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Lestrimelitta niitkib Ayala 1999 (Hymenoptera: Apidae): A thief bee raiding hives in Cerro Mirador, Oaxaca

Lestrimelitta niitkib Ayala 1999 (Hymenoptera: Apidae): Una abeja ladrona que saquea colmenas en Cerro Mirador, Oaxaca

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ABSTRACT. Stingless bees play an important ecological and economic role as pollinators, particularly in tropical and subtropical regions. However, some species exhibit cleptobiotic behavior, threatening managed and wild colonies. The genus *Lestrimelitta* comprises a group of stingless bees that rely on resources obtained by invading other bees. This study reports *Lestrimelitta niitkib* Ayala 1999 (Hymenoptera: Apidae) as a species that raids the hives of *Scaptotrigona mexicana* (Guérin-Meneville 1845) and *S. pectoralis* (Dalla Torre 1896) in Cerro Mirador, Oaxaca. The identification of *L. niitkib* was performed through morphological characterization and sequencing of the mitochondrial cytochrome oxidase 1 (COI) gene. This finding is significant because *L. niitkib* not only invades but eliminates the bees in the colonies it attacks, leading to the destruction of 20 hives managed by a local meliponiculturist.

Key words: Cleptobiosis; *Scaptotrigona mexicana; Scaptotrigona pectoralis*

RESUMEN. Las abejas sin aguijón desempeñan un importante papel ecológico y económico como polinizadores, especialmente en las regiones tropicales y subtropicales. Sin embargo, algunas especies exhiben un comportamiento cleptobiótico, lo que representa una amenaza para las colonias manejadas y silvestres. El género *Lestrimelitta* comprende un grupo de abejas sin aguijón que dependen de los recursos obtenidos al invadir otras colonias de abejas. Este estudio reporta a *Lestrimelitta niitkib* Ayala 1999 (Hymenoptera: Apidae), como una especie que saquea las colmenas de *Scaptotrigona mexicana* (Guérin-Meneville 1845) y *S. pectoralis* (Dalla Torre 1896) en Cerro Mirador, Oaxaca. La identificación de *L. niitkib* se realizó mediante caracterización morfológica y secuenciación del gen mitocondrial citocromo oxidasa 1 (COI). Este hallazgo es significativo debido a que *L. niitkib* no solo invade, sino que también elimina a las abejas de las colonias que ataca, provocando la destrucción de 20 colmenas manejadas por un meliponicultor local.

Palabras clave: Abejas sin aguijón; Cleptobiosis; *Scaptotrigona mexicana; Scaptotrigona pectoralis*

INTRODUCTION

Cleptobiosis is an ecological interaction where an individual or a group of individuals from the same species steal food or other valuable resources from another species or colony (Breed *et al*., 2012). The genus *Lestrimelitta* consists of stingless cleptobiotic bee species that invade other bee colonies to acquire resources. These bees have completely flower foraging, relying exclusively on the resources of other stingless bee colonies (Guevara *et al*., 2020; Ricardo *et al*., 2024). Studies on *L. niitkib* (Hymenoptera: Meliponini) reveal morphological traits compared to its victims, including robust mandibles adapted for raiding, along with the release of specific pheromones during these attacks (Quezada-Euán *et al*., 2013; Grüter *et al*., 2016). The mandibular gland pheromones (MGP) released at the onset of raids recruit numerous raiders, while the toxicity of associated semiochemicals further increases the success of *L. niitkib* raids (James *et al*., 2022).

Lestrimelitta niitkib exhibits a preference for the nests of *Frieseomelitta nigra* (Cresson, 1879) and *Nannotrigona perilampoides* (Cresson, 1878), likely due to share chemical profiles that facilitate infiltration (Quezada-Euán *et al*., 2013). However, it has also been observed attacking the nests of *Melipona beecheii* Bennett, 1831, *Apis mellifera* L., and even other *L. niitkib* colonies. Raids on these species often result in the destruction and death of the affected colonies. Interestingly, intraspecific aggression among *L. niitkib* is particularly intense, with only one colony typically surviving when multiple colonies are kept in proximity. This behavior is thought to act as a mechanism to regulate population density and minimize competition for resources within the species (Quezada-Euán & González-Acereto, 2002; Grüter *et al*., 2016).

