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EVALUATION AND MOLECULAR CHARACTERIZATION OF A NATIVE *BEAUVERIA BASSIANA* ISOLATE FOR THE BIOLOGICAL CONTROL OF *RHIPICEPHALUS MICROPLUS* IN TROPICAL CATTLE PRODUCTION

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ABSTRACT

Rhipicephalus microplus is a tick of major veterinary relevance that causes significant economic losses in tropical cattle production due to its blood-feeding behavior and its role as a vector of hemoparasites. The extensive use of chemical acaricides has led to the development of resistant tick populations, highlighting the need for sustainable biological alternatives. Among these, entomopathogenic fungi such as Beauveria bassiana have gained attention for their efficacy against arthropod pests and low environmental impact. This study aimed to evaluate the in vitro and in situ efficacy of a native B. bassiana isolate against engorged females of R. microplus and to confirm its taxonomic identity through multilocus phylogenetic analysis. Two treatments were applied by immersing ticks in 5 mL and 10 mL of a conidial suspension (1×10^8 conidia/mL), along with an untreated control group. Fungal infection was assessed based on the presence of external mycelial growth on tick cadavers. DNA was extracted from the fungal isolate, and the ITS, RPB1, and TEF1-α regions were amplified and sequenced. Phylogenetic analyses using Bayesian inference and maximum likelihood confirmed the identity of the isolate as B. bassiana, showing high similarity to known type strains. The treatments resulted in infection rates of 64.1% (5 mL) and 71.8% (10 mL), while no infection was observed in the control group. These findings demonstrate the potential of this native B. bassiana isolate as a

biological control agent against R. microplus and support its integration into sustainable pest management strategies in tropical livestock systems.

KEYWORDS: Rhipicephalus microplus, Beauveria bassiana, biological control, phylogenetic analysis.

1. INTRODUCTION

Cattle farming is one of the most important agricultural activities in Mexico, due to its contribution to food security and its impact on the economic and social development of rural regions. The Mexican tropics, particularly the warm-humid areas such as the state of Veracruz, offer favorable agroecological conditions for dual-purpose production systems, which are characterized by the simultaneous generation of meat and milk under extensive or semi-intensive management [1]. This activity represents a vital source of income for millions of small- and medium-scale producers, and it is estimated that over 60% of the national herd is concentrated in tropical regions [8]. In addition to providing meat and milk, the sector generates employment and strengthens local value chains.

However, tropical livestock production faces major sanitary challenges, primarily those associated with ectoparasite-borne diseases. Ticks are among the most harmful ectoparasites, as they compromise the health and productivity of cattle herds, causing substantial economic and social impacts [5]. Besides the physical damage caused by their hematophagous activity, ticks are vectors of serious diseases such as babesiosis and anaplasmosis, which lead to weight loss, reduced milk production, and, in severe cases, death of the animal [7].

The traditional control of these ectoparasites relies mainly on chemical acaricides. However, prolonged and intensive use has resulted in the emergence of *Rhipicephalus microplus* resistant strains, leading to increased production costs, reduced treatment efficacy, and environmental contamination risks [5-2]. In response to these challenges, entomopathogenic fungi have emerged as viable and sustainable biological control alternatives. Among them, *Beauveria bassiana* stands out for its ability to infect a wide range of ectoparasite species without affecting non-target organisms or the environment [6]. This approach aligns with the principles of Integrated Pest Management (IPM), as it allows the inclusion of biological control agents within broader and more sustainable strategies.

The tropical region of Veracruz has been identified as a priority area for implementing such strategies due to its relevance in national cattle production and the high incidence of *R. microplus* infestations [3-4]. Moreover, factors such as climate change, soil degradation, and overgrazing have increased the vulnerability of livestock systems in the region. Studies conducted in this area have shown that the application of B. bassiana can significantly reduce tick populations under field conditions, particularly when combined with other management practices such as pasture rotation and the selection of tick-resistant cattle breeds [4]. This integrated approach helps reduce dependence on chemical products and promotes the long-term sustainability of livestock production systems.

