Brief Communication / Comunicación Breve

Fungivorous butterflies (Lepidoptera: Nymphalidae): A rare record of feeding behavior

Mariposas fungívoras (Lepidoptera: Nymphalidae): Un registro poco común de comportamiento alimentario

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Abstract. In this work, we report a rare feeding behavior, where adults of two species of frugivorous butterflies were feeding on fungi, *Hamadryas februa februa* (Hübner) on *Auricularia cornea* Ehrenb., and *Pierella lena brasiliensis* (Felder & Felder) on *Phallus indusiatus* Vent. These events occurred between April and May 2024 in two distinct areas: the first at *Campus* I of the Instituto Nacional de Pesquisas da Amazônia (INPA) and the second at the Museu da Amazônia (MUSA), both in Manaus, State of Amazonas, Brazil. The rarity of mycophagy events involving Lepidoptera adults, as observed twice here, suggests a potential for the dispersal of fungal spores and underscores the need for more studies to further explore the relationship between these taxonomic groups.

Key words: *Auricularia*; Basidiomycota; Central Amazon; fungivory; *Phallus*.

Resumen. En este trabajo reportamos un comportamiento de alimentación poco común, donde los adultos de dos especies de mariposas frugívoras se alimentan de hongos: *Hamadryas februa februa* (Hübner) en *Auricularia cornea* Ehrenb. y *Pierella lena brasiliensis* (Felder y Felder) en *Phallus indusiatus* Vent. Estos hechos fueron observados entre abril y mayo de 2024 en dos áreas distintas: el primero en el *Campus* I del Instituto Nacional de Pesquisas da Amazonia (INPA) y el segundo en el Museo da Amazonia (MUSA), ambos en Manaus, Estado de Amazonas, Brasil. La rareza de los eventos de micofágia que involucran adultos de Lepidoptera, observada dos veces aquí, sugiere un potencial para la dispersión de esporas de hongos y subraya la necesidad de más estudios para explorar más a fondo la relación entre estos grupos taxonómicos.

Palabras claves: Amazonía Central; *Auricularia*; Basidiomycota; fungivorio; *Phallus.*

Biotic interactions play a central role in the biological processes of the planet, as no species exists in complete isolation (Del-Claro 2012). Thus, the evolutionary histories of the organisms involved in the interactions may be interconnected, characterizing coevolution (Ehrlich and Raven 1964). Biological interactions are temporally and spatially dynamic;

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at certain times, organisms may benefit each other, while at other times, they may suffer losses (Hagen *et al*. 2012). These interactions can generally be classified as mutualism, commensalism, competition, predation, parasitism, and parasitoidism (Biedermann and Vega 2020).

Mycophagy, or fungivory, may involve interactions such as parasitism and predation for the fungus. The diversity and complexity of fungal-insect associations is poorly understood (Barreto *et al.* 2002). Records of mutualism are frequently documented, where the consumption of macrofungi by various animal groups facilitates the dispersal of spores (Claridge *et al.* 1999; Elliott *et al.* 2019a, b, 2022; Kitabayashi *et al.* 2022).

In a review of mycophagy by invertebrates, Santamaria *et al.* (2023) highlighted that despite the abundance of literature, there is limited knowledge regarding species interactions. There is a lack of information about geographic regions, fungal phyla, and invertebrate orders; however, the vast majority of 6,093 observations involved ascomycete and basidiomycete fungi (around 98% of the observations) and insects of the orders Coleoptera, Diptera (around 70% of the observations). The insect orders Hemiptera, Hymenoptera, and Lepidoptera were less frequently observed, with around 1% each. The order Lepidoptera appears in few references (*e.g*., Grehan 1984; Komonen *et al.* 2003; Yoshimatsu and Nakata 2003, 2006; Fritz 2004; Moskowitz and Haramaty 2012; Dobrzański *et al.* 2016; Jaworski *et al.* 2016; Jonsell *et al.* 2016; Nishida and Robbins 2020), with only one event involving an adult butterfly (see Yamashita *et al.* 2018).

