Therya notes Advance Access published June 7, 2024

THERYA NOTES 2024, Vol. 5 : xx-xx DOI: 10.12933/therya_notes-24-168 ISSN 2954-3614

Agave obscura new record in the diet of nectarivorous bats *Leptonycteris nivalis*, *Choeronycteris mexicana* and *Anoura geoffroyi* in the mountains of central Veracruz, México

Agave obscura nuevo registro en la dieta de los murciélagos nectarívoros *Leptonycteris nivalis*, *Choeronycteris mexicana* y *Anoura geoffroyi* en las montañas del centro de Veracruz, México

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We report the interaction of the nectarivorous bats *Leptonycteris nivalis*, *Choeronycteris mexicana* and *Anoura geoffroyi* with the flowers of *Agave obscura* Schiede (Asparagaceae). In this interaction we verified the consumption of nectar and pollination of *A. obscura* flowers, which is why we report for the first time *A. obscura* as part of the diet of these nectarivorous bats. *Agave obscura* is an endemic species of México with distribution in the states of Tamaulipas, San Luis Potosí, Veracruz, Puebla and Oaxaca. Through video recordings and analysis of pollen samples collected directly from the bats, we recorded the interactions between May and June 2018, in the mixed pine-oak forest of Toxtlacoaya, municipality of Las Vigas de Ramírez, Veracruz, México (2,426 m). Eighteen nectarivorous bats were captured: *L. nivalis* ($n = 9$), *C. mexicana* ($n = 3$) and *A. geoffroyi* ($n = 6$), foraging inflorescences of *A. obscura*, the bats presented agave pollen on their snouts, chest, head, or wing membranes. This is the first known record of the interaction of these bats with *A. obscura*, which adds conservation value to this endemic species as a food source for Mexican nectarivorous bats.

Key words: Agavoideae; Asparagaceae; inflorescence; plant-pollinator interaction; pollen samples; Phyllostomidae; pollination.

Reportamos la interacción de los murciélagos nectarívoros *Leptonycteris nivalis*, *Choeronycteris mexicana* y *Anoura geoffroyi* con las flores de *Agave obscura* Schiede (Asparagaceae). En esta interacción comprobamos el consumo de néctar y la polinización de las flores de *A. obscura*, por lo que reportamos por primera vez a *A. obscura* como parte de la dieta de estos murciélagos nectarívoros. *Agave obscura* es una especie endémica de México con distribución en los estados de Tamaulipas, San Luis Potosí, Veracruz, Puebla y Oaxaca. Mediante grabaciones de video y análisis de muestras de polen colectados directamente sobre los murciélagos, registramos las interacciones entre mayo y junio de 2018, en el bosque mixto de pino encino de Toxtlacoaya, municipio de las Vigas de Ramírez, Veracruz, México (2,426 m). Se capturaron 18 individuos de murciélagos nectarívoros: *L. nivalis* ($n = 9$), *C. mexicana* ($n = 3$) y *A. geoffroyi* ($n = 6$), forrajeando inflorescencias de *A. oscura*, los murciélagos presentaron polen del agave en hocico, pecho, cabeza, o membranas alares. Éste es el primer registro conocido de la interacción de estos murciélagos con *A. oscura*, lo que añade valor de conservación a esta especie endémica como fuente de alimento para murciélagos nectarívoros mexicanos.

Palabras clave: Agavoideae; Asparagaceae; inflorescencia; interacción planta-polinizador; muestras de polen; Phyllostomidae; polinización.