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However, to ensure the efficacy and safety of B. bassiana-based bioproducts, it is essential to conduct precise taxonomic identification of fungal isolates and provide experimental evidence supporting their virulence and adaptation to local environmental conditions. In this context, the objective of this study was to evaluate the *in vitro* efficacy of a native *B. bassiana* isolate against engorged females of *R. microplus* and to confirm its identity through multilocus phylogenetic analysis. The results of this research will contribute to the development of adapted biological control strategies for tropical livestock systems in Veracruz, aiming for more sustainable, profitable, and environmentally responsible cattle farming.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in five dual-purpose cattle production units in Tantoyuca, Veracruz, Mexico: Los Olivos, Mirador, Las Conchitas, El Orejón, and Veredas. The farms raise *Bos indicus* and crossbreeds (Cebu, Brahman, Suiz-Bú, and Swiss) under semi-intensive systems.

To avoid interference in the biological control evaluation, farms were selected based on the criterion that cattle had not been treated with chemical acaricides for at least 20 days prior to tick collection.

2.2 Collection of Engorged Female Ticks

In May 2024, 20 engorged females of *Rhipicephalus microplus* were collected from each farm (n = 100). Ticks were manually removed using precision forceps from typical attachment sites (ears, dewlap, dorsum, chest), applying gentle twisting to avoid injury [30]. Specimens were placed in labeled vials and transported to the laboratory at the Instituto Tecnológico Superior de Tantoyuca.

2.3 Tick Disinfection and Handling

Ticks were surface-disinfected by immersion in 1% sodium hypochlorite, rinsed with sterile distilled water, and placed in sterile Petri dishes for subsequent analysis.

2.4 Taxonomic Identification

Morphological identification was performed using a VELAB stereomicroscope and the taxonomic keys of [7] and [26]. Diagnostic features included scutum shape and ornamentation, palp dimensions, capitulum base morphology, presence of festoons, and spiracular plate structure. All specimens were identified as engorged females of *R. microplus.*

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2.5 In Vitro Bioassay with Beauveria bassiana

A native isolate of Beauveria bassiana (source: soil/pathogen; specify) was cultured on PDA medium under controlled conditions (25 ± 2 °C, 70–80% RH, 12 h photoperiod) and stored at 4 °C until use.

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A conidial suspension (1×10^8 conidia/mL) was prepared, and two immersion treatments were applied:

T1: 5 mL conidial suspension

T2: 10 mL conidial suspension

A control group received sterile distilled water with 0.01% Tween® 80. Each tick was immersed for 1 minute, dried on sterile absorbent paper, and incubated individually (27 ± 1 °C, 80 ± 5% RH). Mortality and external mycelial growth were recorded every 48 hours.

2.6 Experimental Design – In Vitro Assay

A completely randomized design was used. Each Petri dish represented an experimental unit, containing three ticks. The variables analyzed were tick mortality and time to death. The distribution of treatments and sample sizes is shown in Table 1.

Table 1: Experimental design of the in vitro assay: treatments with Beauveria bassiana and number of *Rhipicephalus microplus* ticks evaluated per treatment.

Treatment	Conidial concentration (conidia/mL)	Petri dishes	Ticks per dish	Total ticks
T1	8×10^6	13	3	39
T2	1×10^8	13	3	39
Control	—	13	3	39
Total	—	39	—	117

2.7 Collection of Engorged Ticks for Larval Infestation Study

Additional *R. microplus* engorged females were collected at Las Conchitas ranch (Tantoyuca, Veracruz) from Suiz-Bú cattle. Ticks were gently removed using precision forceps, avoiding damage to ensure viability. Specimens (n = 150) were placed in labeled vials and transported to the laboratory.

2.8 Egg Laying and Larval Hatching

Ticks were surface-sterilized with 1% sodium hypochlorite, rinsed with sterile distilled water, and dried on absorbent paper. Their integrity was verified under a stereomicroscope. Morphological identification was confirmed using the keys of [7].

Three females were placed in each Petri dish with a moist chamber and incubated at 27 ± 1 °C for 10 days to allow oviposition [27]. Larval hatching occurred approximately 20 days after the start of oviposition.

