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Mycophagy in the yellow-footed tortoise (*Chelonoidis denticulatus*): feeding trial experiments at Tijuca National Park (Rio de Janeiro, Brazil)

Micofagia de la tortuga de patas amarillas (*Chelonoidis denticulatus*): experimentos de pruebas alimentarias en el Parque Nacional da Tijuca (Río de Janeiro, Brasil)

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Abstract

The act of consuming any part of fungi is termed mycophagy. Certain basidiomes contain highly nutritious elements, such as proteins, vitamins, and minerals, which can be extremely valuable in the animal diet. Mycophagous behavior in animals is well-documented in ethological studies; however, due to the scarcity of macrofungi experts in Brazil, the fungal species consumed are often not identified. The Tijuca National Park (TNP) is a fragment of the Atlantic Forest located in the city of Rio de Janeiro (Brazil), covering an area of 3,953 hectares. The Refauna Association works within TNP to restore ecological interactions in the area through the reintroduction of vertebrate species previously present in the region. One of the reintroduced species is the yellow-footed tortoise (*Chelonoidis denticulatus*), a terrestrial chelonian. Several studies have documented the mycophagous behavior of chelonians; however, it is important to emphasize the lack of studies dedicated to identifying the fungal species comprising their diet. In November 2021, cafeteria-style

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feeding trials were conducted with Refauna's yellow-footed tortoises, in which they were offered the macrofungi collected from TNP were offered to the animals. A total of 21 basidiomycete specimens representing 13 taxa, were collected and offered. The morphology of the offered macrofungi was highly diverse, including agaricoid mushrooms, jelly fungi, gasteroid fungi (earthstar and sequestrate form), and coralloid fungi. The macrofungi were offered to seven individual tortoises, and only one rejected the offered mushrooms. Basidiomes from ten species were consumed by the tortoises, while three were either rejected or only partially accepted.

Keywords: Animal diet; chelonian; fauna reintroduction; fungi; fungivory.

Resumen

El acto de consumir cualquier parte de los hongos se denomina micofagia. Algunos basidiomas contienen elementos altamente nutritivos, como proteínas, vitaminas y minerales, que pueden ser extremadamente valiosos en la dieta animal. El comportamiento micofágico de animales está bien documentado en estudios etológicos; sin embargo, debido a la escasez de expertos en macrohongos en Brasil, las especies fúngicas consumidas a menudo no son identificadas. El Parque Nacional de Tijuca (PNT) es un fragmento de la Mata Atlántica ubicado en la ciudad de Río de Janeiro (Brasil), con una extensión de 3.953 hectáreas. La Asociación Refauna trabaja en el PNT para restaurar las interacciones ecológicas del área mediante la reintroducción de especies de vertebrados que previamente existían en la región. Una de las especies reintroducidas es la tortuga de patas amarillas (*Chelonoidis denticulatus*), un quelonio terrestre. Diversos estudios han documentado el comportamiento micofágico de los quelonios; sin embargo, es importante resaltar la falta de estudios dedicados a la identificación de las especies de hongos consumidas por estos animales. En noviembre de 2021, se realizaron experimentos de prueba alimentaria con las tortugas de patas amarillas de Refauna, a las que se les ofrecieron macrohongos recolectados en el PNT. Se recolectaron y ofrecieron un total de 21 especímenes de basidiomycetes representando 13 taxones. La morfología de los macrohongos ofrecidos fue altamente diversa, incluyendo hongos agaricoides, hongos gelatinosos, hongos gasteroides (estrella de tierra y forma sequestrada) y hongos coraloides. Los macrohongos fueron ofrecidos a siete tortugas individuales, y solo una tortuga no aceptó los macrohongos ofrecidos. Los basidiomas de diez especies fueron consumidos por las tortugas, mientras que tres fueron rechazados o consumidos solo parcialmente aceptados.

Palabras clave: Dieta animal, fungivoría, hongos, quelonio, reintroducción de fauna.

INTRODUCTION

Numerous animals feed on fungi, including invertebrates and vertebrates, and in some cases, fungi constitute their primary food source. Fungi serve as a food resource in forests and woodlands, providing protein and phosphorus, essential substances for many animals. The act of consuming any part of fungi is known as mycophagy (Longvah & Deosthale, 1998; Hanson *et al*., 2003; Trierveiler-Pereira *et al*., 2016; Elliott & Vernes, 2019). In addition to having caloric value, several mushrooms contain organic compounds that are important in the physiology of small mammals. An example of this is ergosterol, which plays a relevant role as a precursor to hormones (Milazzo, 1965; Shivrina *et al*., 1968; Catalfomo & Trappe, 1970). Some fungi may also contain vitamins such as biotin, niacin, pantothenic acid, and riboflavin (Furlani & Godoy, 2007; Cheung, 2010).