Geographically, *L. niitkib* has been recorded across Mexico, from Tamaulipas in the northeast to the Yucatan Peninsula in the south, as well as in Oaxaca, Chiapas, and parts of Central America. Unlike other stingless bees, *L. niitkib* is rarely collected from flowers due to its cleptobiotic behavior, which eliminates the need to forage on floral resources (Michener, 2007; Ayala *et al*., 2013; Guevara *et al*., 2020; Vásquez *et al*., 2021). Recent studies have associated its distribution to various ecosystems, including dry and humid forests (Arnold *et al*., 2018), highlighting its adaptability to diverse environmental conditions.

Although *L. niitkib* has been reported in Oaxaca, there have been no previous accounts of its attacks on stingless bee colonies in the region. This work documents, for the first time, attacks by *L. niitkib* on hives of *Scaptotrigona mexicana* and *S. pectoralis*. Figures 1A–1D illustrate the interior of the affected hives, showing the devastation caused by these raids. This study contributes to understanding the ecological impact of *L. niitkib* and highlights its potential implications for meliponiculture in Mexico, emphasizing the importance of further research into its behavior and management strategies.

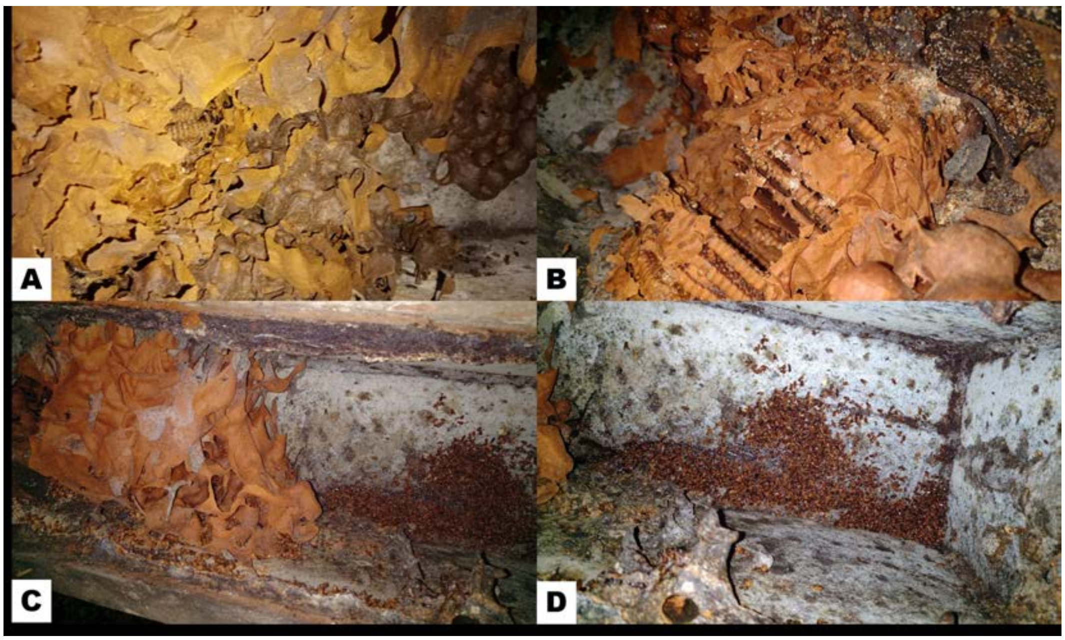

Figure. 1. A-B Interior of *S. mexicana* hives, C-D, interior of *S. pectoralis* hives, D showing dead bees following an attack by *L. niitkib*.

MATERIALS AND METHODS

Biological samples. The bees were collected in the town of Cerro Mirador (17.899444, -96.361667), municipality of Tuxtepec, Oaxaca, on August 18, 2024. The meliponiculturist, Benjamín Gregorio, noticed the attack on his hives and collected some specimens of the killer bee. The specimens were kept in alcohol 96% until their identification at the Laboratorio de Fisiología Molecular y Estructural (Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León).

Ten specimens were analyzed, all apparent workers, and three specimens were dry-mounted on entomological pins and observed under a Leica stereoscopic microscope. The original description of Ayala (1999) was followed to determine this species. The dry-mounted and alcohol-preserved specimens were deposited in the Colección de Insectos Benéficos y Entomófagos (CIBE 24-010) (FCB-UANL).

