

First record and potential biocontrol implication of *Beauveria bassiana* infecting *Lema yerburyi* on *Vanda tricolor* from Bali, Indonesia

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Abstract. Sudiarta IP, Sanjaya DAPK, Dinarkaya SM, Wirya GNAS, Gargita IWD, Yudha IKW, Darmawati IAP, Fitriani Y, Arimbawa IM, Selangga DGW, Dwiyani R, Bakar NKA, Mohd-Yusuf Y. 2025. First record and potential biocontrol implication of *Beauveria bassiana* infecting *Lema yerburyi* on *Vanda tricolor* from Bali, Indonesia. *Biodiversitas* 26: 5755-5762. The application of the entomopathogenic fungus *Beauveria* sp. for biological control has emerged as a viable solution to the problem of excessive insecticide application. Morphological and molecular identification targeting the amplification range of 500-600 bp for entomopathogenic fungal rDNA and 700 bp for insect COI. Pathogenic tests through Koch's postulates, isolated and cultured using Potato Sucrose Agar (PSA) media. The results of the identification of the orchid beetle were identified as *Lema yerburyi*, and the common name "Orchid lema" was proposed to distinguish it from other orchid beetle pests. Macroscopic identification showed that the isolate *Beauveria* sp. has a white colony color, with a pale-yellow underside, aseptate hyphae and conidia, with conidia arranged in chains on conidiophores that show a zigzag pattern. Molecular analysis of *Beauveria* sp. isolates targeting the Internal Transcribed Spacer (ITS) region produced a DNA fragment of ~577 bp. Phylogenetic tree construction showed that this isolate grouped with *B. bassiana* from Malaysia. This study aimed to identify and confirm the entomopathogenic fungus naturally infecting *L. yerburyi* in Bali, and to evaluate its potential as a biological control agent. This finding expands the known host range of *B. bassiana* and provides a scientific basis for its potential application in Integrated Pest Management (IPM) programs for orchids. Future studies should evaluate field efficacy, optimize formulation, and assess its compatibility with existing cultural and biological practices to advance sustainable orchid production.

Keywords: *Beauveria bassiana*, biological control, entomopathogenic fungi, *Lema yerburyi*, orchid pest

INTRODUCTION

Orchids are among the most valuable ornamental plants in global horticulture, prized for their exotic flowers, high aesthetic appeal, and economic value. However, their cultivation presents significant challenges and requires special care, particularly in protecting against pests and diseases. The interaction between pests and pathogens in orchids is complex and can disrupt their sensitive reproductive cycles. Insect pests not only cause direct damage to a wide range of plants, including ornamentals, but also create entry points for pathogens such as fungi, viruses, and bacteria (Wielkopolan and Obrepalska-Stepłowska 2016).

One of the most important pests in orchid cultivation is the orchid beetle (*Lema* sp.), which attacks newly opened flowers of various orchid genera. Its larvae feed on these flowers, causing significant damage that reduces the ornamental value of orchids such as *Micropera*, *Spathoglottis*, *Epidendrum*, and *Papilionanthe* (Meena and Mani 2022; Ragesh and

Gogoi 2023). *Lema yerburyi* (Jacoby, 1908) (Coleoptera: Chrysomelidae) is a florivorous beetle known to infest several commercially cultivated orchid genera, including *Arachnis*, *Ascocenda*, *Dendrobium*, *Mokara*, and *Phalaenopsis* (Suresh and Prathapan 2025). Field observations in Bali in early 2025 identified this beetle species as a pest of *Vanda tricolor* Lindl., indicating that *L. yerburyi* infestations can lead to a decline in the aesthetic and commercial value of affected orchids.

In response to these challenges, strategies needed to manage orchid beetles involve the use of natural enemies, such as entomopathogenic fungi. Insect pathogenic fungi are microorganisms that can cause infections and diseases in insect hosts. The use of biological control products based on beneficial microorganisms has emerged as a practical and environmentally friendly solution to minimize the excessive use of insecticides (Köhl 2025). Biological pest control strategies are an attractive alternative method for pest control. Furthermore, these strategies integrate practices

aligned with the goals of sustainable agricultural systems (Galli et al. 2024).