According to the review presented by Elliott *et al*. (2019) on mycophagy in reptiles, 32 species of chelonian consume fungi. Tortoises have the ability to store water inside their bodies during dry periods and then reabsorb the liquid gradually. Since the basidiomas of fungi can be made up of up to 90% water, the consumption of mushrooms by the tortoises may assist with body hydration (Elliott *et al*., 2019). It has also been observed that some species of the genus *Kinixys* (Bell, 1827) (African tortoises) showed an increase in growth rate when regularly consuming macrofungi (Mifsud & Stapleton, 2014).

In Brazil, two native species of land tortoises occur in the Atlantic Forest, namely: *Chelonoidis carbonaria* (Spix, 1824) and *C. denticulatus* (Linnaeus, 1766) (Sobral-Souza *et al.*, 2017). Jerozolimski (2005), in a study on the diet of wild populations of yellow-footed tortoise (*C. denticulatus*) and red-footed tortoise (*C. carbonaria*) in the state of Pará (Northern Brazil), found that macrofungi are considered the most important food for adult *C. denticulatus* individuals during the wettest season of the year. Additionally, fragments of macrofungi were found in 50% of the fecal samples, but fungal species were not identified. The yellow-footed tortoise is now locally extinct in many forests in Brazil, such as in the Tijuca National Park (Rio de Janeiro), where it is believed that the extinction occurred more than 200 years ago. In this location, the research project (now Association) 'Refauna' has been working on the reintroduction of the species into the park since 2018, with attempts involving more than 50 individuals (Fernandez *et al*., 2017; Dorigo, 2021; Bragança & Menegassi, 2022; Santos *et al*., 2022; Refauna, 2024).

To ensure the success of reintroductions, monitoring the adaptation of reintroduced animals is of utmost importance, aiming to observe the interactions and functions that may have been lost, since generalist species, such as the yellow-footed tortoise, have many ecological associations (Cid *et al*., 2014; Genes *et al*., 2017).

As knowledge about the animal's diet is crucial for ensuring the success of wildlife reintroduction programs, considering the importance of the mycophagous habit for species of tortoises and the gaps in knowledge about animals that consume macrofungi, this study was developed with the aim of contributing to the understanding of this topic in Brazil, identifying the fungal species consumed by the yellow-footed tortoise.

MATERIAL AND METHODS

The Tijuca National Park (TNP) is located in the central-south region of the State of Rio de Janeiro, within the municipality of Rio de Janeiro, covering an area of 3,953 hectares (Fig. 1). The forest fragment of TNP is part of the Atlantic Forest biome and constitutes the largest restored urban forest in the world (Zago *et al.*, 2020). According to the Köppen-Geiger classification (Peel *et al.*, 2007), the climate is Af (superhumid tropical), which contributes to the development of a considerable number of macrofungal species.

Cafeteria-style feeding trials were conducted with *Chelonoidis denticulatus* individuals over four days in November 2021. While trekking through different areas of TNP in search of tortoises monitored by the project, large