2.9 Field Evaluation of Beauveria spp. on Tick Larvae

The field trial was conducted at Pénjamo ranch in Tantoyuca, Veracruz, a sub-humid tropical zone with an average annual temperature of 23.3 °C and precipitation of 1,236 mm. The experimental area was a pasture of *Cynodon dactylon* divided into sun and shade plots, each with nine 9 m² parcels, bordered with 1.5 m margins to avoid larval dispersion.

Treatments applied:

T1 – B5 mL: 1×10^8 spores/mL

T2 – B10 mL: 8×10^8 spores/mL

T3 – Control: distilled water only

Treatments were applied manually every three days (18:00–19:00 h) to reduce solar exposure and maintain spore viability [28-29]. Weeds were removed biweekly, and calcium hydroxide was applied thrice weekly to reduce larval migration.

Larval counts were conducted weekly using the drag cloth method [25]. A white flannel cloth was dragged over vegetation in 1-minute transects. Samples were sealed in nylon containers and transported to the lab, where they were incubated for 5 hours prior to counting.

2.10 Experimental Design – Field Trial

A completely randomized design with six replicates per treatment was used. The evaluated variables were:

Larval mortality (%)

Oviposition reduction

Presence or absence of mycelial growth

Mortality was recorded weekly to assess population decline over time.

2.11 Statistical Analysis

All data were processed in Excel and analyzed using one-way ANOVA to determine significant differences between treatments ($p < 0.05$). Results are presented as mean ± standard deviation.

2.12 Molecular Identification

2.12.1 DNA Extraction and Quantification

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DNA was extracted from the fungal isolate using the CTAB 2% protocol [31], with a buffer composed of Tris-HCl (100 mM, pH 8.0), EDTA (2 mM), CTAB (2%), and NaCl (1.4 M). All procedures were performed under sterile conditions in a laminar flow hood disinfected with sodium hypochlorite and 76% ethanol.

Fungal mycelium was scraped with a sterile loop and transferred to a mortar containing 1,000 µL of 2% CTAB solution. The homogenate was incubated at 96 °C for 90 minutes, followed by vortexing and addition of chloroform-isoamyl alcohol (24:1). After centrifugation (11,500 rpm, 15 min), the supernatant was extracted and the step repeated. DNA was precipitated with 100% cold ethanol (−20 °C) and incubated for 12 hours. The pellet was washed with 70% isopropanol, air-dried, and resuspended in 100 µL of HPLC-grade water. DNA was stored at −20 °C.

DNA concentration and purity were measured by spectrophotometry using 2 µL of sample.

2.12.2 PCR Amplification and Gel Electrophoresis

The ITS, RPB1, and RPB2 regions were amplified using specific primers. The PCR mix included HPLC-grade water, buffer, dNTPs, Taq polymerase, and primers. Each reaction contained 3 µL of DNA and 12 µL of reaction mix.

PCR products were separated via 1.5% agarose gel electrophoresis in 1× TAE buffer. Gels were stained and visualized under UV light to confirm the presence of bands corresponding to expected molecular weights.

3. RESULTS AND DISCUSSION

3.1 Taxonomic Identification of Ticks

The ectoparasite identified in this study corresponds to *Rhipicephalus (Boophilus) microplus*, commonly known as the cattle tick. Morphologically, *R. microplus* presents an oval body. Males exhibit a visible dorsal scutum with ornamentation, while females significantly expand during blood feeding, reaching up to 12 mm in length. Diagnostic features include short palps, a specialized piercing-sucking mouthpart, robust legs, and a forward-oriented capitulum—characteristic of the Boophilus subgenus.

R. microplus is a monoxenous tick, completing its entire life cycle on a single host, typically cattle. It holds high relevance in veterinary medicine due to its role as a vector of hemoparasites such as *Babesia bovis*, *Babesia bigemina*, and *Anaplasma marginale*, causative agents of bovine babesiosis (“tristeza bovina”).