Entomopathogenic fungi are host-specific, easy to produce and apply, have relatively low control costs, and are safe for humans and natural enemies (predators and parasitoids), making them a potential pest control strategy. The potential of entomopathogenic fungi has been extensively studied and reported to control several types of insect pests (Sudiarta et al. 2019, 2024a, 2024b). *Beauveria* sp. is one potential entomopathogenic fungus that has successfully controlled several pests naturally (Singh et al. 2017). Several reports relate to the succession of uses of the entomopathogenic fungus *B. bassiana* which is able to control several important pests such as *Spodoptera frugiperda* (Apirajkamol et al. 2023), *Helicoverpa armigera* (Boulamtat et al. 2025), *Tuta absoluta* (Vivekanandhan et al. 2024), *Odoiporus longicollis* (Viswakethu et al. 2022), *Spodoptera litura* (Fitriana et al. 2021; Gunjan and Srivastava 2024), *Leptocoriza acuta* (Sari and Kumalasari 2022), *Gonipterus platensis* (Mejia et al. 2024), *Ips typographus* (Fora et al. 2022), *Rhynchophorus ferrugineus* (Irshaid et al. 2024). The use of *Beauveria* sp. as a biological agent to suppress pest populations has several important advantages, including being highly effective in controlling plant pests, safe for humans and pets, and environmentally friendly (Wahjono et al. 2024).

The identity of *Beauveria* sp. fungal isolates associated with the *Lema* sp. orchid beetle remains poorly understood. Therefore, to determine the potential of *Beauveria* sp. fungal isolates, additional identification was carried out through morphological and molecular characteristics of *Beauveria* sp. (Hibbett et al. 2011). Confirmation of the *Lema* sp. orchid beetle species also requires morphological and molecular characterization using cytochrome C Oxidase I (COI) (Monti et al. 2020; Abdalla et al. 2022).

In recent years, breakthroughs in molecular techniques, particularly those based on DNA analysis using the Polymerase Chain Reaction (PCR), have enabled the development of rapid, accurate, and scalable methodologies for detecting and identifying various fungi. Ribosomal DNA (rDNA) and Internal Transcribed Spacer (ITS) genes enable the study of phylogenetic relationships among several groups of entomopathogenic fungi. Based on these considerations, this study aimed to identify and confirm the entomopathogenic fungus naturally infecting *L. yerburyi* in Bali, and to evaluate its potential as a biological control agent.

MATERIALS AND METHODS

Sample collection and identification of orchid beetles

Orchid beetles primarily target blooming orchid flowers; however, instances of attacks on leaves, young roots, and stems have also been documented. The beetles show a preference for the orchid genera *Vanda*, *Dendrobium*, *Aerides*, *Arachnis*, *Epidendrum*, and *Rhynchostylis*. The orchid species most frequently affected in Bali is *V. tricolor*. The fragrant aroma of *Vanda* orchid flowers is a key factor in attracting orchid beetles for feeding. Orchid beetles were collected

from *V. tricolor* orchid plantations in several areas in Bali at altitudes of around 228.67 to 1,281.92 m above sea level (masl), with coordinates between 8°15'05"S 115°09'05"E to 8°27'56"S 115°04'44"E. The samples were taken 5-10 per location. Samples were identified morphologically and molecularly. Morphological identification was conducted by examining the characteristics of adult orchid beetles (imago) and comparing them with previous studies (Suresh and Prathapan 2025), followed by molecular characterization (Selangga et al. 2023).

Exploration, isolation, and identification of insect pathogenic fungi associated with orchid beetles

A directed method was employed to investigate the presence of insect pathogenic fungi infecting orchid beetles, concentrating on various life stages of the insects: larvae, pupae, and imago collected from the field. The insects were subsequently observed for any signs of fungal infection. The pathogenic fungi found in the orchid beetles were isolated and cultured in the laboratory using a Potato Sucrose Agar (PSA) medium. Identification is achieved by examining morphological characteristics and molecular traits. Macroscopic and microscopic morphological identification of the fungus *Beauveria* sp. was performed based on the identification guide for entomopathogenic fungi by Humber (2005). Molecular identification was conducted using the modified method described by Sudiarta et al. (2024a, 2024b).