Pollinator–plant interactions play a crucial role in the ecological and evolutionary processes of species in ecosystems (Bascompte 2009; Trejo-Salazar *et al.* 2023). Pollination, mediated by animals, is essential in the reproductive process of many flowering plants. Pollinators transfer pollen from a male flower (or structure) to a female flower (or structure), producing fruits and seeds, while the pollinator obtains nectar as a reward. This process is essential in the survival and evolution of many plant species since it promotes genetic diversity and variability within populations (Sahli and Conner 2006; Bascompte and Jordano 2007). The more genetic diversity presents in a population, the greater the species' ability to cope with environmental changes, pathogens, and other challenges (Mayr 2001; Bascompte and Jordano 2007). Many plant species and their pollinators develop a mutualistic relationship over time, some becoming dependent, leading to processes of coevolution, where the characteristics of plants and pol-

Therya notes Advance Access published June 7, 2024

linators evolve in response to mutual selection pressures, giving rise to morphological, physiological and behavioral adaptations in both interacting species, which provide them with adaptive advantages (Sahli and Conner 2006; Bascompte and Jordano 2007; Bascompte 2009; Johnson and Anderson 2010; Kevan *et al.* 2013).

Agaves are succulent plants characterized by rosette-shaped growth, with fleshy leaves and conspicuous inflorescences; they are native to the desert regions of America (Gentry 1982; García-Mendoza 2007, 2011), distributed from the southern United States to Colombia and Venezuela and on Caribbean islands such as Trinidad and Tobago, Aruba, Bahamas, and Curaçao (Gentry 1982; Good-Avila *et al.* 2006). They are grouped in the Asparagaceae family, with a total of 200 species. In México, among 150-159 species are recorded with 69 % endemism, being the country with the highest number of species and considered the center of origin and diversity of the *Agave* genus (García-Herrera *et al.* 2010; García-Mendoza 2007, 2011). Plants of the genus *Agave* are considered key species in communities in the arid and semiarid zones of México; they are essential for nectarivorous pollinators due to their important nectar production (Good-Avila *et al.* 2006; Fleming *et al.* 2009; Eguiarte *et al.* 2013), the main pollinators are bats and hummingbirds as well as insects from the Order Hymenoptera, Lepidoptera and Diptera.

Agave obscura, is a little-known endemic species of México, which is just beginning to be studied (Cházaro Basáñez 1981; Cuéllar-Martínez 2020; Cuéllar-Martínez *et al.* 2024); its inflorescence produces flowers with nectar which is used by various day and night floral visitors; however, nocturnal cross-pollination, in which pollen is transported during the night to different plants, produces the greatest number of fruits and seeds, which shows the efficiency of nocturnal pollinators for this species (Cuéllar-Martínez *et al.* 2024). The aim of this study was to register interactions (*A. obscura*-pollinator) and determine its nocturnal visitors, which led us to identify *A. obscura* as a new species in the diet of nectarivorous pollinating bats.

Study area. On the slopes of the Cofre de Perote volcano at an altitude of 2,426 m, in the town of Toxtlacoaya, municipality of Las Vigas de Ramírez, in the center of the state of Veracruz, México (19º 31' — 19º 41' N and 96º 39' — 97º 05' W; Figure 1a), a wild population of *A. obscura* is presented. The site is part of an irregular lava spill (called "malpaís", an area composed of fragmented rocks of volcanic origin), 50 km long and 3 km wide, covering an area of 100.85 km^2. It forms a soft hillock covered by a continuous layer of ashes (Cuéllar-Martínez *et al.* 2024). The climate is cold-humid with an average annual temperature of 12.7 ºC, an annual minimum of 7.2 ºC and an annual maximum of 18.4 ºC; the average annual rainfall is 1,842 mm (CONAGUA

