

First record of *Bassania amethystata* Walker, 1860 as a potential defoliating insect on the invasive species *Ulex europaeus* L. in Colombia (Lepidoptera: Geometridae)

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Abstract

Understanding the association between species biology and their host enables comprehension of the mechanisms underlying species adaptation and evolution, as well as their potential application in biological control. During a population structure study of the invasive plant *Ulex europaeus* L. in the Cordillera Central of Colombia, we documented a larva of the Lepidoptera order feeding on the flower buds. The larva was captured and its development was monitored until it reached the adult stage for identification purposes. Morphological identification revealed the larval as *Bassania amethystata* Walker, 1860. However, molecular analyses showed taxonomic uncertainties for this species and others in the genus. Consequently, the larva of *B. amethystata* could potentially serve as a biological control agent for the invasive plant. By attacking the buds, the larva could affect flowering, fruiting, and formation of the seed bank of the populations. However, additional research is necessary to investigate the larva's specificity and preferences for flower buds and its adaption capacity in other regions where invasions occur. Finally, studies on the taxonomic relationships within the genus *Bassania* Walker, 1860 are needed.

Keywords: Lepidoptera, Geometridae, natural history, herbivory, natural enemy, Colombia.

**Primer registro de *Bassania amethystata* Walker, 1860 como un insecto defoliador potencial en la especie invasora *Ulex europaeus* L. en Colombia
(Lepidoptera: Geometridae)**

Resumen

Entender la asociación entre la biología de las especies y sus hospederos permite comprender los mecanismos subyacentes a la adaptación y evolución de las especies, así como su potencial aplicación en el control biológico. Durante un estudio de la estructura poblacional de la planta invasora *Ulex europaeus* L. en la Cordillera Central de Colombia, se documentó una larva del orden Lepidoptera alimentándose de los botones florales de la especie. La larva fue capturada y su desarrollo fue monitoreado hasta alcanzar la etapa adulta. Se identificó morfológicamente a la larva como *Bassania amethystata* Walker, 1860. Sin embargo, los análisis moleculares revelaron incertidumbres taxonómicas tanto para esta especie como para otras del mismo género. En consecuencia, la larva de *B. amethystata* podría desempeñar potencialmente un papel como agente de control biológico para la especie invasora. Al atacar los botones florales, la larva podría afectar la floración, la fructificación y la formación del banco de semillas de las poblaciones. No obstante, es necesario realizar

investigaciones adicionales sobre la especificidad y preferencias de la larva para los botones florales, así como su capacidad de adaptación en otras regiones donde se producen las invasiones. Finalmente, se requieren estudios sobre las relaciones taxonómicas dentro del género *Bassania* Walker, 1860.

Palabras clave: Lepidoptera, Geometridae, historia natural, herbivoría, enemigo natural, Colombia.

Introduction

The genus *Bassania* Walker, 1860, is a small genus, currently consisting of 13 described species, distributed from Mexico to Argentina in tropical and subtropical forests (Pitkin, 2002; Rajaei et al. 2022). Species of *Bassania* are considered economically important pests and have been reported attacking plantations of *Pinus patula* Schltd, *Eucalyptus* sp., and *Cupressus* sp. (Madrigal, 1981; Sociedad Colombiana de Entomología y Fundación Nacional de Entomología Forestal, 1983). Currently, four species of *Bassania* are reported in Colombia: *B. crocallinaria* Oberthür, 1883, *B. schreiteri* Schaus, 1923, *B. hilaris subturgis* Dognin, 1913 and *B. amethystata* Walker, 1860 (Bernal & Martínez, 2023). However, the species *B. crocallinaria* was excluded from the genus (Brehm et al. 2019; Pitkin, 2002) and *B. schreiteri* (Chalup, 2011) seems to be an endemic species of the Yungas ecoregion, therefore, the record of this species for Colombia could be a misidentification. Consequently, there is lack of studies focused on the biology, morphology, and descriptions of the species. Additionally, there is an absence of research focused on interactions with their hosts and their potential role in biological control processes.