Total DNA extraction and DNA amplification with PCR

The total DNA extraction procedure refers to the Cetyl Trimethylammonium Bromide (CTAB) method, which has been modified by Aboul-Maaty and Oraby (2019). PCR amplification was conducted using MyTaq HS Red Mix (Bioline), targeting amplification ranges of 500-600 bp for entomopathogenic fungal rDNA and 700 bp for insect COI. The PCR process commenced with the preparation of the PCR master mix, which included 9.5 µL of ddH₂O, 12.5 µL of MyTaq Red Mix (2x), 20 µmol of forward primer, 20 µmol of reverse primer (detailed in Table 1), and the DNA template (Sudiarta et al. 2024a, 2024b). The amplified DNA, known as PCR products, was subsequently subjected to electrophoresis on a 1% TBE agarose gel. The DNA bands were subsequently visualized and analyzed with a UV transilluminator.

DNA sequencing

DNA samples of orchid beetles and *Beauveria* sp. fungi obtained from PCR amplification were sent to PT. Genetika Science Indonesia for sequencing. The sequencing data from the forward and reverse primers were combined using CLC Sequence Viewer 8 software, assembled, and analyzed in the National Center for Biotechnology Information (NCBI) database with the BLAST nucleotide-to-nucleotide program.

Sequences with the highest similarity and an outgroup were then aligned using the ClustalW method in Bioedit software version 7.7.1. The similarity was evident in the percent identity obtained from the BLAST analysis of the sequence in question.

Table 1. Primers used in this study

Primer name	Sequence (5'-3')	DNA Target	References
ITS1-Forward	TCCGTAGGTGAACCTGCGG	ITS rDNA of <i>Beauveria</i> sp.	(Sudiarta et al. 2024a)
ITS4-Reverse	TCCTCCGCTTATTGATATGC		
LCO-Forward	GGTCAACAAATCATAAAGATATTGG	COI of <i>Lema</i> Orchid Beetle	(Folmer et al. 1994)
HCO-Reverse	TAAACTTCAGGGTGACCAAAAAATCA		

Phylogenetic analysis

Phylogenetic tree construction for samples was performed using MEGA 11 software, using the Neighbor-Joining tree method and the bootstrap method, with 1,000 replications or repetitions, transition-transversions, and pairwise deletion gaps.

Koch's postulate test

Koch's postulate test begins with the dissolution of *Beauveria* sp. fungus that has been incubated for 21 days before being applied to orchid beetles. Petri dishes containing *Beauveria* sp. fungus were dissolved in distilled water and mixed with 0.05 mL Tween 80 until thoroughly combined. The resulting suspension was tested for its infectivity by inoculating pure isolates into the insects' bodies using the immersion method. After immersion, the test insects were placed in clear glass containers with food and covered with gauze. The dead test insects were observed until *Beauveria* sp. fungus grew on the test insects' bodies and documented using a stereo microscope with magnification of 0.8× to 1.5×.

RESULTS AND DISCUSSION

Morphological characteristics of orchid beetles

The morphological characteristics of orchid beetles in Bali suggest that the species may be *L. yerburyi*. "Orchid Lema" is proposed as the common name to distinguish it from other orchid beetle pests. Eggs, newly hatched larvae, pupae, and adults were yellow. Larvae feed immediately after hatching and then take on the color of their food. The larvae were soft, fleshy, and covered by mucus exudate and fecal material expelled during molting. Larvae underwent four growth stages. Before pupation, mature larvae secreted a meringue-like substance for their pupae. Upon the emergence of the pupae, well-formed legs and distinct contours of the wings and antennae were observed (Figure 1). Development from egg to adult took approximately 24 days. Newly emerged adult insects remained inside the pupae for several hours, after which they emerged by chewing a hole with a diameter of about 1 cm. The adult beetles reached sexual maturity 2-3 weeks after emergence. Mating occurred at any time during the day, and beetles were capable of mating multiple times throughout their lifespan.

Molecular characteristics of orchid beetles

The orchid beetle species identified in Bali was presumed to be *Lema* sp., based on its morphological characteristics.

Further testing through molecular characterization was conducted to confirm the accuracy of this identification. Molecular identification of the *Lema* sp. was conducted with PCR and followed by sequence analysis. The PCR results were successfully visualized using electrophoresis, measuring approximately 700 bp DNA fragments (Figure 2). The phylogenetic tree constructed from the COI sequence of the Bali orchid beetle (PV467117.1) showed that this specimen clustered tightly with *Lema yerburyi* (PP667398), with short branch lengths and strong nodal support, confirming that the orchid beetle in Bali belongs to *L. yerburyi*. This result is congruent with the recent redescription and biological study of *L. yerburyi* infesting orchids in India, which also relied on a combination of detailed morphology and COI-based barcoding to stabilize the species concept and host records (Suresh and Prathapan 2025). Their study documented *L. yerburyi* on several commercial orchid genera, including *Arachnis*, *Ascocenda*, *Dendrobium*, *Mokara*, *Oncidium*, and *Phalaenopsis*, emphasizing its specialization as a florivorous orchid pest. The close genetic match between the Bali population and Indian *L. yerburyi* sequences suggests that the orchid beetle damaging *Vanda tricolor* in Bali is conspecific with populations reported from other orchid-growing regions in Asia.