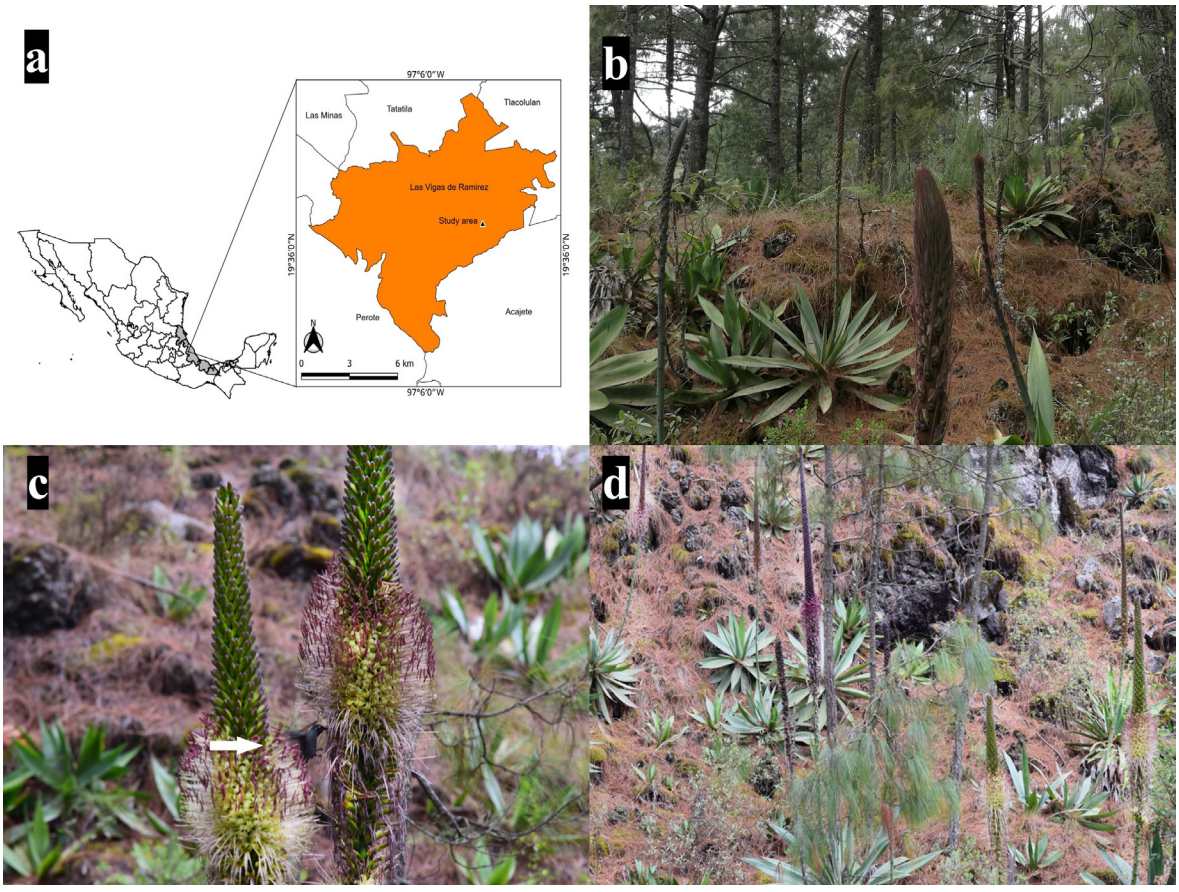

Figure 1. Location map of the study area in the state of Veracruz. a) *Agave obscura* in its natural environment; b) developing inflorescences; c) inflorescences in their mid-flowering stage (note the presence of a hummingbird showed with white arrow); d) group of individuals in flower, the area with outcrops due to the lava spill called malpaís is observed. Photographs M. Cuellar Martínez.

2019). The soil is made up of volcanic ashes and permeable calcareous sediments, with good drainage. Xerophyte species and tree vegetation dominated by *Pinus pseudostrobus*, *P. patula*, *P. teocote*, *Quercus mexicana*, *Q. candicans*, *Arbutus xalapensis* and deciduous forest species such as *Liquidambar macrophylla*, *Clethra mexicana*, *Carpinus caroliniana* and *Alnus jorullensis* are present (Cuéllar-Martínez 2020).