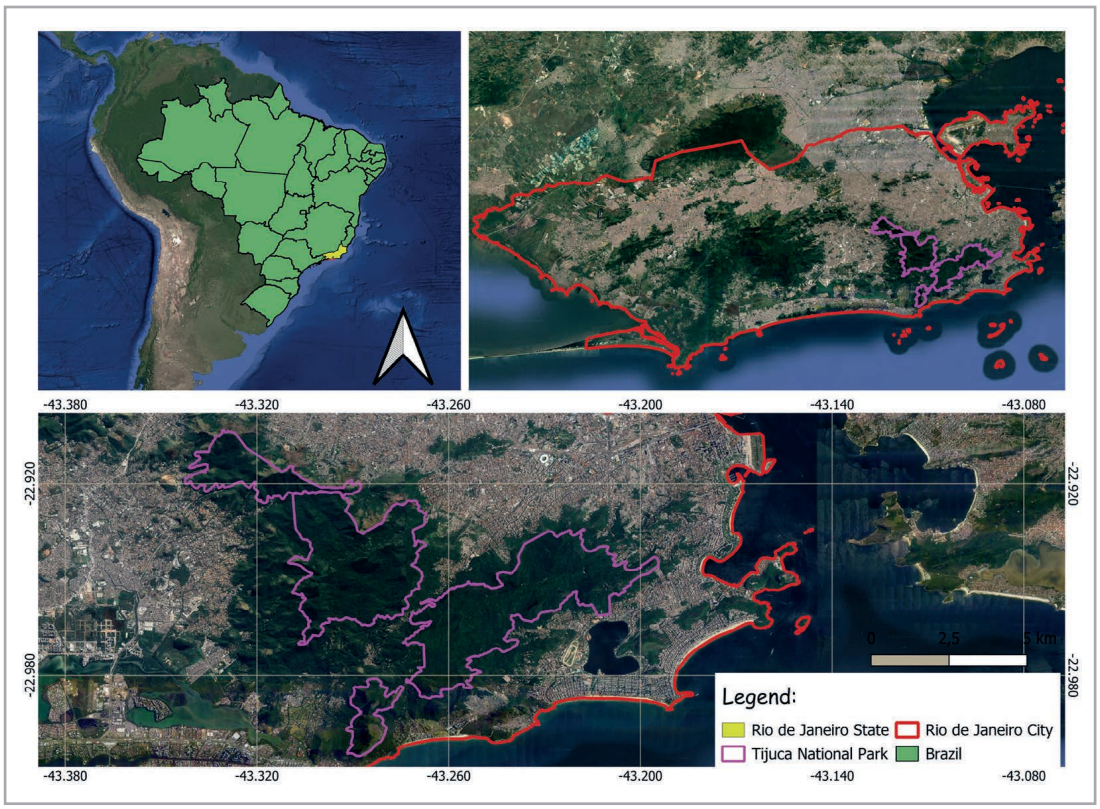

Fig. 1. Study area, Tijuca National Park (Rio de Janeiro, Brazil).

Fig. 1. Área de estudio, Parque Nacional Tijuca (Río de Janeiro, Brasil).

basidiomes found were photographed from different angles (both i*n situ* and *ex situ*), collected from the substrate with the aid of a pocket knife, and placed into collection bags and/or plastic boxes with individual compartments (Lodge *et al*., 2004). Duplicates of the collections were separated to be offered to the tortoises on the same day (fresh basidiomes). The macrofungal species were offered one at a time, and no other food was provided except for the macrofungi.

The reintroduced tortoises (six adults, four females and two males) in TNP were located in the forest with the aid of radio transmitters. One male individual (Xuanwu, No 63) that had not yet been reintroduced in the forest participated in the experiment within an enclosure. All tortoises involved in the study (seven in total) were identified by a name and a number. After locating the tortoises, the basidiomes collected during the trails were offered to them (Fig. 2), and their behavior in response to the offering was observed. The study used direct observation of mycophagic events to gather data (classified as consumed, partially consumed, or not consumed, irrespective of time), and Refauna holds all necessary permits to conduct the experiments. Data analysis was carried out qualitatively, aiming to identify the fungal species consumed.

Duplicates of the specimens offered to the tortoises were dehydrated, analyzed macro- and microscopically in the laboratory (LEMic-UFSCar), and then prepared for herbarium preservation (Wu *et al*., 2004). Morphological analyses were carried out following traditional methods for studying macrofungi (Largent *et al*., 1977; Largent, 1986; Singer, 1986; Pegler, 1997). The vouchers are deposited in the Mycological Collection of the SPSC Herbarium at UFSCar.

Fig. 2. Fresh basidiomes were offered directly from our hands (A) or were left in front of the tortoises (B). Photos: A. Larissa Trierveiler-Pereira; B. Amanda Prado-Elias.

Fig. 2. Basidiomas frescos fueron ofrecidos directamente desde nuestras manos (A) o fueron dejados frente a las tortugas (B). Fotos: A. Larissa Trierveiler-Pereira; B. Amanda Prado-Elias.