Taken together, the concordance between morphological observations and COI-based molecular data strengthens the reliability of *L. yerburyi* identification in this study. It also illustrates the broader usefulness of DNA barcoding in resolving taxonomic uncertainties in economically important insect pests, particularly within Chrysomelidae, where cryptic diversity and color variation frequently complicate morphology-based taxonomic identification (Ojha and Venkatesan 2014).

Macroscopic and microscopic characteristics of *Beauveria* sp.

Observations conducted 21 days post-isolation revealed that the *Beauveria* sp. fungal isolate exhibited a white coloration on the PSA medium on the top of the agar. Underneath the petri dish, the colony exhibited a pale to yellow coloration. The colony growth pattern was diffuse, without a pattern, and the edges were irregular. The texture of the colony was fine, like powder, which was more evident as the colony grew (Figure 3). Research by Bich et al. (2021) states that the diffuse, irregular growth pattern, irregular edges, and powdery texture of the colony are characteristic macroscopic features of the genus *Beauveria*.

Fungi that had been isolated for 21 days were observed under an Olympus CX21 binocular microscope connected directly to a computer using ImageFocus software at 40x magnification. Microscopic observations of the *Beauveria*

sp. isolate revealed aseptate hyphae and conidia. Chain-like conidia were abundantly present on the conidiophores in a zig-zag arrangement. The conidia were spherical with a length of 1.6-2.1 µm in diameter (Figures 4.C, 4.D). The reproductive structure of the fungus, characterized by its size and shape, is indicative of the species *B. bassiana*. This is consistent with the entomopathogenic fungus identification guide by Humber (2005), which states that the hyphae and conidia of the fungus are aseptate, the

conidia are round to nearly round in shape, the conidial chains are often long, and the conidial heads are spread out, with conidial lengths ranging from 1.5-3.5 µm. Based on macroscopic and microscopic morphological characteristics, the fungus *Beauveria* sp. isolated from the orchid beetle was suspected to be the species *B. bassiana*. The accuracy of this identification was further confirmed through molecular characterization.

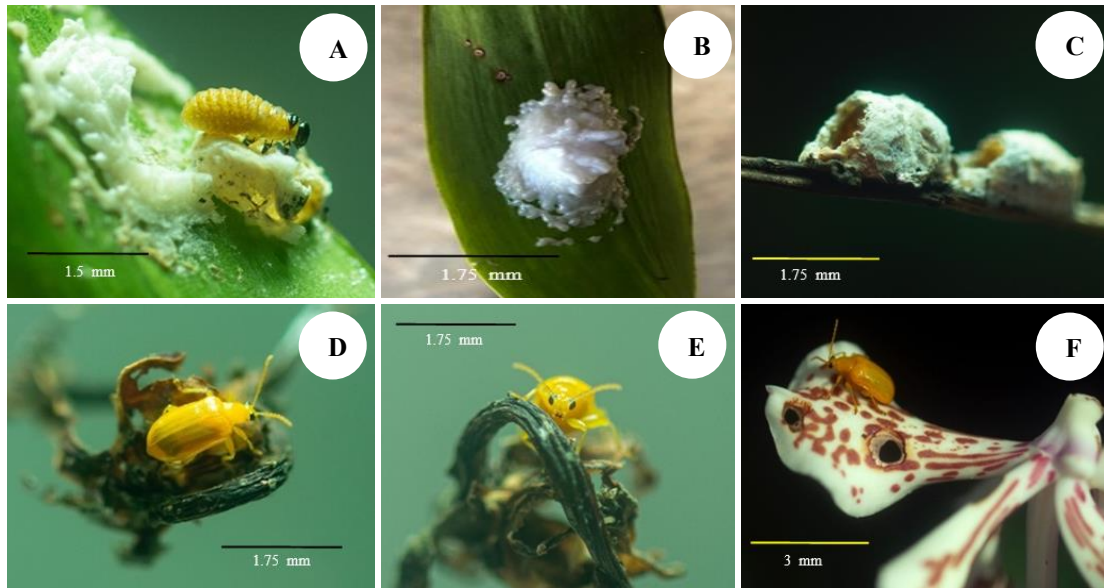


Figure 1. Living individuals of the orchid beetle are in the host plant (*Vanda tricolor*). A. Lava, B, C. Cocoon, D-F. Imago