The host association and species biology have a great potential to understanding the mechanism underlying species adaptation and evolution (Menken et al. 2010). However, we often lack natural history data, specially to comprehend the close association between insects and their hosts specificity and their potential role in biological control (Lenteren et al. 2006). Among insects, the Geometridae could serve as a model group to understanding such species interactions due to their status as a mega diverse group of herbivorous insects in the Andean forests (Brehm et al. 2003, 2005). Nevertheless, in the order Lepidoptera and in most insect species, our knowledge of their natural history is limited (Wagner et al. 2021). Therefore, their diversity patterns, host association, and specificity need to be further explored (Dyer et al. 2007).

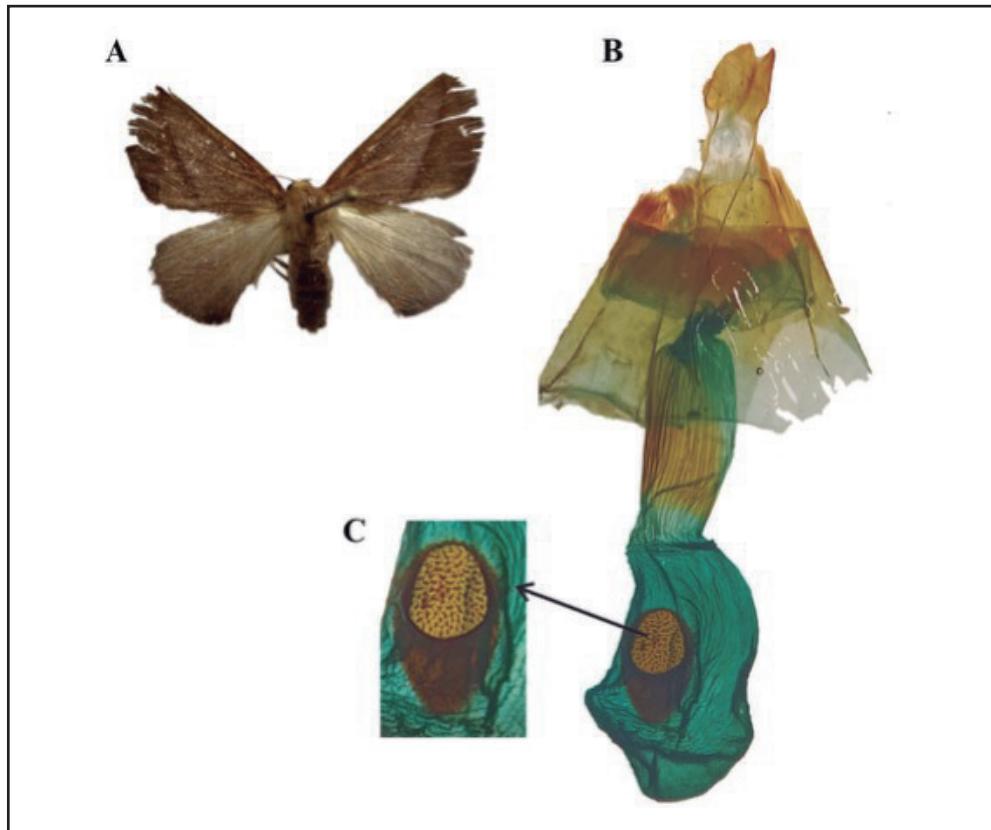
In this study, we report a case of herbivory by *B. amethystata* feeding on flower buds of the invasive species *Ulex europaeus* L. in Colombia. *U. europaeus* is a shrub native to Western Europe listed as one of the 100 most invasive species worldwide (León Cordero et al. 2016). In Colombia, this species was introduced in the 1950s to be used as a living fence in the departments of Cundinamarca, Antioquia, Boyacá and Tolima (Amaya-Villarreal & Renjifo, 2010; Barrera-Cataño et al. 2019; Ocampo-Zuleta & Solorza-Bejarano, 2017). However, due to its temperature requirements and life history characteristics, such as high growth and reproduction rates, this species is invading Andean, high Andean and paramo ecosystems (Barrera-Cataño et al. 2019; Hill et al. 2008). Considering all the above, this study aims to investigate the taxonomic and molecular identification, biology, and behavior of *B. amethystata* in Colombia. Additionally, considering the importance of identifying potential natural enemies of the invasive plant *U. europaeus*, we present the first record of larva of *B. amethystata* feeding on flower buds.