RESULTS AND DISCUSSION

During the feeding trials, six individuals of *C. denticulatus* consumed the offered basidiomes: Magali (No 38), Venus (No 15), Farol (n° 17), Grizzy (No 07), Iara (No 22), and Xuanwu (No 63) (Fig. 3). Individual n° 63 was the one that consumed the greatest diversity of macrofungi (six taxa in total), partially rejecting *Geastrum* cf. *saccatum* (consuming only the exoperidium – 'rays') and completely rejecting the coralloid fungus *Phaeoclavulina* sp. Similar to the previous one, No 07 also rejected *Geastrum* cf. *saccatum*, expelling part of it after chewing. Individual No 32 completely rejected the mushroom *Hygrocybe* cf. *conica*, the only fungus offered to it (Fig. 4).

All other offered fungi were consumed completely, as shown below. In total, 13 specimens were offered to the tortoises and were identified (at least to generic level). Table 1 presents the names of the taxa, along with other relevant information about the morphology of the basidiomes and the individuals that consumed them.

Table 1. Results of feeding trials conducted with yellow-footed tortoises (*Chelonoidis denticulatus*) individuals at the Tijuca National Park, RJ, Brazil.

Tabla 1. Resultados de experimentos de prueba alimentaria con las tortugas de patas amarillas (*Chelonoidis denticulatus*) en el Parque Nacional de Tijuca, RJ, Brasil.

Fungal taxon	Morphological group	Water Content*	Consumption**	Tortoise name and number
Auricularia fuscosuccinea (Mont.) Henn.	jelly fungus	HWC	CC	Venus (15), Farol (17), Grizzy (07)
Clitocybula azurea Singer	agaricoid mushroom	HWC	CC	Farol (17), Grizzy (07)
Collybia violacella Speg.	agaricoid mushroom	HWC	CC	Venus (15)
Entoloma cf. *bloxamii* (Berk. & Broome) Sacc.	agaricoid mushroom	HWC	CC	Iara (22)
Favolus brasiliensis (Fr.) Fr.	fleshy wood-ear	HWC	CC	Venus (15), Grizzy (07), Xuanwu (63)
Geastrum cf. *saccatum* Fr.	earthstar (gasteromycete)	MWC (exoperidium) / LWC (endoperidium)	PC	Grizzy (07), Xuanwu (63)
Hygrocybe cf. *conica* (Schaeff.) P. Kumm.	agaricoid mushroom	HWC	R	Esmeralda (32)
Lactifluus sp. (Pers.) Roussel	agaricoid mushroom	HWC	CC	Xuanwu (63)
Lepiota sp. ("La princesa") (Pers.) Gray	agaricoid mushroom	HWC	CC	Xuanwu (63)
Leucopaxillus gracillimus Singer & A.H. Sm.	agaricoid mushroom	HWC	CC	Magali (38)
Oudemansiella cubensis (Berk. & M.A. Curtis) R.H. Petersen	agaricoid mushroom	HWC	CC	Magali (38), Xuanwu (63)
Phaeoclavulina sp. Brinkmann	coralloid fungus	LWC	R	Xuanwu (63)
Protubera maracuja Möller	sequestrate phalloid (gasteromycete)	HWC	CC	Farol (17)

*HWC (High Water Content); MWC (Medium Water Content); LWC (Low Water Content); **CC (Complete Consumption); PC (Partial Consumption); R (Rejected).

*HWC (Alto Contenido de Agua); MWC (Contenido Medio de Agua); LWC (Bajo Contenido de Agua); **CC (Consumo Completo); PC (Consumo Parcial); R (Rechazado).