Molecular identification. Genomic DNA was non-destructively extracted following the protocol described by Giantsis *et al*. (2016) and modified by Ramírez-Ahuja *et al*., (2020). Polymerase chain reaction (PCR) was carried out to amplify the DNA barcode region of cytochrome oxidase subunit I (COI) using primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 5 '-

TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994). PCR was performed in a 30 µL reaction volume containing 3 µL of DNA, 15 µL of Taq DNA polymerase master mix (Qiagen, Hilden, Germany), 1 µL Fw primer, 1 µL Rv primer, 1.5 µL MgCl2 and 8.5 µL of H_2O. The thermal cycling conditions were as follows: 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 sec, 52 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were visualized by 1% agarose gel electrophoresis and sequenced in both directions at Lanbama IPICYT (San Luis Potosí, Mexico). Voucher specimens were deposited at "Colección de Insectos Benéficos Entomófagos" (FCB-UANL).

The sequences were assembled and edited using GeneStudio (Professional Edition, V 2.2.0), a Windows-based molecular biology program that utilizes the Contig Editor.

Orthologous sequences from closely related organisms, available in GenBank, were identified using our seq-like hook and were downloaded. After trimming 24 nucleotides from the 5´ end and 22 nucleotides from the 3´ end, all sequences were standardized to a length of 571 bp. These aligned sequences were subsequently used to construct a phylogenetic tree with GSTree.

RESULTS

Figure. 2. *Lestrimelitta niitkib*, female in lateral view

Morphological identification. **Diagnosis**: Black bees with apparently smooth, bright without pubescence integument; body length of 5.5 mm (Fig. 2). Mandibles are dark brown with a black basal end; the labrum is brown (Fig. 3) with a narrow line at the basal end. The clypeus is black, with brown inferolateral angles; this clear area continues submarginally towards the anterior end, with a marginal black line. The vertex has well-defined, short, erect hairs; the scutum is covered with fine, prominent pilosity and longer black hairs, mainly concentrated at the anterior and posterior ends and posterolateral angles. The scutellum has abundant hairs across the entire surface, with longer hairs at the posterior end (Ayala, 1999).

Figure. 3. Mandibles dark brown with basal end black

Molecular identification. The CO1 barcode sequences generated in this study were deposited in GenBank under ID PQ509929. The phylogenetic analysis revealed that *Lestrimelitta niitkib* has a close relationship with other species such as *Plebeia frontalis* (94.93 %), *Scaptotrigona xanthotricha* (93.53 %), *Cephalotrigona capitata* (94.23 %), and *Tetragona clavipes* (93.71 %) (Fig. 4).

DISCUSSION

Although *L. niitkib* is believed to nest near the attack site, locating the nest was unsuccessful; previous reports indicate that nests of this species are difficult to find in rural areas (Quezada-Euán & González-Acereto, 2002). This characteristic aligns with broader observations of the genus *Lestrimelitta*, where colonies are often elusive due to their preference for secluded nesting sites and their highly mobile scouting behavior during raids (Grüter *et al*., 2016).

In this study, *L. niitkib* displayed a notable capacity for coordinated attacks, resulting in the destruction of 20 hives of *Scaptotrigona mexicana* and *S. pectoralis*, leaving only the hives of *Melipona beecheii* intact. This pattern of selective attack mirrors findings from Quezada-Euán *et al*. (2013), who observed that *L. niitkib* tends to preferentially target host species based on chemical similarity, as well as resource availability and defense capabilities. In particular, *S. pectoralis* and *S. mexicana* might present chemical profiles or colony attributes that render them more vulnerable to *L. niitkib* raids, compared to *M. beecheii*, which may possess stronger or more effective defensive adaptations.