Materials and methods

STUDY AREA

The study area is situated on the Alaska property, Pajonales rural district of the municipality of Murillo - Tolima, in the center of Colombia (04°52'28.6"N, 075°08'45.0"W), at an altitude of 2667 meters. The orobiomes found in the region include nival, paramo and Andean ecosystems (Morales-Rivas et al. 2007). However, these ecosystems are under threat due to the presence of several invasive processes of *U. europaeus* along roads, productive land, and in the upper part of the Lagunilla River basin, located at an altitude of 3700 meters. In the study area, there has been an invasion of *U. europaeus* for 33 years, extending for approximately 1 km. The shrubs within the invasion reach heights of up to 3.40 meters and are surrounded by exotic species such as patula pine and eucalyptus planted nearby. Additionally, agricultural, fish farming, and livestock activities are conducted in both the municipality and the study area, leading to significant anthropic intervention (Alcaldía Municipal Murillo, 2019).

Figure 1. Adult of *Bassania amethystata*. **A)** dorsal view of female adult reared in this study. **B)** we display the genitalia dissection. We can observe the Corpus bursae subtriangular. **C)** signum large, hollow, denticulate, and oval as showed in Pitkin (2002) for *B. amethystata*.



SAMPLING AND BREEDING

As part of the research project “Population status, reproductive biology and genetic diversity of the invasive plant *Ulex europaeus*, in populations of the Colombian Central Cordillera”, three 60-meter transects were installed in the study area to measure the population structure of the species. Additionally, visual inspections were carried out on plants, buds, flowers, and fruits to look for signs of herbivore, aiming to identify potential animals that consume the plant and could act as natural enemies. Subsequently, a larva from the order Lepidoptera was manually captured and stored in a plastic container with *U. europaeus* buds, which were replaced every three days. The larva’s development was monitored until its adult stage for identification purposes.

IDENTIFICATION

Genitalia dissection was prepared following methods outlined by Sihvonen (2005). The abdomen was briefly immersed in 10% KOH solution and heated for 5 minutes to remove fat and soft tissues, it was then cleaned in water and ethanol. The larva was identified as female, and the structures were stained with Chlorazol black before being mounted in Euparal (Figure 1). Morphological identification was carried out by comparing our genitalia dissection with photos of *Bassania* type species published in the Neotropical

review of Ennominae (Pitkin, 2002), from the Smithsonian National Museum of Natural History (USNM), and dissections published by Chalup (2011). Moreover, we consulted specialist Vitor O. Becker to further confirm our identifications. The reared adult specimen was deposited in the Entomological Collection of the Biology Program at Universidad de Caldas CEBUC (registration number RNC 188).

Additionally, molecular analyses were conducted on a fragment of the mitochondrial gene Cytochrome Oxidase subunit I (COI). Genomic DNA (gDNA) was extracted from one leg of the specimen using the saline extraction protocol (Miller et al. 1988). Subsequently, the concentration and quality of the extracted gDNA were evaluated using the NanoDrop ND-2000 spectrophotometer (Thermo Scientific™). The primers LCO1490 (5'-GGTCAACAAATCATAAGATATTGG-3') (Folmer et al. 1994) and C1-N-2191 (5'-CCCGGTAAAATTAAAATATAACTTC-3') (Simon et al. 1994) were used to amplify the 5' end of the COI gene. The amplification reaction included 2 µl of gDNA, 1 U of GoTaq DNA polymerase, 1X reaction buffer, 200 µM of dNTP Mix, 2.5 mM of MgCl₂, and 0.3 µM of each primer in a final volume of 20 µL.

A negative control was included to detect possible contaminations, and PCR was performed under the following conditions: initial denaturation at 95°C for 5 minutes, followed by 35 denaturation cycles at 95°C for 30 seconds, hybridization at 50°C for 35 seconds, and extension at 72°C for 45 seconds, followed by a final extension of 10 minutes at 72°C. The amplified product was visualized by electrophoresis in a 2.0% agarose gel stained with Midori Green, using the 100bp Opti-DNA Marker molecular weight marker. Subsequently, the amplified fragment was purified and sequenced in both directions by Macrogen, Inc., Korea. The obtained electropherograms were edited with the SeqMan II 4.0 software (DNASTAR Inc.), and the consensus sequence was deposited in the GenBank (access number: OR502635).

