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First record of cinnamon coloration in opossum *Didelphis* sp. in México

Primer registro de coloración canela en la zarigüeya *Didelphis* sp. en México

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Pigmentation plays a vital role in vertebrate coloration, influenced by genetic and environmental factors. Albinism, leucism, melanism, and flavism are notable color anomalies in vertebrates. This manuscript documents the first record of flavism in *Didelphis* sp. in México, captured in Yucatán Península. In July 2023, 2 orphaned young male opossums were rescued from a backyard in an urbanized area in Chuburná Hidalgo, in Yucatán, México, and transported to a rehabilitation shelter in Mérida. One, displayed a typical dark fur, while the other showed a cinnamon coloration. Following hand-rearing, the opossums were released into the wild after reaching appropriate size and weight. Individual cinnamon maintained the coloration and exhibited an orange-brown pelage, distinguishing him from the other one. The cinnamon pigmentation in the opossum is likely not only the result of a genetic mutation affecting melanin metabolism but also influenced by other evolutionary pressures in this region. Anomalous coloration in vertebrates can be linked to genetic diversity, habitat quality, or environmental contamination. Additionally, population isolation due to habitat fragmentation could play a significant role. Comprehensive genetic and population studies are needed to fully understand the impact of these factors on the population. Despite risks, opossums' adaptability may ensure survival, as seen in similar cases.

Key words: Flavism; marsupial; México; tlacuache; Yucatán Península.

La pigmentación desempeña un papel vital en la coloración de los vertebrados, influenciada por factores genéticos y ambientales. El albinismo, leucismo, melanismo y flavismo son anomalías de color en los vertebrados. Este manuscrito documenta el primer registro en México de flavismo en *Didelphis* sp., capturado en la Península de Yucatán. En julio de 2023, se rescataron 2 crías de zarigüeya machos de un jardín en una zona urbanizada en Chuburná Hidalgo, en Yucatán, México, y se transportaron a un refugio de rehabilitación en Mérida. Uno de ellos mostraba un pelaje oscuro típico, mientras que el otro mostraba una coloración canela. Tras la crianza, las zarigüeyas fueron liberadas en la naturaleza después de alcanzar el tamaño y peso apropiados. El individuo con coloración canela mantuvo la coloración y exhibió un pelaje de tono naranja-marrón. La pigmentación canela observada en la zarigüeya probablemente no sea solo el resultado de una mutación genética que afecta el metabolismo de la melanina, sino que también esté influenciada por otras presiones evolutivas en esta región. La coloración anómala en vertebrados puede estar vinculada a factores como la diversidad genética, la calidad del hábitat o la contaminación ambiental. El aislamiento poblacional por la fragmentación del hábitat podría jugar un papel significativo. Se necesitan estudios genéticos y poblacionales para comprender completamente el impacto de estos factores en la población. A pesar de los riesgos asociados, la adaptabilidad de las zarigüeyas parece garantizar su supervivencia, como se ha visto en otros casos documentados.

Palabras clave: Flavismo; marsupial; Península de Yucatán; tlacuache.

Animal pigmentation is highly noticeable and has a significant effect on the observable coloration traits within groups of vertebrates. A variety of colors and patterns observed on the skin, fur, and feathers of vertebrates, serves as a crucial tool in species identification (Álvarez-Castañeda *et al.* 2017). Environmental and biotic factors may affect in pigmentation variation within populations (Cuthill *et al.* 2017; Nigenda-Morales *et al.* 2018). However, pigment production has a molecular mechanism basis, and it is the result of the action of more than 150 genes, where genetic alterations may result in color anomalies mainly due to excesses or deficits in melanin or other pigments, affecting an animal's body partially or totally (Hubbard *et al.* 2010). According to Gong *et al.* (2021), anomalous col-

oration, due to a deficiency of melanin, can be classified as albinism characterized by a total lack of melanin in skin, fur, and eyes. Leucism, is marked by the lack of melanin while maintaining the original eye color, piebaldism often is classified as partial leucism when melanin is absent in specific areas, resulting in spotting patterns. Melanism is characterized by a dark phenotype, with an elevated concentration of eumelanin. Finally, flavism is a hypopigmentation condition that produces different shades of red, yellow or "cinnamon" coloration hair with unchanged color of eyes, is linked to the synthesis of pheomelanin (Canády 2016; Holcová-Gazárková *et al.* 2017).