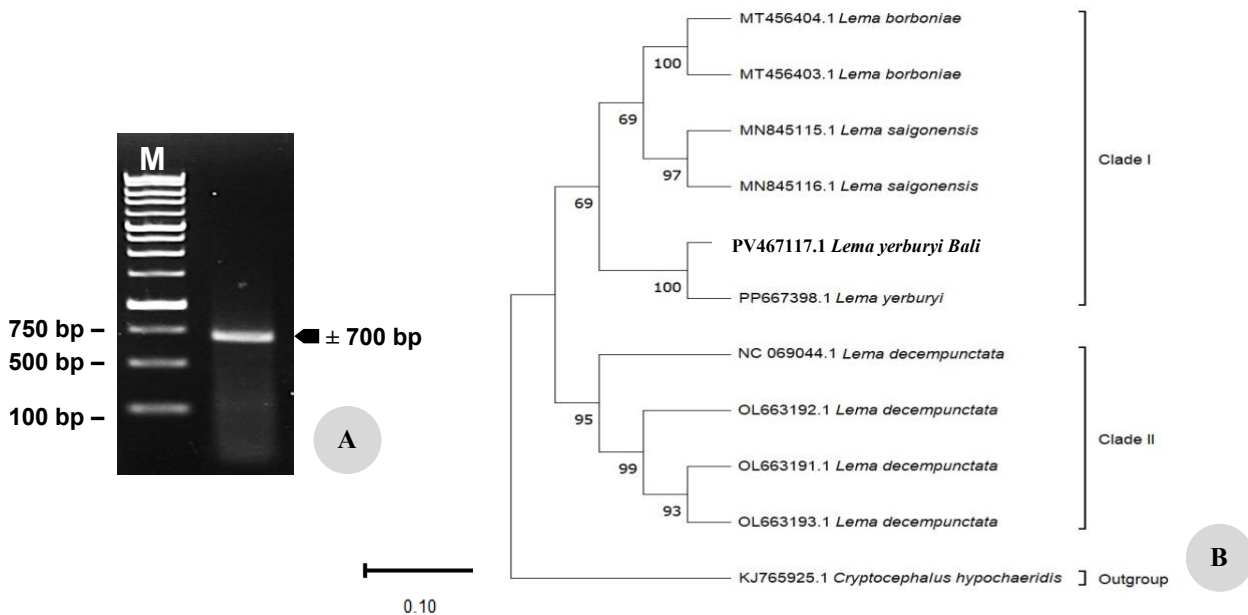


Figure 2. The molecular characterization of the orchid beetle *Lema yerburyi*. A. Agarose gel electrophoresis of PCR. The marker was a 100 bp DNA ladder (M), B. Phylogenetic tree arranged based on DNA composition of orchid beetle *Lema yerburyi* with the Maximum Parsimony method. The number in the branch was the percentage of the level of trust in the group