For molecular analyses, a BLAST similarity search was initially performed through the NCBI database (National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/>). Subsequently, an alignment of 639 bp was obtained using the Clustal W algorithm implemented in the Mega 7.0 program (Kumar et al. 2016). This alignment included, in addition to the sequence of the specimen obtained in this study, 39 sequences from the genus *Bassania* and a sequence from the genus *Isochromodes* available in the BOLD (Barcode of Life Database, www.barcodinglife.org). The alignment was visually inspected and translated to identify both editing errors and the presence of stop codons that could indicate the amplification of NuMts (nuclear mitochondrial sequences). From the generated alignment, the Kimura-2-Parameter (K2P) substitution model was used to estimate genetic distances between species or monophyletic clades in the Mega software, and phylogenetic relationships were reconstructed through the maximum likelihood method using the IQ -Tree program (Nguyen et al. 2015; Trifinopoulos et al. 2016; <http://iqtree.cibiv.univie.ac.at/>). The substitution model TPM2u+F+G4 was determined by the BIC criteria implemented in ModelFinder (Kalyaanamoorthy et al. 2017), and 1000 ultrafast bootstrap replicates were performed (Hoang et al. 2018).

Results and discussion

The adult larva, which feeds on flower buds of *Ulex europaeus*, was morphologically identified as *Bassania amethystata* (Figure 1). Additionally, a crypsis (camouflage) was observed at the time of capture. When the larva felt threatened, it positioned itself horizontally, holding onto its prolegs and stretching its entire body, mimicking a branch (Figure 2). This behaviour could be observed for 50 minutes. Particularly in the species of the Geometridae family, this cryptic behaviour is well-developed, their larvae assume feeding or resting postures that imitate a part of the host plant (Greeney et al. 2012).

Regarding the larva's development, on the ninth day after its capture, it entered the prepupa stage: its body shortened and thickened, taking on a dark brown hue. Subsequently, on the 11th day, the larva formed the chrysalis, which lasted for 22 days before the adult metamorphosis occurred. In total, the larva's development from capture to its adult stage lasted 35 days. This duration of the life cycle documented here is similar to that reported for *B. schreiteri* under laboratory conditions, where the prepupa stage lasts from 2 to 5 days and metamorphosis into the adult is reached after 25 days (Madrigal, 1981).

Molecularly, the BLAST analysis of the COI gene sequence obtained for the adult larva confirmed a high similarity to sequences from the genus *Bassania* (Identity = 93.3-94.7%; E-value = 0). However, phylogenetic reconstruction by maximum likelihood (Figure 3) and estimates of K2P genetic distance (Table 1) revealed that the adult morphologically identified as *B. amethystata* is more closely related to BOLD sequences taxonomically identified as *B. schreiteri* collected in Antioquia, Colombia (0.9%

genetic divergence) than to sequences identified as *B. amethystata* collected in Cuzco, Peru (5.6% genetic divergence). Additionally, a monophyletic clade was recovered with strong statistical support for the sequences taxonomically identified as *B. amethystata* and *B. olivacea* collected in Zamora Chinchipe, Ecuador (Figure 3). The species *B. amethystata* is distributed from Costa Rica to Peru (GBIF, 2023) while the species *B. schreiteri* appears to be exclusive to the Yungas ecoregion in Peru, Bolivia, and Argentina (Chalup, 2011). Taxonomic uncertainties and the limited number of studies and specialists on Neotropical moths suggest that the taxonomic identifications of the BOLD data for this group are not reliable.

Figure 2. Larva of *Bassania amethystata*. **A)** feeding on flower buds of *Ulex europaeus*. **B)** Crypsis behaviour.



