Bardales, J. M., Leiva-Espinoza, S. T., Oliva-Cruz, C., Mendez-Fasabi, L. D., and Juarez-Contreras, L. (2024). Compatibility of Native Strains of Beauveria Peruviensis and Metarhizium sp. As Strategy for Biological Control of Coffee Berry Borer (Hypothenemus hampei, Ferrari). *Agronomy* 14 (5), 904. doi: 10.3390/agronomy14050904

Ortiz-Urquiza, A., and Keyhani, N. (2013). Action on the surface: entomopathogenic fungi versus the insect cuticle. *Insects* 4, 357–374. doi: 10.3390/insects4030357

Palmieri, L., Lourdes Chamorro, M., and Sharma, P. P. (2022). Phylogenetic assessment of the Metamasius hemipterus species complex (Coleoptera, Curculionidae, Dryophthorinae). *Mol. Phylogenet. Evol.* 175, 107589. doi: 10.1016/j.ympev.2022.107589

Pedrini, N., Crespo, R., and Juárez, M. P. (2007). Biochemistry of insect epicuticle degradation by entomopathogenic fungi. *Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol.* 146, 124–137. doi: 10.1016/j.cbpc.2006.08.003

Pillai, S., Kobayashi, K., Michael, M., Mathai, T., Sivakumar, B., and Sadasivan, P. (2021). John William Trevan's concept of Median Lethal Dose (LD50/LC50) – more misused than used. *J. Pre-Clinical Clin. Res.* 15, 137–141. doi: 10.26444/jpccr/139588

Qazi, S. S., and Khachatourians, G. G. (2007). Hydrated conidia of *Metarhizium anisopliae* release a family of metalloproteases. *J. Invertebrate Pathol.* 95, 48–59. doi: 10.1016/j.jip.2006.12.002

Qu, S., and Wang, S. (2018). Interaction of entomopathogenic fungi with the host immune system. *Developmental & Comparative Immunology. 83*, 96–103. doi: 10.1016/j.dci.2018.01.010

Reyes-Figueroa, O., Ortiz-García, C. F., Torres-de La Cruz, M., Lagunes-Espinoza, L. D. C., and Valdovinos-Ponce, G. (2016). Especies de Trichoderma del agroecosistema cacao con potencial de biocontrol sobre Moniliophthora roreri. *Rev. Chapingo Serie Cienc. Forestales y del Ambiente* 22, 149–163. doi: 10.5154/r.rchscfa.2015.08.036

Risco, S. (1967). Metamasius hemipterus L. «gorgojo rayado de la caña de azúcar»: Control del insecto en los ingenios «San Carlos»y «Valdez». *Rev. Peruana Entomol.* 10, 82–95. Available online at: https://sisbib.unmsm.edu.pe/BVRevistas/entomologia/v10/pdf/a14v10.pdf (Accessed August 13, 2025)

Rivera, G., and Pinto, L. (1999). *Evaluación de patogenicidad de aislamientos nativos de hongos entomopatógenos sobre el gusano blanco de la papa*, Premnotrypes vorax (Hustache). *Rev. Colombiana Biotecnol.* III, 53–65.

Rodríguez, M., Gerding, M., and France, A. (2006). Selección de Aislamientos de Hongos Entomopatógenos para el Control de Huevos de la Polilla del Tomate, Tuta absoluta Meyrick (Lepidoptera: Gelechiidae). *Agric. Técnica* 66, 151–158. doi: 10.4067/S0365-28072006000200005

Rubio-G., J. D., and Acuña-Z., J. R. (2006). Anatomía comparada del tracto digestivo en imagos del complejo picudo (Coleoptera: Curculionidae) asociados al cultivo del plátano. *Rev. Colombiana Entomol.* 32, 67–72. doi: 10.25100/socolen.v32i1.9363

Rustiguel, C. B., Fernández-Bravo, M., Guimarães, L. H. S., and Quesada-Moraga, E. (2018). Different strategies to kill the host presented by *Metarhizium anisopliae* and *Beauveria bassiana*. *Can. J. Microbiol.* 64, 191–200. doi: 10.1139/cjm-2017-0517

Salazar, J., and Chávez, S. (2013). *Estrategias modernas de manejo y control de plagas en el cultivo de la caña de azúcar*. San José, Costa Rica: Costa Rican Sugar Cane Industry League (LAICA), Sugar Cane Research and Extension Directorate (DIECA).