Agave obscura Schiede, Linnaea (Asparagaceae: Agavoideae), is a medium-sized plant (40-60 cm high), which can grow solitary or tussock. It has lanceolate-acuminate to oblong succulent leaves, arranged in a rosette, with 35-65 leaves that have small reddish-brown spines (Cuéllar-Martínez 2020). It is found in tropical deciduous forests, tropical savannahs and cloud forests with abundant rainfall (Gentry 1982). It is endemic to México, distributed in the states of Tamaulipas, San Luis Potosí, Veracruz, Puebla, and Oaxaca; its altitudinal distribution ranges from 1,200 to 2,500 m (Cházaro Basáñez 1981; Gentry 1982; Espejo-Serna 2012). The inflorescence is spiked, 2 to 3 m high, densely covered by flowers starting from the upper half or third of the inflorescence (Figure 1 b, c, d); flower anthesis is sequential from bottom to top. The flowers are up to 51 mm long with reddish tepals and stamens; flowering occurs between May and June in the study area. The lifespan of a flower is on average 5 days and the flowers are protandrich, that is, they first develop the staminate phase (anther maturation and pollen release), followed by the pistillate phase (stigma receptivity; Cházaro Basáñez 1981; Gentry 1982; Cuéllar-Martínez 2020; Cuéllar-Martínez *et al.* 2024).

Floral visitors. During the middle flowering stage of *A. obscura*, a video camera was installed for 5 nights in order to record nocturnal floral visitors. Video recordings were made with a digital camera (Sony DCR-SR65, Sony Corporation, Tokyo, Japan), equipped with infrared light (HVL-HILR) in night vision mode, placed 1 m in front of the inflorescences from 20:00 to 22:00 hr. Interactions were recorded when visitors contacted the inflorescence.

In order to identify floral visitors, 4 mist nets of 3 x 9 m were installed near the inflorescences for 6 nights without the presence of the moon. The nets remained open from 19:00 to 24:00 hr., checked every 30 min. Captured bats were identified using field guides (Medellín *et al.* 2008). With a brush, pollen samples were taken from the captured specimens; samples were placed in Eppendorf tubes with 70 % ethanol. Preparations of each sample were made with fuchsin and glycerinated gelatin and, through an optical microscope (Iroscope MOD. MG-11TF), they were compared with reference samples of pollen grains collected from *A. obscura*. After identifying the specimen and taking samples of pollen grains, the animals were released at the collection site.

A total of 11 hr, 53 min of night video was recorded. Nectarivorous bats (Phyllostomidae) and nocturnal moths (Sphingidae) were recorded visiting agave flowers. A higher visitation rate (mean visitation frequency hr/inflorescence) was recorded by bats compared to moths (mean ± standard deviation; 23.6 ± 6.97 *vs* 9.4 ± 2.49), although the differences were not significant (Mann Whitney U, W = 68, *P*-value = 0.08; Figure 2).

Eighteen nectarivorous bats belonging to 3 species were captured: *Leptonycteris nivalis* (*n* = 9), *Choeronycteris mexicana* (*n* = 3) and *Anoura geoffroyi* (*n* = 6). Ten individuals had *A. obscura* pollen grains on their snout, chest, head, or wing membranes (*L. nivalis* 4, *C. mexicana* 1, and 3 individuals of *A. geoffroyi*). The pollen samples obtained from the bats were identified as *A. obscura* pollen grains (Figure 3).

For the first time, we recorded the interaction between the nectarivorous bats *L. nivalis*, *C. mexicana* and *A. geoffroyi* with the endemic agave *A. obscura*. The results of these interactions showed legitimacy since when comparing the number of fruits and seeds produced by the floral visitors, nocturnal cross-pollination produced a greater number of viable fruits and seeds than diurnal pollination (Cuéllar-Martínez *et al.* 2024). The above coincides with several works that report that pollination of agaves by insects and birds generally results in lower fruit and seed production, and lower values of genetic diversity than with pollination by bats (Silva-Montellano and Eguiarte 2003; Rocha *et al.* 2006), showing the efficiency of nectarivorous bats as legitimate pollinators of *A. obscura*. A recent study identified the interactions of the nectarivorous bats, *Leptonycteris yerbabuenae* and *L. nivalis*, with 96 species of agaves in México (Trejo-Salazar *et al.* 2023), and the interaction with *A. obscura* was not found, which reiterates the novelty of this research.