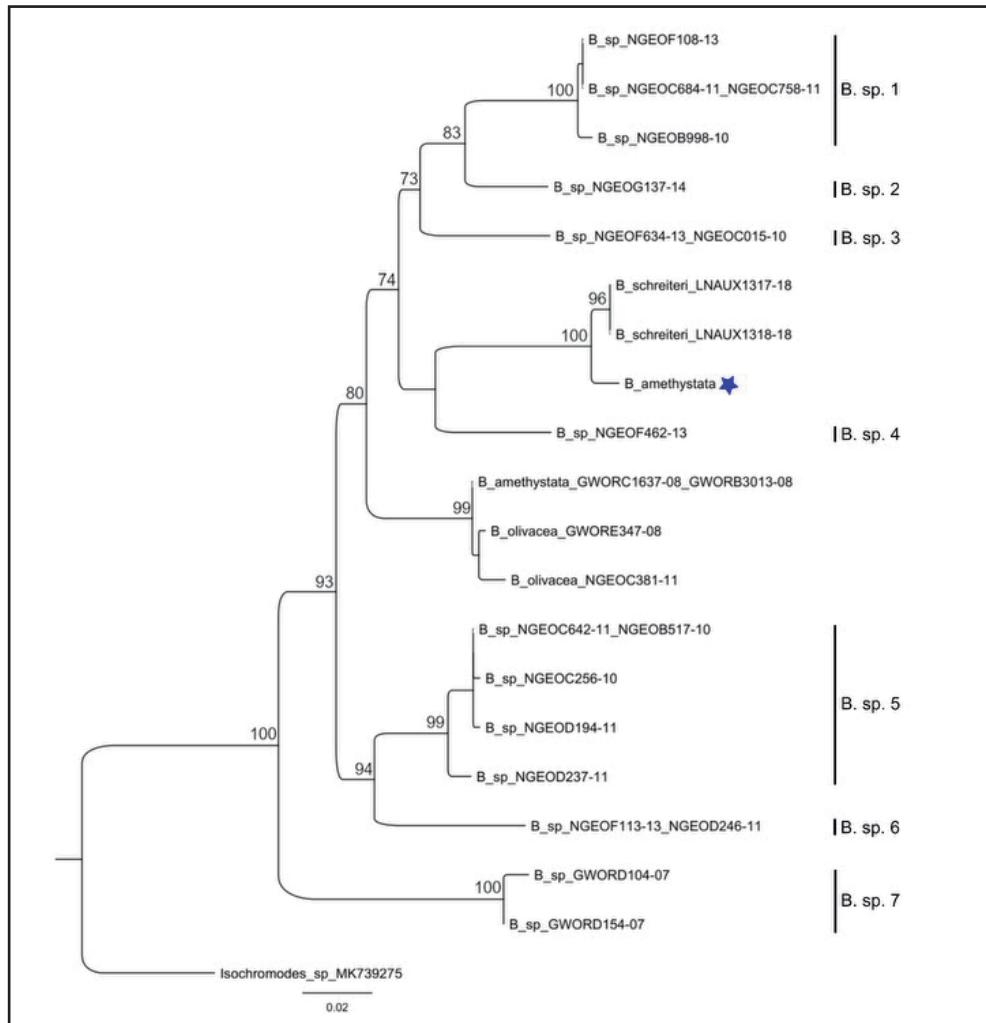
Table 1. Kimura-2-Parameters genetic distance (%) for *Bassania* monophyletic species and clades estimated from a COI fragment. *Sequence recovered for the adult of larva feeding on the flower buds of *Ulex europaeus*.

	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>B. amethystata</i> *	—												
<i>B. amethystata</i>	5.6	—											
<i>B. crocallinaria</i>	11.9	10.9	—										
<i>B. olivacea</i>	5.6	0.6	11.0	—									
<i>B. schreiteri</i>	0.9	4.8	12.3	4.9	—								
<i>B. sp. 1</i>	6.8	5.6	10.9	5.8	6.4	—							
<i>B. sp. 2</i>	7.2	5.0	10.7	5.2	7.1	4.4	—						
<i>B. sp. 3</i>	5.8	4.9	11.0	5.0	6.0	5.2	4.9	—					
<i>B. sp. 4</i>	5.7	5.2	11.5	5.2	5.0	5.8	4.5	5.4	—				
<i>B. sp. 5</i>	6.8	5.6	10.5	5.6	6.5	6.3	5.9	5.8	5.3	—			
<i>B. sp. 6</i>	8.2	5.7	10.9	5.7	7.9	7.2	6.2	7.5	6.4	4.9	—		
<i>B. sp. 7</i>	8.7	7.6	11.9	8.1	7.7	7.9	8.9	8.7	9.0	8.0	9.0	—	
<i>B. sp. 8</i>	8.8	8.3	7.9	8.5	9.0	7.9	7.5	8.5	8.2	7.7	8.1	9.1	—
<i>B. sp. 9</i>	9.5	8.6	9.3	9.0	10.1	8.7	9.0	8.8	9.0	7.3	8.6	9.6	4.5