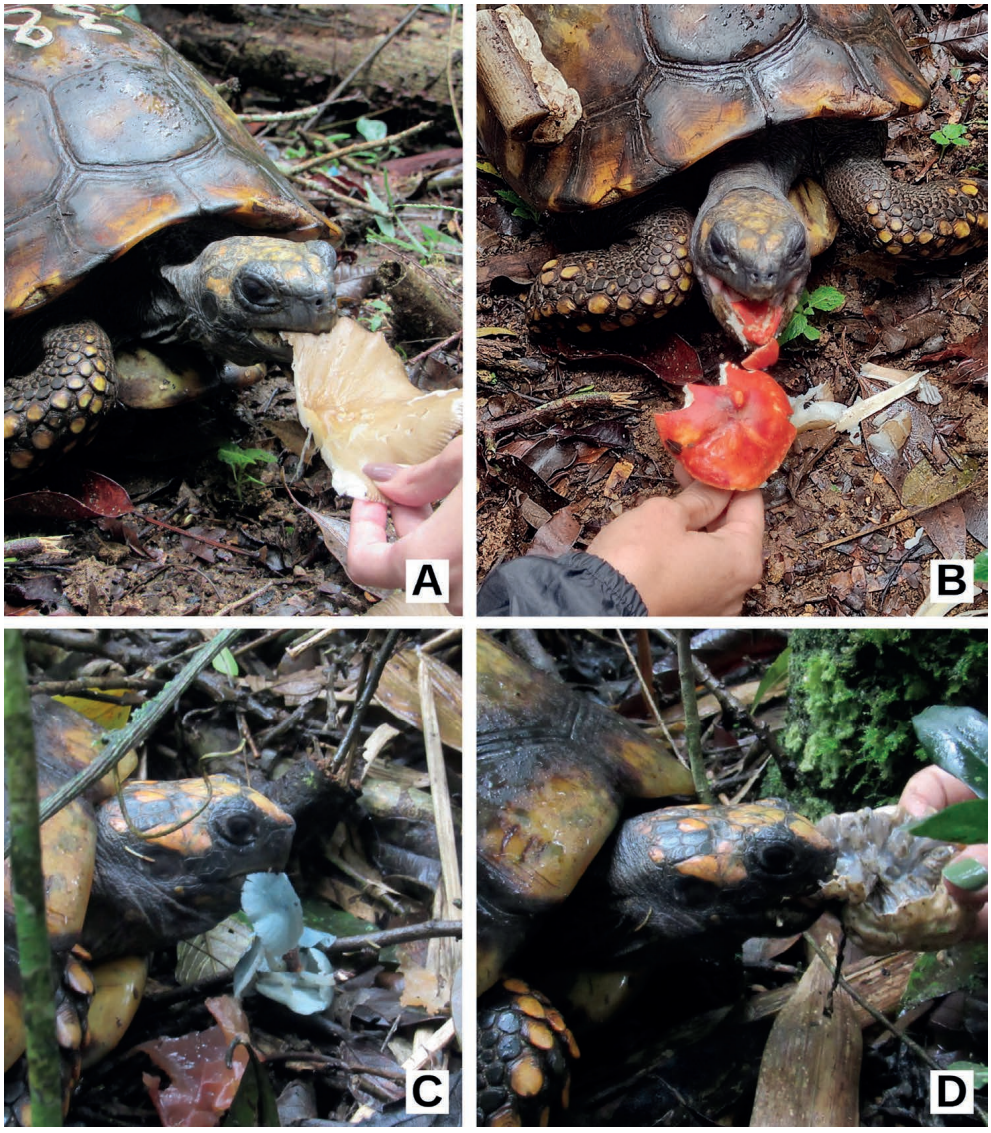

Fig. 3. Individuals of *Chelonoidis denticulatus* eating on the offered macrofungi (Tijuca National Park, RJ, Brazil). A-B) No. 38 eating on *Oudemansiella cubensis* (A) and *Leucopaxillus gracillimus* (B). C-D) No. 17 eating on *Clitocybula azurea* (C). and *Protubera maracuja* (D). Photos: A, C, D. Larissa Trierveiler-Pereira; B. Amanda Prado-Elias.

Fig. 3. Individuos de *Chelonoidis denticulatus* alimentándose de los macrohongos ofrecidos (Parque Nacional da Tijuca, RJ, Brasil). A-B) Nº 38 alimentándose de *Oudemansiella cubensis* (A) y *Leucopaxillus gracillimus* (B). C-D) Nº 17 alimentándose de *Clitocybula azurea* (C) y *Protubera maracuja* (D). Fotos: A, C, D. Larissa Trierveiler-Pereira; B. Amanda Prado-Elias.

Fig. 4. Refused basidiomes. A) Individual No. 63 only ate the exoperium ('rays') of *Geastrum* cf. *saccatum.* B) Individual No. 32 completely rejected *Hygrocybe* cf. *conica*. Photos: A. Larissa Trierveiler-Pereira; B. Amanda Prado-Elias.

Fig. 4. Basidiomas rechazados. A) Individuo Nº 63 sólo consumió el exoperidio ("rayos") de *Geastrum* cf. *saccatum.* B) Individuo Nº 32 rechazó completamente *Hygrocybe* cf. *conica*. Fotos: A. Larissa Trierveiler-Pereira; B. Amanda Prado-Elias.