Lepidoptera larvae are predominantly herbivores, primarily consuming green leaves but also bark, seeds, dry leaves, flowers, fruits, roots, and fungi sporocarp parts like stipe, pileus, hymenium, and spores (Carneiro *et al.* 2024). Diet preferences for Lepidoptera adults are mostly known from butterflies, divided mainly into two guilds: nectarivores, feeding on nectar or pollen, and frugivorous, feeding on fermented fruit and plant sap (Brown Jr. and Freitas 1999; Cézar *et al.* 2022). Lepidoptera also feed on animal excreta or plant resins (detritivores) and blood (hematophagous). In several groups, especially among moths, adults do not feed, relying on energy reserves accumulated during the larval stage throughout their adult lives (Carneiro *et al.* 2024).

In Lepidoptera, the only groups with mainly fungivorous species appear to be larvae of Oecophoridae and Tineoidea (Dobrzański *et al.* 2016; Birkemoe *et al.* 2018), which inhabit and feed on fungal fruiting bodies (Jaworski *et al.* 2016).

In this work, we report adult individuals from three species of frugivorous butterflies feeding on the fruiting bodies of two species of mushrooms, *Auricularia cornea* Ehrenb and *Phallus indusiatus* Vent.

Lepidopterans were identified at the species/subspecies level based on Constantino (1995), and Warren *et al.* (2024), and fungi were identified at the species complex (Cabral *et al.* 2019) and species (Wu *et al.* 2021) levels.

A male specimen of *Hamadryas februa februa* (Hübner, [1823]) was observed feeding on *Auricularia cornea* Ehrenb. in April 2024 at *Campus* I of the Instituto Nacional de Pesquisas da Amazônia (03°05'42" S - 59°59'16" W). Due to the unexpected nature of the encounter, it was not possible to capture more precise photographic documentation, but the observer photographed the specimen of *H. f. februa* still present in the vicinity (Fig. 1A). The event occurred around 11:15 a.m., on an access trail near the institution's board of directors building, an area with frequent traffic. The fungus was growing on a fallen tree trunk of *paricá* (*Schizolobium amazonicum* Huber ex Ducke (Caesalpiniaceae)) (Fig. 1B). Initially, the specimen of *H. f. februa* was observed with its proboscis in contact with the lower part of the fungal cap. Subsequently, a *Morpho helenor helenor* (Cramer, 1776) approached the same mushroom but exhibited avoidance behavior upon detecting the observer's presence. The butterfly remained near the mushroom momentarily before fleeing (Fig. 1C).

Additionally, two male specimens of *Pierella lena brasiliensis* (Felder & Felder, 1862) were observed feeding on *Phallus indusiatus* Vent. (Fig. 1D). They were observed with their proboscis in contact with the gleba and veil of the mushroom. The mushroom was on fallen leaves on the ground in May 2024 at the Museu da Amazônia (MUSA) (03°00′26″ S - 59°56′24″ W), Manaus. This observation was at 1:30 p.m. when the observer was walking and taking photographs along the trail near Victoria Regia Lake.

Figure 1. Lepidoptera feeding on fungi. **A.** Specimen of *Hamadryas februa februa* (Hübner, [1823]) observed on *Auricularia cornea* Ehrenb. **B.** *Auricularia cornea* on the trunk of a paricá (*Schizolobium amazonicum* Huber ex Ducke (Caesalpiniaceae)). **C.** *Morpho helenor helenor* (Cramer, 1776) close to *A. cornea*. **D.** *Pierella lena brasiliensis* (Felder & Felder, 1862) feeding on *Phallus indusiatus* Vent. / **Figura 1.** Lepidópteros alimentándose de hongos. **A.** Ejemplar de *Hamadryas februa februa* (Hübner, [1823]) observado sobre *Auricularia cornea* Ehrenb. **B.** *Auricularia cornea* sobre el tronco de un paricá (*Schizolobium amazonicum* Huber ex Ducke (Caesalpiniaceae)). **C.** *Morpho helenor helenor* (Cramer, 1776) cerca de *A. cornea*. **D.** *Pierella lena brasiliensis* (Felder y Felder, 1862) alimentándose de *Phallus indusiatus* Vent.