The morphological data of *A. obscura*, such as the length of the flowers, pistil, stamens, corolla, ovary, as well as the nocturnal schedule and the amount of nectar production (Cuéllar-Martínez *et al.* 2024), are very similar to those reported in other species of agaves of the subgenus *Littaea*, which present bat pollination syndrome (Silva-Montellano and Eguiarte 2003; Rocha *et al.* 2005; Flores-Torres

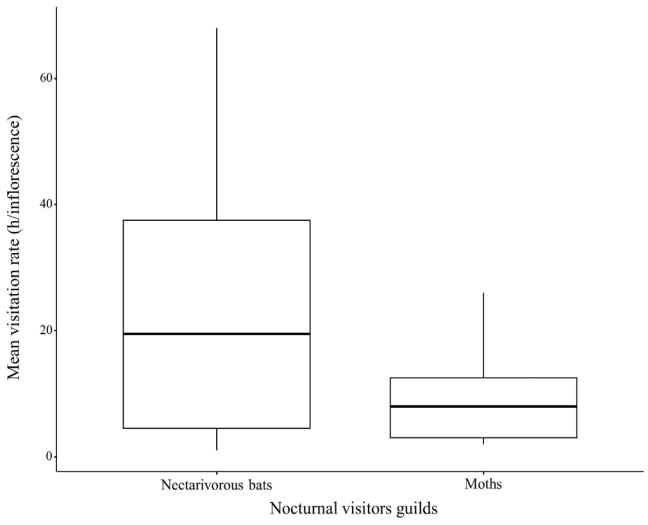

Figure 2. Boxplots showing the average number of visits to *Agave obscura* inflorescences per hr by nectarivorous bats and moths. The differences between these groups were not statistically significant, with a confidence level of 95 %.

and Galindo-Escamilla 2017), the above suggests that *A. obscura* is specialized for pollination by bats, and shows the importance of agaves of the subgenus *Littaea* as a food source for nectarivorous bats.

Although *A. obscura* is also frequented by various daytime visitors (bees, bumblebees, hummingbirds; Cuéllar-Martínez 2020), several investigations have recorded a close ecological relationship between species of the genus *Agave* and the nectarivorous bats *Leptonycteris nivalis*, *L. yerbabuenae* (Álvarez and González-Quintero 1970; Easterla 1972; Howell 1979; Arizaga *et al.* 2000; Molina-Freaner and Eguiarte 2003; Scott 2004; Rocha *et al.* 2005; Sánchez and Medellín 2007; Flores-Torres and Galindo-Escamilla 2017; Flores-Abreu *et al.* 2019), and with *A. geoffroyi* (Álvarez and González-Quintero 1970; Caballero-Martínez *et al.* 2009). The flowering of *A. obscura* occurs between May and June in the study area, and the inflorescences are densely populated with flowers with nectar (Cuéllar-Martínez *et al.* 2024), which represents a good source of food for nectar-eating bats that inhabit the distribution areas of *A. obscura*, or the migratory ones that are visitors.

From the point of view of the ecological niche, the existence of different food sources reduces the possibilities of competition for a limited resource (Begon *et al.* 1990; Soberón and Nakamura 2009), such as flower nectar, which allows the coexistence of different species of nectarivorous bats, as is the case of the study area in Toxtlacoaya, where 3 species of nectarivorous bats cohabit sharing the same resource. This report adds conservation value to this endemic agave species as a food source for Mexican nectarivorous bats.

Acknowledgements

The Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCYT) provided a doctoral scholarship (MC-M No. 340509). A. Castro Luna provided nets and equipment for capturing pollinators and helped in the identification of bats. W. Colorado Durán and the people of the Toxtlacoaya community for their participation and help in the field work. The comments of 2 anonymous reviewers helped improve the first version of this note.