The morphology and composition of the offered macrofungi were quite diverse: of the 13 macrofungi, eight are fleshy agaricoid mushrooms, which have a high water content. In addition to these, fungi with auricularioid, poroid, clavarioid, gasteroid (earthstar), and a fleshy sequestrate form (phalloid) were identified. It is noteworthy that only *Geastrum* cf. *saccatum* (partially consumed) and *Phaeoclavulina* sp. (rejected) have basidiomes with distinct textures, more rigid or papery. Among the completely rejected macrofungi, only *Hygrocybe* cf. *conica* had a high water content; however, it was offered to only one individual (Esmeralda, No 32). We can hypothesize that the tortoise No 07 was not hungry or was not attracted to the slimy pileus.

The mycophagous behavior of different groups of reptiles has been studied, often indirectly. Most studies report consumption based on observation and diet analysis methods, which fail to provide precise results. Studies using fecal or stomach analyses may also generate incomplete data, as fungi may be overlooked due to their digestibility.

For example, Koenig *et al*. (2001) mentioned that fungi were only detected in the diet of the blue-tongued lizard [*Tiliqua scincoides* (White, 1790)] through stomach dissection, but not through fecal analysis. Had the study not employed the dissection method, mycophagy would not have been detected, demonstrating that relying on just one method may lead to partial results. Assessing the importance of fungi as a component of the diet of reptiles is a challenging task, which explains why many diet studies neglect fungi (Elliott *et al*., 2019).

Among studies on fungal consumption by reptiles, the majority focus on terrestrial chelonians, which is the group with the most data on this topic. This allows for more refined analyses of the impacts of these associations. It has been reported that all species of the genus *Kinixys* consume fungi and/or invertebrates (Hailey *et al*., 1997). Elliott *et al*. (2019) compiled studies between 1892 and 2018 that documented mycophagous behavior in chelonians, resulting in reports from five families (Chelidae, Pelomedusidae, Emydinae, Geoemydidae, Testudinidae) across six regions (Africa, Asia, Europe, North America, and South America). The species *Terrapene carolina* (Linnaeus, 1758) has the highest number of mycophagous reports, likely due to its extensive distribution. *Chelonoidis denticulatus* is mentioned in five of these studies, with only one specifying the fungi it consumes. In a study on diet and food preference, Bayona & Rylander (1984) identified three macrofungi in its diet: *Favolus* sp., *Marasmius* sp., and *Auricularia* sp. Specimens of *Favolus* and *Auricularia*, which are abundant in the Atlantic Forest, were also consumed by the tortoises in the present study. From the Colombian Amazon, Vasco-Palacios *et al*. (2008) reported that native communities widely recognize that *C. denticulatus* consumes mushrooms, although no mushroom species were identified in the study.

Tortoises prefer humid areas close to water during the dry season, but during the rainy season they move away from these areas, as rivers and their tributaries may overflow (Bayona & Rylander, 1984). Moskovits & Bjorndal (1990) observed in their study, conducted on Maracá Island (northern Roraima, Brazil), that *Chelonoidis carbonaria* and C. *denticulata* consumed fungi more frequently during the dry season. It is hypothesized that the basidiomes they consume may serve as a hydration method for the tortoises, considering that some are composed of up to 90% water. Tortoises retain water to reabsorb when needed, and regular consumption of mushrooms may indicate an immediate hydration method or a way to store water for future use (Elliot *et al*., 2019).

After evaluating the results, considering that most of the specimens offered were agarics (mushroom form) and had a high water content, it is assumed that the specimens rejected or partially consumed had a lower percentage of water or even a different texture from the others, as being rigid; consequently, they became less attractive compared to the other available options. Also, we must not forget that the tortoises may be attracted to the color and scent of the food, and these characteristics can influence their food choice.

It is known that tortoises are major seed dispersers, being highly effective in increasing germination rates by passing seeds through their digestive tract (Varela & Bucher, 2002; Jerozolimski *et al*., 2009). However, there are not enough studies to understand how they contribute to fungal spore dispersal. To gather this information, studies which identifies the species consumed, are needed.

By identifying the specimens and assessing the viability of the spores, it is possible to measure the effects of disperser agents on the ecosystem. It is important to emphasize that data on animal behavior (e.g. feeding behavior), as well as the understanding of different ecological relationships occurring in nature, are crucial tools for developing strategies for the conservation of natural habitats (Reed, 2002).

This study reinforces the importance of mushrooms in the diet of the yellow-footed tortoise, but further research is needed in this area, over longer periods and using different methodologies, such as quantitative analyses, ecological studies, and spore viability tests.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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