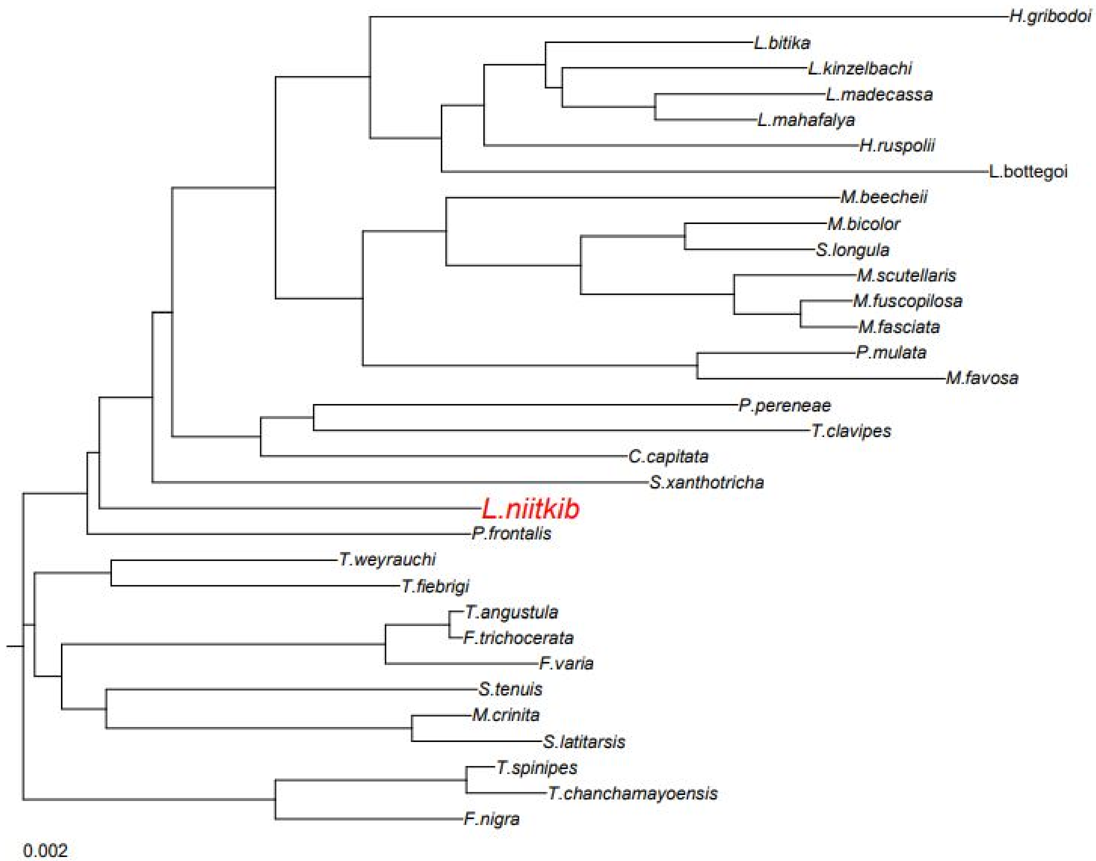

Figure. 4. Phylogenetic tree showing evolutionary relationships between various bee species using the COI gene sequence. The *L. niitkib* sequence generated by us is highlighted in red.

Interestingly, previous studies have shown that when only a few colonies of *Scaptotrigona* are maintained in meliponiculture settings, attacks by *L. niitkib* are rare or nonexistent (Quezada-Euán & González-Acereto, 2002). However, the present findings suggest that the presence of multiple colonies in close proximity may trigger larger-scale raids, as seen in other obligate cleptobiotic species such as *L. limao*, where dense host colony clusters increase the likelihood of attacks (Ricardo *et al*., 2024). This observation highlights the potential role of colony density as a key factor influencing the behavior of *L. niitkib* and its impact on meliponiculture.

The destructive potential of *L. niitkib* raids underscores the need for further research to assess its ecological and economic impact in Mexico. Given the ability of *L. niitkib* to adapt its strategies based on host availability, density, and resistance, studies should also explore the role of chemical cues and defensive adaptations in shaping the dynamics of these interactions (Grüter *et al*., 2016). Moreover, transcriptomic analyses of *L. niitkib* foragers could provide valuable insights into molecular adaptations that support its cleptobiotic behavior, including mechanisms for evading host defenses and coordinating collective attacks (Ricardo *et al*., 2024).

The phylogenetic closeness between *L. niitkib* and *P. frontalis* species indicates that they share a recent common ancestor. This nearness is consistent with the hypothesis that differences in behavior, specifically the aggressive and cleptobiotic behavior of *L. niitkib*, could have developed rapidly in its evolutionary history. The aggressive behavior of *L. niitkib* towards other bees and its tendency to steal resources appear to be specific adaptations to its environment, where competition for resources is high. These behaviors could have provided selective advantages, allowing *L. niitkib* to thrive in its ecological niche.

Finally, the presence of *L. niitkib* in Cerro Mirador, Oaxaca expands the known distribution range of this species, previously reported in Yucatán and other areas of Mesoamerica (Quezada-Euán & González-Acereto, 2002; Rivero *et al*., 2023). This report highlights the importance of continued monitoring and documentation of *Lestrimelitta* species across Mexico to better understand their ecological roles and their potential influence on native bee populations and meliponiculture practices. Collaborative efforts that combine field studies, behavioral experiments, and molecular approaches could help mitigate the risks associated with *L. niitkib* and preserve the viability of stingless beekeeping in affected regions.

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