Identification was confirmed morphologically using the taxonomic keys proposed by [9], based on features such as the shape and ornamentation of the scutum, palp length and arrangement, hypostome dentition, and the morphology of legs, spiracles, and genital plates. All specimens were accurately identified as *R. microplus* females.

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3.1 In Vitro Efficacy of Beauveria bassiana Against Engorged Females of Rhipicephalus microplus

The *in vitro* bioassay consisted of two treatments: immersion of ticks in 5 mL and 10 mL of a conidial suspension (1×10^8 conidia/mL), plus a control group with no fungal application. Fungal infection was confirmed by the presence of external mycelial growth.

Fungal infection was observed in 64.1% of ticks treated with 5 mL and in 71.8% of those treated with 10 mL, while no infection was detected in the control group (Table 2). A chi-square test ($\chi^2 = 19.185$; $p < 0.0001$) indicated significant differences among treatments, confirming the effectiveness of B. bassiana in colonizing *R. microplus* females.

Table 2: Mycelial growth in engorged females of *R. microplus* treated with B. bassiana

Treatment	Mycelium Present (P^+)	Mycelium Absent (A^-)
B5 mL	25	14
B10 mL	28	11
Control	0	39

These findings support the infective capacity of *B. bassiana*, even at the engorged stage, one of the tick's most resistant phases. Similar results were reported by [10], who evaluated *B. peruviensis* and observed up to 92% mortality in engorged females at concentrations of 1×10^9 conidia/mL, along with a 60% reduction in oviposition. Although mortality was not directly measured in this study, the high incidence of visible mycelium suggests lethal or sublethal infection.

In Mexico, [11] also demonstrated over 75% mortality in adult females of *R. microplus* and more than 25% reduction in viable egg numbers when exposed to native strains of *B. bassiana* and *Metarhizium anisopliae*. More recently, [12] evaluated native fungal isolates against *Amblyomma mixtum* larvae and reported clinical mortality rates exceeding 24%, highlighting the versatility of entomopathogenic fungi across tick genera.

Similarly, [24] observed over 90% mortality in *Amblyomma maculatum* exposed to 10^8 conidia/mL of *M. anisopliae*, with fungal colonization in more than 70% of treated individuals. These findings reinforce the feasibility of entomopathogenic fungi as viable alternatives to chemical acaricides in tropical tick control.

Overall, the results of this study confirm the potential of *B. bassiana* as a sustainable alternative to chemical acaricides. Fungal infection of engorged females may disrupt the reproductive cycle by reducing oviposition capacity and egg viability [11], thus contributing to long-term tick population management.

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3.2 In Situ Evaluation of Beauveria bassiana Against Larvae of Rhipicephalus microplus on Cynodon dactylon

Field trials evaluated two concentrations of B. bassiana (1×10^8 and 8×10^6 conidia/mL) under sun and shade conditions over three evaluation periods. Two variables were analyzed: number of live larvae and presence of external mycelium.

The Shapiro-Wilk test indicated non-normal data distribution ($p < 0.0001$); therefore, mixed models and analysis of variance (ANOVA) were applied. ANOVA results for mycelial presence showed significant effects of treatment, light condition, and evaluation date, as well as their interactions. However, Student's t-test detected no significant differences between sun and shade conditions ($p = 0.4646$), although a slightly higher mean mycelial presence was recorded under shade (7.65) compared to full sun (6.67).

For the variable "number of live larvae," neither the dose nor the application condition alone showed a statistically significant effect. However, the evaluation period was a key factor ($F = 308.1$; $p < 2.2 \times 10^{-16}$), suggesting that treatment success was strongly influenced by temporal and environmental conditions.

These findings are consistent with previous reports highlighting the influence of abiotic factors such as humidity, temperature, and solar radiation on fungal conidial viability and infection rates in natural environments. For instance, [23] demonstrated that UV exposure and low humidity significantly reduced the efficacy of entomopathogenic fungi under open-field conditions.

The significant interactions between dose, application condition, and evaluation period underscore that fungal efficacy does not rely on a single factor but rather on a combination of environmental and operational variables. This highlights the importance of designing application strategies that consider seasonality and microenvironmental variation in tropical livestock systems.