Adults of *B. amethystata* typically exhibit a brown or reddish coloration, characterized by a semi-

parallel dark line on the outer margin of the forewings and a thorax covered in a dense layer of scales (Madrigal, 1981; Pitkin, 2002). In the male genitalia, the uncus features an inverted T-shape with a sharp end, small and circular socii, gnathos with an expanded middle area, thin branches, several rows of spines arranged in the middle region, valves with processes absent, furcation deviated to the left, arched, or undulating (Chalup, 2011). In females, the genitalia have a subtriangular and elongated corpus bursae. The signum can be large, hollow, denticulate, and oval (Pitkin, 2002). Species of the genus *Bassania* have been reported on multiple native and introduced host plants in the Neotropics, including plants of the Ericaceae, Euphorbiaceae, Melastomaceae, and Solanaceae families, as well as in patula pine and cypress plantations, among others (Madrigal, 1981).

Figure 3. Maximum likelihood tree for *Bassania* from a fragment of the COI. The numbers in the nodes represent the bootstrap. Bootstraps < 70% are not shown. Identical sequences were collapsed. The star indicates the sequence recovered for the adult of larva feeding on the flower buds of *Ulex europaeus*.



Although *U. europaeus* is one of the most aggressive and widely distributed invasive species worldwide, there are few biological control programs targeting it. Fourteen biological controllers have been

tested since 1940, specifically specialist seed predators and foliage-consuming species aimed at suppressing plant reproduction and growth (Broadfield & McHenry, 2019). Particularly, as a biological control strategy, natural enemies of *U. europaeus* have been introduced into invaded areas, such as *Cydia succedana* ([Denis & Schiffermüller], 1775) (Lepidoptera) in New Zealand and *Exapion ulicis* (Forster, 1771) (Coleoptera) and *Agonopterix ulicetella* (Fabricius, 1794) (Lepidoptera) in Chile (Hill & Gourlay, 2002; Norambuena et al. 2000, 2001). However, the mobilization and introduction of exotic natural enemies may have unforeseen impacts that could potentially lead future biological invasions (Thomas & Reid, 2007).

Due to the invasions of *U. europaeus* in Colombia, *B. amethystata*, as a native natural enemy, should not be introduced into the country. Moreover, as a pest species, there is a significant availability of individuals (Bustillo, 2008). By attacking the flower buds of *U. europaeus*, the larva affects both flowering and fruiting in populations, thus limiting recruitment to the existing seed bank and favoring seed depletion (Hill et al., 2001). Reducing the seed bank is crucial in control programs as it directly correlates with decreasing population growth. Invasive populations of *U. europaeus*, older than 40 years, can produce approximately 15,000 seeds m², which remain viable and persistent for more than 50 years (Aguilar-Garavito, 2015; Beltrán & Barrera-Cataño, 2014), making control efforts for preventing their establishment. However, due to the species' capacity for vegetative propagation through runners and root cuttings, complementary control and eradication strategies should be employed (Roberts & Florentine, 2021).

In conclusion, the larva of the *B. amethystata* shows potential as a candidate for biological control of invasive populations of *U. europaeus* in Colombia. However, further research is needed to determine the specificity and preference of the larva for *U. europaeus* flowers, as well as the actual percentage of flowers predated. Additionally, it is crucial to evaluate the larva's adaptation capacity in different geographical areas where invasions occur. Finally, taxonomic reviews of the genus are necessary to delimit its species and clarify its evolutionary relationships, as the limited number of studies and specialists in Neotropical moths render BOLD data for this group unreliable.

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Conflict of Interest

The authors declare that there is no known financial interest or personal relationship that could have influence the work presented in this article.

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