Within Marsupials, anomalous coloration as leucism or albinism have been documented in Australian species like wallabies (*Macropus rufogriseus*; Guillery *et al.* 1999; Hayashi *et al.* 2022), tasmanian devil (*Sarcophilus harrisi*), and brush possum (*Trichosurus vulpécula*, Le Souef 1916). In American marsupials reports of atypical colorations are scarce. Mc Cardle (2012) described albinism in *D. virginiana* from Texas. Abreu *et al.* (2013) and Hoyos *et al.* (2020) reported individuals from the *Didelphis* sp. and *D. marsupialis* with leucism in the South of Brazil and Colombia, respectively. Recently, Cuxim-Koyoc *et al.* (2019) reported the first record in México of complete albinism in a female individual of *D. virginiana* captured in Yucatán Península. Furthermore, Hartman (1922) reported 3 "cinnamon" specimens of *D. virginiana* from Missouri and Illinois of United States, while Cuyler (1924) reported a cinnamon female and an albino male in Austin, Texas. However, until now, there have been no documented cases of cinnamon coloration of an opossum specimen in Latin America. In this paper, we report the first case of flavism in *Didelphis* sp. in México, specifically in the Yucatán Península.

In México, the genus *Didelphis* (Linnaeus, 1758) is present in nearly all states of the country, except Baja California Península, arid areas bordering the United States and the Central Plateau (Ceballos *et al.* 2002; Gardner 2005). *Didelphis marsupialis* and *D. virginiana* coexist in México and have a broad overlapping in the Yucatán Península region. In the northern part of this region, *D. virginiana* populations predominate over those of *D. marsupialis* (Jones *et al.* 1974; Ruiz-Piña and Cruz-Reyes 2002).

Although various morphological characteristics can differentiate species within the *Didelphis* genus, these traits can be subjective. Identifying species based on these morphological traits can be challenging, as they often appear very similar to one another. This is especially difficult in this region where both species coexist (Ruiz-Piña and Cruz-Reyes 2002). One characteristic that may help distinguish between the 2 species is the coloration in the cheek region: white in *D. virginiana*, and pale yellow to buffy orange *in D. marsupialis.* Nowadays, genetic and molecular techniques are used for precise identification. The hair in *Didelphis* sp. has 2 layers (pelage and guard hair) and its coloration has 2 phases: gray and black, depending on the color of the guard hair (Hartman 1922; Ceballos 2014). Gray is the most common color pattern. The black phase is uncommon to rare north of Georgia and the Gulf coastal states (including Texas), but it is common in the southeastern United States and southward through México to Costa Rica. In the gray phase, the pelage is interspersed with long white guard hairs, while in the black phase, the guard hairs have broadly black tips (Gardner 1973; Gardner and Sunquist 2003; Álvarez-Castañeda *et al.* 2017). In México, populations typically exhibit a gray or whitish dorsal coloration, darker coloration are rare. The guard hairs feature white tips, and the ventral area is white, cream, or yellowish. Additionally, the middle section of the tail and legs are black (Gardner 1973; Gardner and Sunquist 2003).

On July 2023, 2 orphaned young opossum specimens were rescued from a backyard in Chuburná Hidalgo (21° 00′ 44″ N, 89° 37′ 59″ W); an urbanized population situated in the north of Mérida city, in Yucatán, México (Figure 1). No information was available about the color of the mother's hair. The animals were transported to the rehabilitation shelter, Ooch, located in Mérida, Yucatán. Ooch is a small facility (< 30 animals a year) that focuses on caring and rehabilitation for local wildlife opossum (*Didelphis* spp.).

The specimens rescued corresponded to 2 young male opossums. The body weight was: 80 g for one individual named "Harry", which displayed the typical of gray phase coloration and did not appear to present any anomalous pigmentation. The other one, named "Ron" weighed 110 g and presented a cinnamon coloration: an orange-brown variety corresponding to the gray phase; that is, the pelage was orange-brown, with long white guard hairs throughout his body (Figure 2). This individual was initiating pigmentation in ears, showing brown pigmented irises, the feet and the base of the tail were brown, and albinism was discarded (Figure 2). Coloration characteristics corresponded to the cinnamon color, according to the descriptions previously reported by Hartman (1922) and Cuyler (1924) for opossum populations in the United States of America.