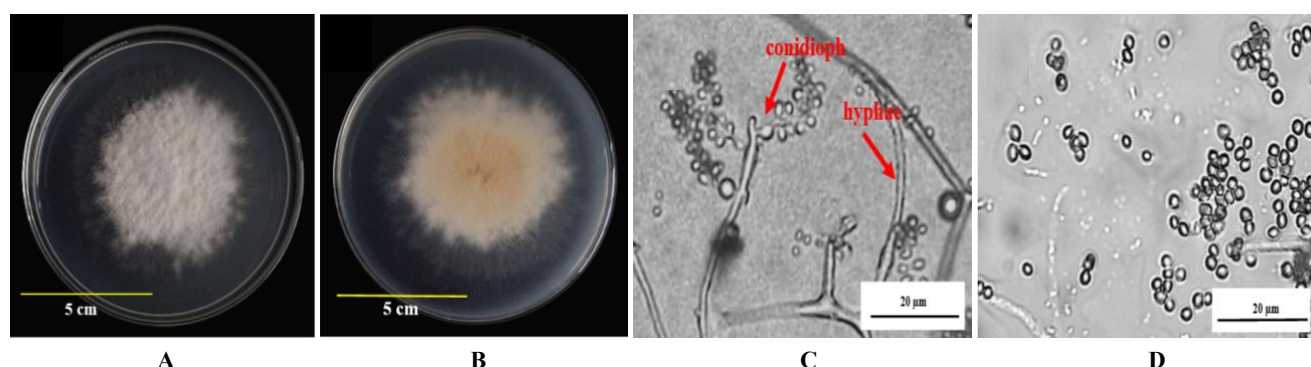


Figure 3. Results of macroscopic and microscopic morphological identification of *Beauveria* sp. isolate *Lema yerburyi*: A. The top part of the agar, B. The underside of Petri dish conidiophore, C. Hyphae magnification 40 \times , and D. Conidia magnification 40 \times

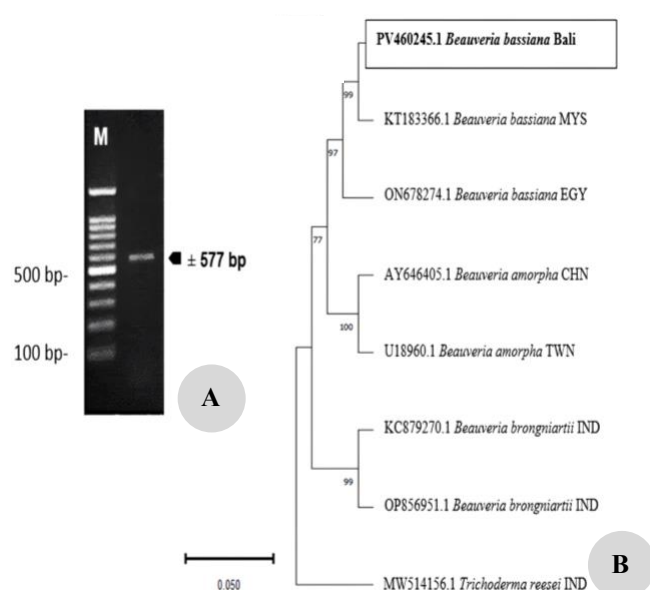


Figure 4. The molecular characterization of the fungus *Beauveria bassiana*: A. Agarose gel electrophoresis of PCR. The marker was a 100 bp DNA ladder (M), B. Phylogenetic tree arranged based on the DNA composition of *Beauveria bassiana*

Molecular characteristics of *Beauveria* sp.

Molecular identification of the species *Beauveria* was conducted, starting with PCR and followed by sequence analysis. The PCR results were successfully visualized using electrophoresis with ITS primers on a 1% agarose gel. The presence of distinct DNA bands indicated successful amplification. Figure 4 presents the PCR visualization. The nucleotides combined from each sequence of *Beauveria* sp. isolated from the orchid beetle *L. yerburyi* did not exceed the ITS gene region, measuring approximately 500 bp, specifically 577 bp DNA fragments. This is in accordance with the research by White et al. (1990), who stated that universal ITS primers used to amplify ribosomal DNA from all fungal species will produce specific fragments ranging in size from 400 bp to 900 bp. DNA band size of approximately 600 bp is a characteristic feature of *B. bassiana* (Wartono et al. 2016; Sari and Rosmeita 2019;

Lakshita et al. 2024). The PCR results were subsequently sequenced to ascertain the nucleotide sequence.

The DNA sequencing results of *Beauveria* sp. orchid beetle isolates yielded a query length of 550 bp following the analysis of the chromatogram data. The combined sequence results were then used as target sequences to search for organisms with the same coding genes in the NCBI database using the BLAST feature. The investigation of organisms through the acquired target sequences revealed multiple species of *Beauveria* present in the NCBI GenBank database. The reference sequences exhibiting the highest Percent Identity, highest Query Cover, and identical forward and reverse primer types were chosen for comparison against the *Beauveria* sp. sequences.

Based on homology analysis, the sequence of *Beauveria* sp. Bali (PV460245.1) was found. The orchid beetle isolate *L. yerburyi* exhibited a percentage greater than 90%, specifically 95.00% with the *B. bassiana* isolate from Egypt (ON678274) and 94.20% with the *B. bassiana* isolate from Malaysia (KT183366). A phylogenetic tree was constructed to analyze the molecular relationship between the sequence of *Beauveria* sp. Bali and the reference sequences available in the GenBank database, NCBI.