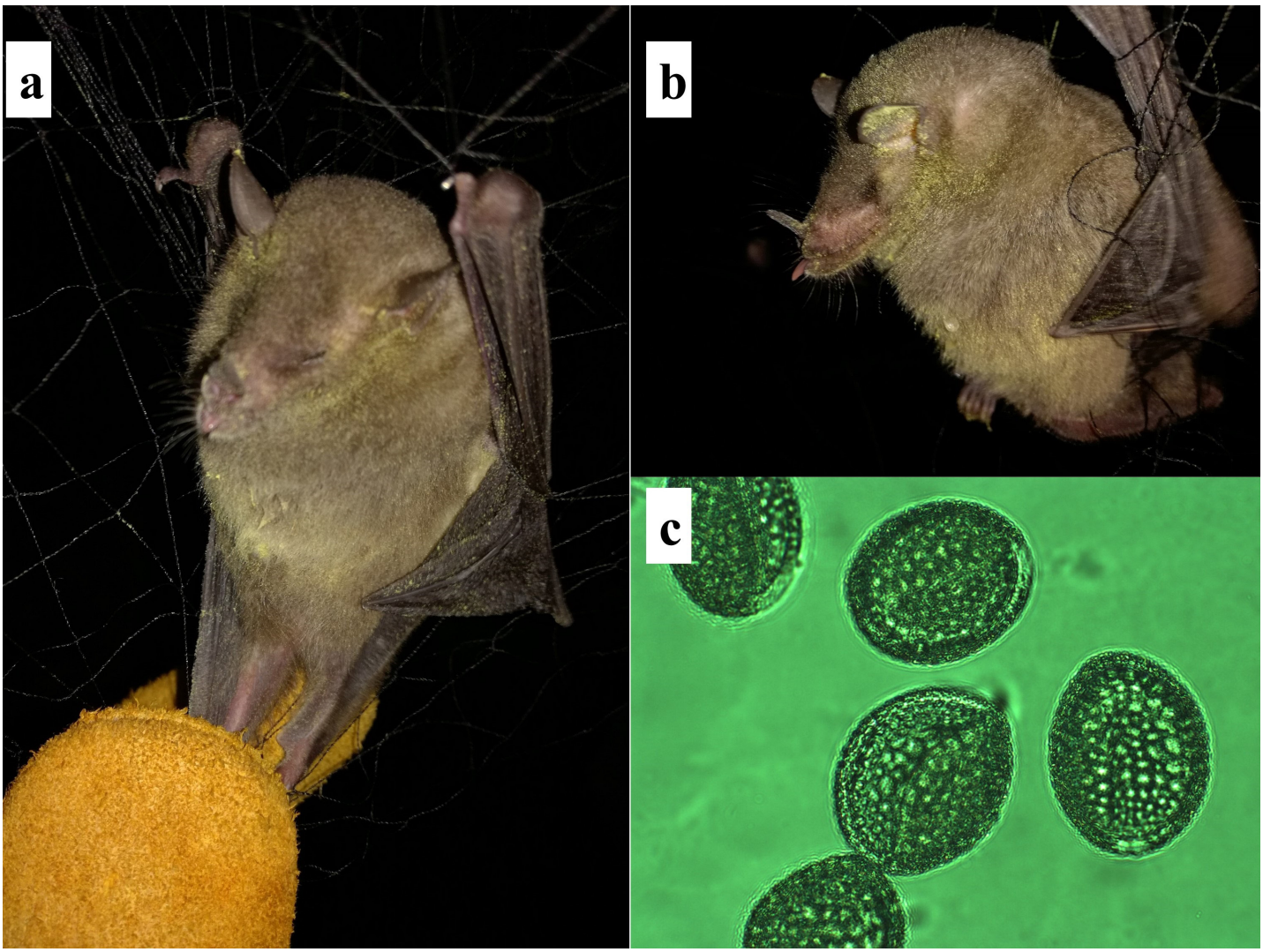

Figure 3. Bats captured with the presence of pollen (golden color) on the snout, head, chest and wings. a) *Leptonycteris nivalis*; b) *Anoura geoffroyi*; c) *Agave obscura* pollen grains observed in an optical microscope at 40X. Photographs M. Cuellar Martínez. Images available at jorgegalin@gmail.com.

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Associated editor: Cristian Kraker Castañeda.
Submitted: February 21, 2024; Reviewed: May 10, 2024.
Accepted: May 31, 2024; Published on line: June 7, 2024.