Hand-rearing was attempted following to the guidelines of the American Society of Mammalogists (Sikes and The Animal Care and Use Committee of the American Society of Mammalogists 2016), and it was successful. The juvenile specimens were maintained in rehabilitation for 4 months until they reached a young adult body size. Prior to their release into the wildlife, a thorough assessment of the individuals' health status was conducted by a wildlife biologist considering the general condition of the opossums, weight, and total length (Krause and Krause 2006; Arcangeli 2014). Both opossums attained appropriate size and weight corresponding to subadult males, measuring at 475 mm total length and 857 g for Harry; and 497 mm total length and 923 g for Ron. The last-mentioned individual exhibited pelage, with white guard hairs, and maintained the feet and the base of the tail brown. The ears were lighter compared to the black ears observed in the gray phase and tended toward distinctly and unmistakably brown (Figure 2 c, d). Opossums exhibited the ability to instinctively hunt insects

and grubs, relying on their sense of smell and hearing. The 2 opossums were subsequently released near a water source at the cenote Noc Ac (21° 4′ 26″ N, 89° 43' 15" W) near to a small village located 29 km north of Mérida (Figure 1). This site was selected because it presented a body of water, as well as good habitat quality.

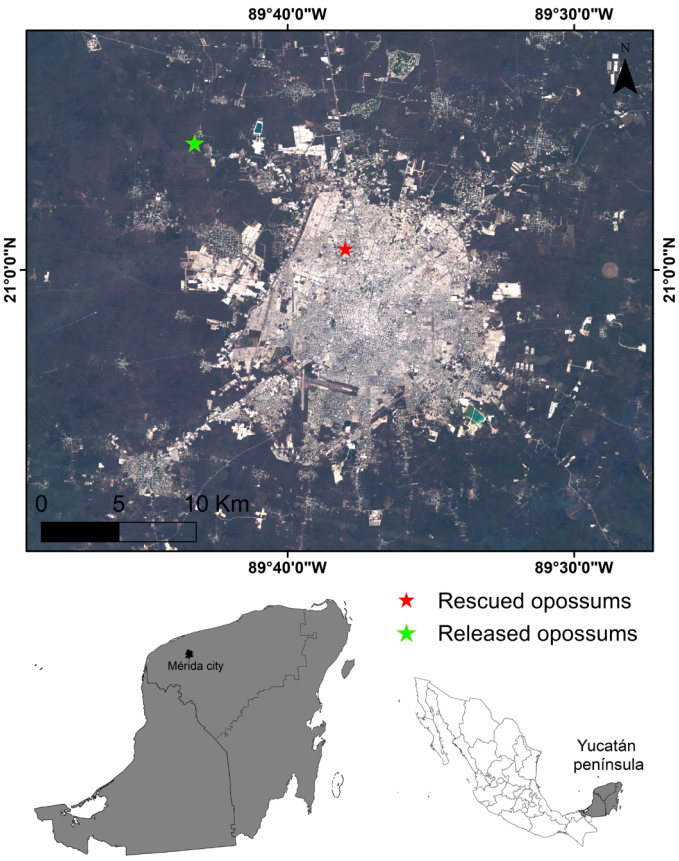

Figure 1. Geographic location of the first record of flavism in marsupials (*Didelphis* sp.) in México.

Generally, opossums distributed in tropical latitudes (< 26° N), exposed to higher precipitation and temperature, have darker coats and a higher percentage of pigmented skin on their ears, hind feet, and tails compared to opossums inhabiting drier and cooler temperate environments (Nigenda-Morales *et al.* 2018; Cerezer *et al.* 2020). In contrast, those distributed in northern latitudes show a greater proportion of depigmented skin on the naked ears, feet, and tails, along with lighter pelage coloration on the face and dorsal part of the torso (Gardner 1982; Gardner and Sunquist 2003; Nigenda-Morales *et al.* 2018).

The cinnamon pigmentation observed in the opossum "Ron" indicates anomalous coloration resulting from a genetic modification affecting melanin metabolism, unlike his brother, who maintains typical pigmentation. The inheritance pattern of this condition is established as autosomal recessive, signifying that an individual inherits 2 copies of the altered gene from each parent (Griffiths *et al.* 2000; Schmidt-Küntzel *et al.* 2005; Puckett *et al.* 2023). This work represents the first report of opossums with flavism in México. Anomalous coloration in vertebrates have been related with low genetic diversity (Sexton *et al.* 2009), poor habitat quality or poor diet (Holyoak 1978; Bensch *et al.* 2000; Castillo-Guerrero *et al.* 2005) and other factors such as chemical contamination or radiation (Moller and Mousseau 2001). While biological and demographic processes linked to deleterious variation can impact the occurrence of anomalous phenotypes, they are not the only factors involved. Here, we reported a single record, therefore it is not possible to determine the causes of the variation or whether it is an isolated case in the area.