The phylogenetic tree was constructed from the sequence of the fungus *Beauveria* sp. Bali, isolated from the orchid beetle *L. yerburyi*, indicates a close relationship with *B. bassiana* (KT183366) from Malaysia, supported by a bootstrap value of 99 in 1,000 repetitions (Figure 5.B). This strong statistical support indicates that the Bali isolate is genetically very similar to Southeast Asian *B. bassiana* populations. Regional connectivity is plausible, as orchid trading and the movement of planting materials between Indonesia and neighboring countries may facilitate the dispersal of fungal strains through contaminated substrates or infected insect hosts. Additionally, the shared humid tropical climates of Indonesia and Malaysia may select for similar fungal lineages adapted to these environments.

The phylogenetic tree shows that this species is part of a monophyletic group alongside *B. bassiana* (KT183366) and *B. bassiana* (ON678274). Both species are part of a monophyletic group that includes the fungal sequence *Beauveria* sp. isolate orchid beetle *L. yerburyi*, suggesting that the members of this group share a close relationship and are believed to have originated from a common ancestor

(Hidayat and Pancoro 2008). Consequently, the molecular analysis results indicate that *Beauveria* sp. isolates were derived from the orchid beetle *L. yerburyi*. Bali is a species that is closely related to *B. bassiana*.

Koch's postulate test

Beauveria sp. was successfully isolated from the pupa of the orchid beetle *L. yerburyi* found on the orchid plant *Vanda* in Bali (Figure 5). After discovery, the fungus growing on the body of the orchid beetle *L. yerburyi* was isolated on a PSA medium and incubated for >14 days. The fungus growing on the medium was then subjected to Koch's postulate test. Koch's postulate test involved two essential stages: inoculation and isolation. The fungal isolate was inoculated onto insect larvae. Koch's postulate test of *Beauveria* sp. on *L. yerburyi* larvae demonstrated successful infection, with the fungus on the larvae displaying macroscopic characteristics identical to those observed on *V. tricolor* orchid plants (Figure 6).

Two imagoes of *L. yerburyi* were inoculated with the *Beauveria* sp. isolate, while one imago served as a control. The pathogenicity test results indicated that *L. yerburyi* died on the fourth Day Post-Inoculation (DPI). On the sixth day post-inoculation, white hyphae began to develop on the body of the orchid beetle *L. yerburyi*. In relation to this, *B. bassiana* isolated from the orchid beetle *L. yerburyi* has pathogenicity against the orchid beetle *L. yerburyi*.

Research by Sharma et al (2023) shows that some species of the genus *Beauveria* are host-specific. The ability

of entomopathogenic fungi to successfully infect selected insect species is governed by a unique set of genetic factors related to virulence (Valero-Jiménez et al. 2016), which includes pathogen variants adapted to either a narrow or broad range of hosts and their capacity to evade the host's immune responses and other defensive mechanisms (Boston et al. 2020). The orchid beetle identified based on morphological and molecular characteristics that attacked the *V. tricolor* was *L. yerburyi*. The entomopathogenic fungus isolated from infected *L. yerburyi* beetle pupae was identified as *B. bassiana*, marking the first such report in Indonesia.

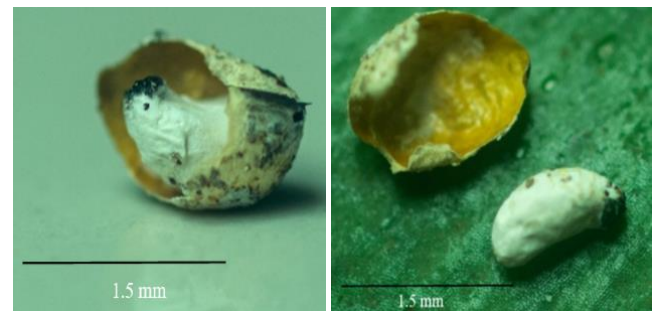


Figure 5. Pupae of the orchid beetle *Lema yerburyi* infected with the fungus *Beauveria* sp. found on *Vanda tricolor* orchid plants