Together with findings from previous studies, the results from this trial support the integration of native *B. bassiana* strains into Integrated Tick Management (ITM) programs, particularly in tropical regions like Tantoyuca, Veracruz, where agroecological conditions favor spore persistence and activity.

3.3 Molecular Identification of the Native Beauveria Isolate

A consensus sequence was obtained by assembling the full ITS region and partial fragments of RPB1 and TEF1-α using BioEdit v7.0.5 [17]. Multiple sequence alignment was performed with MAFFT v7.475 [19], and sequences were compared with type strains in public databases (GenBank, UNITE, BOLD Systems), using Cordyceps javanica (NR_111172) as an outgroup. Gene alignments were concatenated in Mesquite v3.6 [20].

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Evolutionary model selection was conducted with jModelTest v2 [21]. Phylogenetic analyses were performed using Bayesian inference MrBayes v3.2.1 [32] and maximum likelihood raxmlGUI 1.5b2 [22]. For MrBayes, four Markov chains were run for two million generations, with a 25% burn-in. For ML analysis, a rapid bootstrap with 1,000 replicates under the GTR+G model was used. Resulting trees were visualized with FigTree v1.4.4 [18] and MEGA7 (Figure 1).

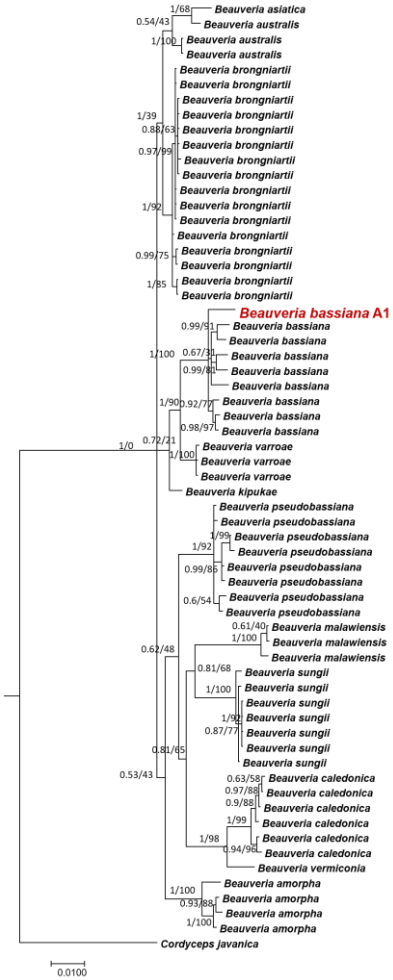

Figure 1. Phylogenetic tree of *Beauveria* strains constructed using concatenated multilocus data.

The use of multiple markers (ITS, TEF1-α, RPB1) is well supported in the literature for resolving species boundaries within Beauveria. MAFFT provides accurate alignments for both ribosomal and protein-coding regions [19], and BioEdit is reliable for raw sequence editing [17]. Studies by [15] and [16] have shown that multilocus datasets including ITS, TEF, RPB1, RPB2, and Bloc resolve robust clades within the *B. bassiana* complex.

In a recent comprehensive study, [14] applied multilocus phylogeny and five DNA-based species delimitation methods, based on genetic distance, coalescent theory, and genealogical concordance, demonstrating that polyphasic approaches are essential for robust species delineation in Beauveria. Their results supported the delimitation of 20–28 species, revealed cryptic diversity in *B. bassiana* and related taxa, and led to the formal description of *B. peruviensis* from northeastern Peru. Notably, their findings emphasized that congruence among methods, especially multilocus phylogeny, genetic distance, and coalescent analyses, provides reliable support for species boundaries in this genus.

These insights reinforce the approach adopted in the present study and highlight the importance of integrating multilocus phylogenetic frameworks when characterizing native isolates for potential biocontrol applications.

Notably, [15] demonstrated that combining RPB1, Bloc, and TEF1-α provides strong statistical support for cryptic species delimitation within Beauveria, using both Bayesian and ML approaches. Similarly [13], using six loci including ITS and RPB1, confirmed the existence of numerous cryptic species in the genus.