Genetic drift can play a significant role in small or isolated populations, allowing anomalous phenotypes such as flavism to increase in frequency due to random fluctuations in gene frequencies. However, natural selection is typically the dominant evolutionary force in large populations, maintaining rare phenotypes at low frequencies unless they confer a significant advantage (*e.g.*, thermoregulation, reproduction, or camouflage; Nigenda-Morales *et al.* 2018). Furthermore, it should be noted that the current population size of opossums in the area where "Ron" was found is unknown. This lack of demographic data makes it more difficult to determine the exact evolutionary pressures at

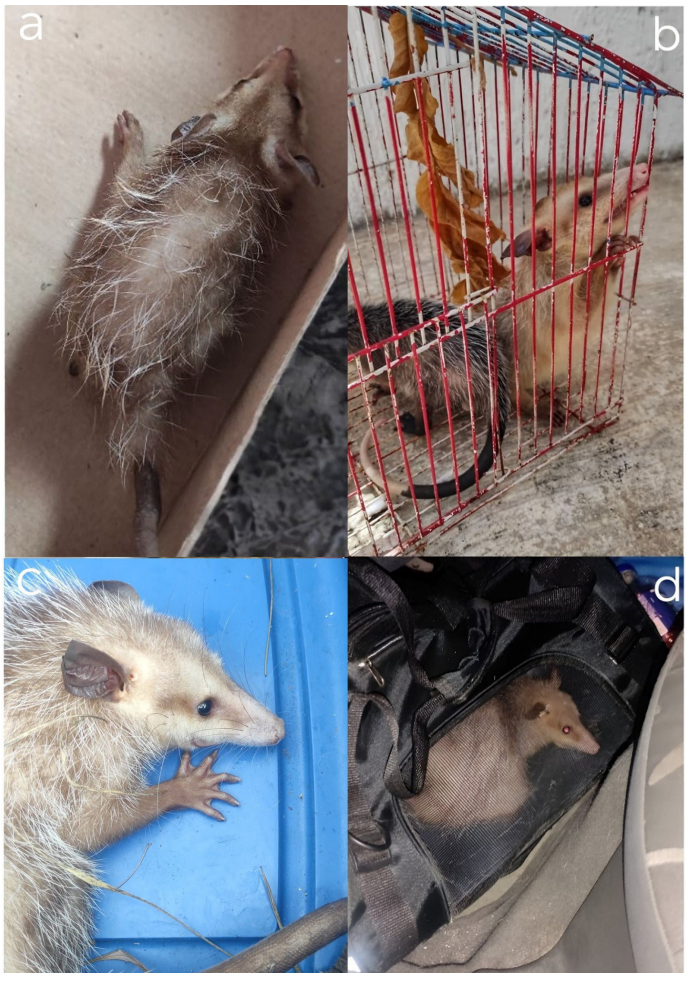

Figure 2. First specimen of *Didelphis* sp. with flavism in México. a) Juvenile individual shortly after rescued; b, c) specimen during its rehabilitation; d) the subadult individual prior to release in to the wild. Photography: P. A. Tenorio-Rodríguez.

play. Hence, genetic and population studies are necessary to further investigate the causality that this condition may be inducing in the population of this region. Anomalous pigmentation may have negative consequences for these individuals, as they face a higher risk of predation due to their lack of camouflage in their habitat or even may be unattractive for reproduction (Cerezer *et al.* 2020; Tenorio-Rodríguez *et al.* 2024). However, opossums have nocturnal habits, omnivorous diet, and are extremely adaptable to different conditions; their physiology allows them to live in a wide variety of habitats (Oceguera-González and González-Romero 2008), it seems possible that Ron may survive in the wild. Recently, an albino specimen of *D. virginiana* with pups in the marsupium has been documented in the same region (Cuxim-Koyoc *et al.* 2019), indicating successful reproduction. Consequently, it is reasonable to expect that Ron, exhibiting flavism, also can reproduce successfully.

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