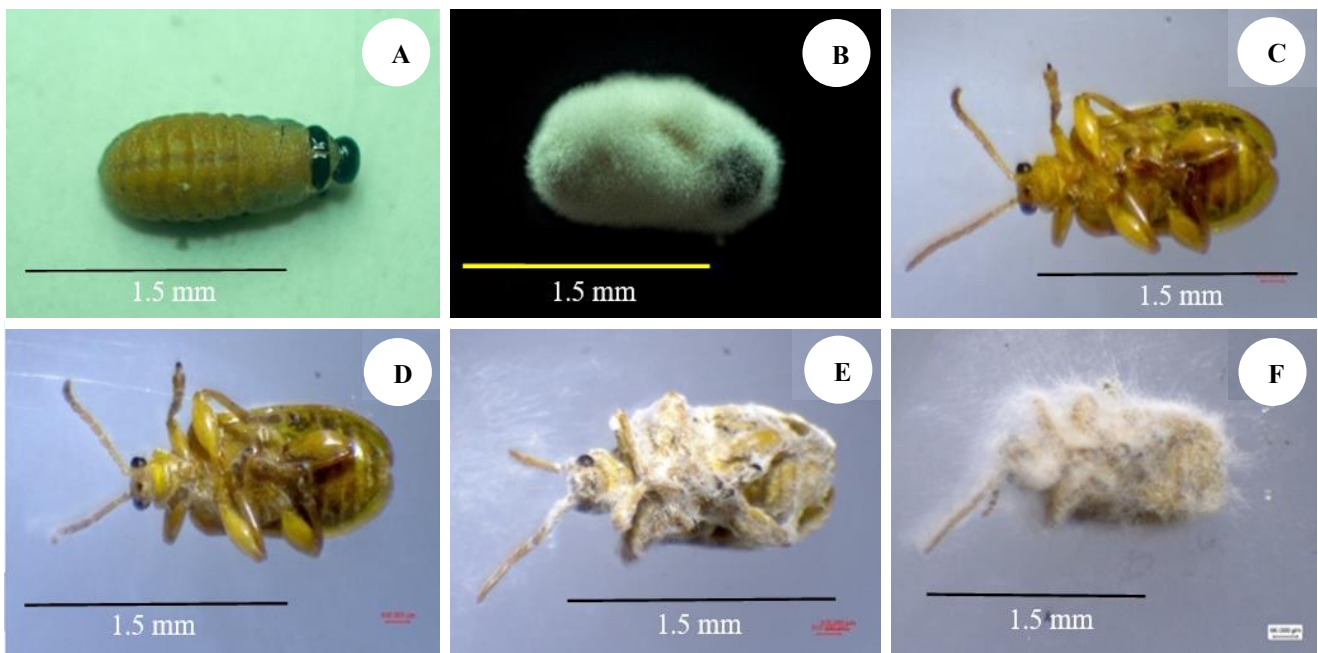


Figure 6. Results of Koch's postulate test on orchid beetle larvae *Lema yerburyi*. Larvae of *Lema yerburyi* before inoculation with: A. *Beauveria* sp., B. *Lema yerburyi* larvae 7 days after inoculation with *Beauveria* sp., C. Imago of *Lema yerburyi* larvae 4 days after inoculation, D. 6 days after inoculation, E. 8 days after inoculation, and F. 10 days after inoculation, magnified using a 0.8× stereo microscope

Limitations and future prospects

While these findings are significant, several limitations should be acknowledged. The sample size was modest, and pathogenicity was tested under laboratory rather than field conditions. Environmental factors such as humidity, temperature, and interactions with other microbes can influence fungal efficacy and should be addressed in future field trials. Additional work is also needed to evaluate formulation methods, persistence, and compatibility with cultural practices and other natural enemies.

In conclusion, this study reports, for the first time, the natural infection of *L. yerburyi* by *B. bassiana* on *V. tricolor* orchids in Bali, Indonesia. Morphological and molecular analyses confirmed the identity of the pathogen, while pathogenicity tests demonstrated its ability to cause rapid mortality in adult beetles. These findings expand the known host range of *B. bassiana* and highlight its potential as a biological control agent against orchid pests. Beyond confirming a new host-pathogen record, this work provides a foundation for developing sustainable pest management strategies in orchid cultivation. Future research should focus on field evaluations of fungal efficacy, formulation and delivery methods, and integration into broader Integrated Pest Management (IPM) programs. Such approaches will be essential for reducing chemical pesticide dependence, preserving orchid biodiversity, and supporting environmentally responsible orchid production in Indonesia and beyond.

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