Observations of butterflies feeding on fungi were occasional. The events occurred in fragmented urban forests, on trails with relatively large traffic, and during the rainiest period in the Amazon region. The climatic normals for Manaus show over 315 mm of precipitation from January until May, indicating the regional wet season. During this period, the climate is hot and humid.

Auricularia cornea is a lignocellulolytic basidiomycete with a wide distribution and is observed across a large number of host species. In tropical regions, durable fruiting bodies can be produced almost year-round. They belong to the polyphyletic group of gelatinous fungi, characterized by an extracellular matrix composed of a loosely organized filamentous structure filled with hydrophilic components rich in proteins, complex

carbohydrates, polysaccharides, and exopolysaccharides arranged on the surface of the mushrooms (Yang *et al.* 2022; Ye *et al.* 2024). Caterpillars and adults of Coccinelidae coleopterans, adults of *Drosophila* Fallén, 1823, as well as caterpillars of Geometridae lepidopterans have been observed interacting with this fungus (Paulo Coutinho, pers. comm.). Despite these interactions (at least Coccinelidae and lepidopteran caterpillars eat fruiting bodies), the primary mode of spore dispersal in *A. cornea* is active (ballistospory), in which the fungus ejects the spore autonomously (Money 2023).

Phallus indusiatus are widely distributed basidiomycetes of the order Phallales. Unlike the active dispersal in *A. cornea*, these fungi are of the gasteroid type, characterized by passive dispersal of their spores (statismosporia), so the presence of dispersing animals, such as insects, is expected. In the case of the genus *Phallus*, the adopted dispersal strategy involves attracting insects through the gleba, a gelatinous mass where spores are produced. This gleba exudes a strong fetid odor (Yamashita *et al.* 2018) and undergoes deliquescence by absorbing water, a process facilitated by the rainy season of the region.

In the study of mycophagy in gasteroids of the genus *Dictyophopra* (= *Phallus*) conducted by Yamashita *et al.* (2018), a great variation in the structure of communities of insect dispersers is highlighted around the world: drosophilid and muscid flies in Japan; stingless bees (Meliponini) in the Neotropics; and Nitidulidae beetles in Southeast Asia. In Borneo, the authors also observed a significant abundance of nitidulid beetles; however, most of these beetles fed on the veil, suggesting they were not dispersers of these fungi. The primary insects feeding on the gleba were drosophilid flies and the frugivore nymphalid butterfly *Neorina lowii* (Doubleday, [1849]).

According to Santamaria *et al*. (2023), similar to plants that send signals to attract pollinators, fungi have developed secondary metabolites to attract mycophagous invertebrates for spore dispersal and other mutualisms. This means fungal odors attract insects that live and/or feed on fungi or substrates deteriorated by fungi (Fäldt *et al*. 1999).

Since frugivorous butterflies are attracted to fermented fruits, it is possible that the odor emitted by fungi similarly attract them, as fermentation of fruits also involves various microorganisms, including fungi.

The dispersion of Phallales fungal spores is discussed in relation to the viability of germination of ingested spores and the transport of these spores adhered to insect bodies (Burr *et al.* 1996; Tuno 1998; Yamashita *et al.* 2018). Focusing on Phallales mycophagy in the Amazon, Oliveira and Morato (2000) used the expression "robbery or dispersal?" and proposed that meliponids are occasional spore dispersers, based on the absence of spores and traces of gleba on their bodies. However, Santana and Couceiro (2024) recently observed a greater quantity of *P. indusiatus* spores adhered to the body surface of meliponids (and other insects) than in their stomachs.

In our study, *P. lena brasiliensis* was observed feeding on the gleba and veil of *P. indusiatus*. The rarity of mycophagia events involving butterfly adults suggests the possibility of occasional dispersal of fungal spores. Observing three individuals from two butterfly species feeding on mushrooms of two fungal species in Manaus over one month (between April and May 2024) highlights the need for systematic studies on the ecological interactions and Natural History between these taxonomic groups.

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Author Contributions

GML: Investigation, Writing - Original Draft. **ASF:** Investigation, Writing - reviewing and editing. **PEGC:** Investigation, Writing - reviewing and editing. **RSC:** Investigation, Writing - reviewing and editing. **KFSC:** Investigation, Writing - reviewing and editing.

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