Saleem, A.-R, and ChálIbrahim, R. A. (2019). Assessment of the virulence and proteolytic activity of three native entomopathogenic fungi against the larvae of Oryctes agamemnon (Burmeister) (Coleoptera: Scarabaeidae). *Egypt. J. Biol. Pest Control.* 29, 21. doi: 10.1186/s41938-019-0120-1

Sepúlveda-Cano, P. A., Entomología, M., Rubio-Gómez, J. D., and Entomología, M. (2009). ESPECIES DE DRYOPHTHORINAE (COLEOPTERA: CURCULIONIDAE) ASOCIADAS A PLÁTANO Y BANANO (Musa *spp.*) EN COLOMBIA. Bogotá, Colombia: Universidad Nacional de Colombia.

Shang, Y., Feng, P., and Wang, C. (2015). Fungi that infect insects: altering host behavior and beyond. *PloS Pathog.* 11, e1005037. doi: 10.1371/journal.ppat.1005037

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomological Soc. America* 87, 651–701. doi: 10.1093/aesa/87.6.651

Stephenson, G. R., Ferris, I. G., Holland, P. T., and Nordberg, M. (2006). Median Lethal Concentration: From: Glossary of Terms Relating to Pesticides *[Dataset]* (Berlin, Germany: De Gruyter). doi: 10.1515/iupac.78.0408

Suárez, H. (2009). Patogenicidad de Beauveria bassiana (Deuteromycotina: Hyphomycetes) sobre Sitophilus zeamais motschulsky (Coleoptera: Curculionidae) plaga de maíz almacenado. *Intropica* 4, 47–53.

Sujithra, M., Prathibha, V. H., Daliyamol, M., Madhu, T. N., Josephrajkumar, A., and Govindharaj, G.-P.-P. (2025). Biological control of the red palm weevil, Rhynchophorus ferrugineus olivier (coleoptera: Curculionidae) using indigenous Beauveria bassiana (Bb-045) in coconut palms. *Egyptian J. Biol. Pest Control* 35, 21. doi: 10.1186/s41938-025-00856-1

Takiya, D. M., Tran, P. L., Dietrich, C. H., and Moran, N. A. (2006). Co-cladogenesis spanning three phyla: Leafhoppers (Insecta: Hemiptera: Cicadellidae) and their dual bacterial symbionts. *Mol. Ecol.* 15, 4175–4191. doi: 10.1111/j.1365-294X.2006.03071.x

Valle, S., and Caicedo, W. (2019). "Efficacy of native isolates of Metarhizium spp. In the control of the weevil (Metamasius hemipterus) of sugarcane in laboratory conditions," in *Proceedings of MOL2NET 2019, International Conference on Multidisciplinary Sciences, 5th Edition*. MDPI, Basel, Switzerland. doi: 10.3390/mol2net-05-06720

Vásquez, M. (2015). Efecto de *Beauveria bassiana* e *Isaria fumororosea* sobre, *Oligonychus punicae en condiciones de laboratorio* (Trujillo-Perú: Universidad Nacional de Trujillo (UNT)).

Vaurie, P. (1967). A revision of the Neotropical genus Metamasius (Coleoptera, Curculionidae, Rhynchophorinae). Species group 3. Bulletin of the AMNH ; v. 136, article 4. (New York : American Museum of Natural History). Available online at: http://hdl.handle.net/2246/1990.