The selection of these markers and bioinformatics tools aligns with current standards in fungal phylogenetics. MrBayes provides detailed posterior probability estimates, while RAxML is known for computational efficiency and high bootstrap support. The congruence between both phylogenetic methods in this study strengthens the reliability of the final tree and taxonomic resolution.

The results validate the taxonomic identification of the isolate, positioning it within a well-supported Beauveria clade. Its phylogenetic placement confirms its identity and supports its candidacy as a genetically coherent and ecologically relevant biocontrol agent.

4. CONCLUSIONS

This study demonstrates the potential of a native isolate of *Beauveria bassiana* as an effective biological control agent against *Rhipicephalus (Boophilus) microplus* under both laboratory and field conditions in tropical environments. *In vitro* bioassays confirmed the pathogenicity of the fungus, with infection rates exceeding 70% in engorged females, highlighting its virulence even in the most resistant stage of the tick's life cycle.

Field trials revealed that although environmental variables such as light exposure and spore concentration influenced fungal activity, the evaluation period was the most critical factor affecting larval population dynamics. These findings underscore the importance of optimizing application strategies according to seasonal and microclimatic conditions to enhance fungal efficacy in pasture systems.

REFERENCES

[1] Rojo-Rubio, R., Vázquez-Armijo, J. F., Pérez-Hernández, P., Mendoza-Martínez, G. D., Salem, A. Z. M., Albarrán-Portillo, B., et al. (2009). Dual purpose cattle production in Mexico. Tropical Animal Health and Production, 41(5), 715–721.

[2] George, J. E., Pound, J. M., & Davey, R. B. (2004). Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitology, 129(S1), S353–S366.

[3] Instituto Nacional de Estadística y Geografía (INEGI). (2019). Encuesta Nacional Agropecuaria 2019. https://www.inegi.org.mx/programas/ena/2019/

[4] Lucero, J., Manzano, J., Loaiza, I., & Orellana, Y. (2024). Producción de *Beauveria bassiana* para la formulación de bioplaguicidas. La Granja, 113–129.

[5] Rodriguez-Vivas, R. I., Jonsson, N. N., & Bhushan, C. (2018). Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. Parasitology Research, 117(1), 3–29.

[6] Alonso-Díaz, M. A., & Fernández-Salas, A. (2021). Entomopathogenic fungi for tick control in cattle livestock from Mexico. Frontiers in Fungal Biology, 2, 657694. https://doi.org/10.3389/ffunb.2021.657694

[7] Guglielmone, A. A., Robbins, R. G., Apanaskevich, D. A., Petney, T. N., Estrada-Peña, A., & Horak, I. G. (2014). The hard ticks of the world (Vol. 10, pp. 978–994). Dordrecht: Springer.

[8] Rojas Sandoval, L.A., Albarrán Portillo, B., Mondragón Ancelmo, J., Martínez García, C.G., & García Martínez, A. (2024). La Ganadería de doble propósito en trópico seco: diversidad de unidades de producción y orientación productiva. Tropical and Subtropical Agroecosystems, 27(2). https://doi.org/10.56369/tsaes.5459

[9] Walker, A. R. (2003). Ticks of domestic animals in Africa: a guide to identification of species (Vol. 74). Edinburgh: Bioscience Reports.

[10] Zumaeta, V., Bardales, W., & Oliva, S. (2022). Patogenicidad in vitro de *Beauveria peruviensis* en hembras adultas de garrapatas *Rhipicephalus microplus*. Revista de investigación Agropecuaria Science and Biotechnology, 2(2), 01–14.

[11] Fernández-Salas, A., Rodríguez-Vivas, R. I., & Alonso-Díaz, M. A. (2012). First report of a Rhipicephalus microplus tick population multi-resistant to acaricides and ivermectin in the Mexican tropics. Veterinary Parasitology, 183(3-4), 338-342. 10.1016/j.vetpar.2011.07.028

[12] Alonso-Díaz, M. A., Jiménez-Ruíz, M., & Fernández-Salas, A. (2022). First evidence of the tickicide effect of entomopathogenic fungi isolated from Mexican cattle farms against *Amblyomma mixtum*. The Journal of Parasitology, 108(6), 539–544.