Wang, M., Li, A.-M., Pan, Y.-Q., Chen, Z.-L., Qin, C.-X., Su, Z.-L., et al. (2025). Gibberellin biosynthesis gene ScGA20 oxidase enhances sugarcane growth by modulating genes associated with phytohormone and growth processes. *Plant Physiol. Biochem.* 221, 109652. doi: 10.1016/j.plaphy.2025.109652

Wang, C., and St Leger, R. J. (2007). The MAD1 adhesin of *Metarhizium anisopliae* links adhesion with blastospore production and virulence to insects, and the MAD2 adhesin enables attachment to plants. *Eukaryot. Cell* 6, 808–816. doi: 10.1128/EC.00409-06

Weissling, T., and Giblin-Davis, R. (2003). *Silky Cane Weevil,* Metamasius hemipterus sericeus *(Olivier) (Insecta: Coleoptera: Curculionidae): EENY-053/IN210, 5/2003* Vol. 2003 (Gainesville, FL, United States: EDIS). doi: 10.32473/edis-in210-1998

Weissling, T., and Giblin-Davis, R. (2010). *Silky Cane Weevil, Metamasius hemipterus* sericeus (Olivier) (Insecta: Coleoptera: Curculionidae). Available online at: https://edis.ifas.ufl.edu/pdffiles/IN/IN21000.pdf.

Weissling, T., Giblin-Davis, R., Center, B., Heath, R., and Peña, J. (2003). Oviposition by METAMASIUS HEMIPTERUS SERICEUS (COLEOPTERA: DRYOPHTHORIDAE: RHYNCHOPHORINAE). *Florida Entomol.* 86, 174–177. doi: 10.1653/0015-4040(2003)086%255B0174:OBMHSC%255D2.0.CO;2

Xiao, G., Ying, S.-H., Zheng, P., Wang, Z.-L., Zhang, S., Xie, X.-Q., et al. (2012). Genomic perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. *Sci. Rep.* 2, 483. doi: 10.1038/srep00483

Xu, W., Gan, Q., Pu, J., Pan, Y., Cai, B., Ao, S., et al. (2021). *Gene Sequence Analysis of Mitochondrial COI in Genus Saperda (Coleoptera: Cerambycidae: Lamiinae)* (ZooKeys). ARPHA Preprints. doi: 10.3897/arphapreprints.e70782

Yasin, M., Wakil, W., Ghazanfar, M. U., Qayyum, M. A., Tahir, M., and Bedford, G. O. (2019). Virulence of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against red palm weevil, *Rhynchophorus ferrugineus* (Olivier). *Entomological Res.* 49, 3–12. doi: 10.1111/1748-5967.12260

Zamora-Avilés, N., Orozco-Flores, A. A., Cavazos-Vallejo, T., Romo-Sáenz, C. I., Cuevas-García, D. A., Gomez-Flores, R., et al. (2024). Intra-Phenotypic and -Genotypic Variations of Beauveria bassiana (Bals.) Vuill. Strains Infecting Aedes aEgypti L. Adults. *Int. J. Mol. Sci.* 25, 8807. doi: 10.3390/ijms25168807

Zárate, H. (2016). Adaptación de Metarhizium *sp como entomopatógeno de garrapatas* (México: Universidad Autónoma Agraria Antonio Narro (UAAAN)).

Zare, M., Talaei-Hassanloui, R., and Fotouhifar, K.-B. (2014). Relatedness of proteolytic potency and virulence in entomopathogenic fungus *Beauveria bassiana* isolates. *J. Crop Prot.* 3, 425–434. doi: 10.48311/jcp.2014.1151

Zhang, D., Qi, H., and Zhang, F. (2025). Parasitism by entomopathogenic fungi and insect host defense strategies. *Microorganisms* 13, 283. doi: 10.3390/microorganisms13020283

Zhao, L., Ran, M., Zhang, J., Zhao, P., Zan, F., Zhao, J., et al. (2025). Comparative analysis of ratoon-competent and ratoon-deficient sugarcane by hormonal and transcriptome profiling. *Agronomy* 15, 1669. doi: 10.3390/agronomy15071669

Zorzenon, F. J., Bergmann, E. C., and Bicudo, J. E. A. (2000). Primeira ocorrência de metamasius hemipterus (Linnaeus 1758) E metamasius ensirostris (Germar 1824) (Coleoptera, Curculionidae) em palmiteiros dos gêneros euterpe e bactris (arecaceae) no Brasil. *Arquivos do Instituto Biológico* 67, 265–268. doi: 10.1590/1808-1657v67n2p2652000.