[13] Wang, Y., Fan, Q., Wang, D., Zou, W. Q., Tang, D. X., Hongthong, P., & Yu, H. (2022). Species diversity and virulence potential of the *Beauveria bassiana* complex and Beauveria scarabaeidicola complex. Frontiers in Microbiology, 13, 841604.

[14] Bustamante, D. E., Oliva, M., Leiva, S., Mendoza, J. E., Bobadilla, L., Angulo, G., & Calderon, M. S. (2019). Phylogeny and species delimitations in the entomopathogenic genus Beauveria (Hypocreales, Ascomycota), including the description of B. peruviensis sp. nov. MycoKeys, 58, 47.

[15] Rehner, S. A., & Buckley, E. (2005). A Beauveria phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologia, 97(1), 84–98. https://doi.org/10.1080/15572536.2006.11832842

[16] Rehner, S. A., Minnis, A. M., Sung, G. H., Luangsa-ard, J. J., Devotto, L., & Humber, R. A. (2011). Phylogeny and systematics of the anamorphic, entomopathogenic genus Beauveria. Mycologia, 103(5), 1055–1073.

[17] Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95–98.

[18] Rambaut, A. (2010). FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh.

[19] Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution, 30(4), 772–780.

[20] Maddison, W. P. (2008). Mesquite: a modular system for evolutionary analysis. Evolution, 62, 1103–1118.

[21] Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. Nature Methods, 9(8), 772–772.

[22] Silvestro, D., & Michalak, I. (2012). raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution, 12(4), 335–337.

[23] Inglis, G. D., Goettel, M. S., Butt, T. M., & Strasser, H. (2001). Use of hyphomycetous fungi for managing insect pests. In: Fungi as Biocontrol Agents: Progress, Problems and Potential (pp. 23–69). Wallingford, UK: CABI Publishing.

[24] Kirkland, B. H., Westwood, G. S., & Keyhani, N. O. (2004). Pathogenicity of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* to Ixodidae tick species. Journal of Medical Entomology, 41(4), 705–711.

[25] Salomón, J., Hamer, S. A., & Swei, A. (2020). A beginner's guide to collecting questing hard ticks (Acari: Ixodidae): a standardized tick dragging protocol. Journal of Insect Science, 20(6), 11.

[26] Bautista-Garfias, C. R. (2006). Entomología veterinaria esencial. Ed. INIFAP, México DF, México.

[27] Jaén-Torrijos, M., Álvarez-Calderón, V., Quintero-Noriega, R., Espinales, K., Rangel-Tapia, G., & Quintero-Vega, N. (2015). Sensibilidad al clorpirifos y cipermetrina en la garrapata *Rhipicephalus microplus* en fincas ganaderas de Panamá. Ciencia Agropecuaria, (22), 70–77.

[28] Nchu, F., Maniania, N. K., Hassanali, A., & Eloff, K. N. (2010). Optimizing modes of inoculation of Rhipicephalus ticks (Acari: Ixodidae) with a mitosporic entomopathogenic fungus in the laboratory. Experimental and Applied Acarology, 51(4), 373–382.

[29] Polar, P., Kairo, M. T., Moore, D., Pegram, R., & John, S. A. (2005). Comparison of water, oils and emulsifiable adjuvant oils as formulating agents for Metarhizium anisopliae for use in control of *Boophilus microplus*. Mycopathologia, 160(2), 151–157.

[30] Servicio Nacional de Sanidad y Calidad Agroalimentaria. (2025). Instructivo para la toma de muestras de interés en garrapata del bovino. SENASA. https://biblioteca.senasa.gob.ar/items/show/4409

[31] Doyle, J. (1991). DNA protocols for plants. In: *Molecular Techniques in Taxonomy* (pp. 283–293). Berlin: Springer.

[32] Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61(3), 539–542. https://doi.org/10.1093/sysbio/sys029