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SHORT COMMUNICATION - Conservation of nature

Anurida granaria (Collembola) and *Peziza arvernensis* (Ascomycota) in Mycophagy Association at Riparian Zone in Southern Brazil

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Abstract

Riparian zones are important areas for biodiversity and preservation, where springtails and fungi contribute to nutrient cycling and organic matter decomposition. This study aimed to report a mycophagy interaction involving a colony of *Anurida granaria* with *Peziza arvernensis*. In the riparian zone, the use of apothecia as a nutritional source for springtail colonies was investigated. The collected data were analyzed, considering the fungal structures and the number of springtails. A colony of 515 springtails found in the apothecium was observed over a period of 5 weeks. Feeding events, courtship behavior, molting, and oviposition were recorded. The survival and mortality rates of the colony fluctuated during the observation period but remained stable between weeks 4 and 5. This study presents the first report involving *P. arvernensis* and *A. granaria* in a mycophagic association, highlighting the significance of this dynamic in benefiting the life cycle and fungal dispersion in riparian zones.

Keywords: Fungi, springtails, food resource, population dynamics, survival location.

Riparian zones are an important site for the protection of water resources, maintaining diversity in fauna and flora (Del Cid et al., 2023). *Peziza* sp. (Ascomycota, Pezizomycetes) is a saprobic fungi that grows in soil, typically in forested areas with litterfall. Additionally, the genus is widely distributed throughout the American continent, including several edible species (Pfister et al., 2022).

Springtails are cosmopolitan, displaying a high dispersal capacity and can be found in various layers of soil, but they primarily inhabit leaf litter. Regarding their diet, they are herbivorous, detritivorous, and omnivorous, with a diet consisting of organic matter, bacteria, and fungi (Deharveng & Bedos, 2019). *Anurida* sp. is a type of edaphic springtail, widely distributed in the American continent, playing a fundamental role in the dynamics of organic matter decomposition in the soil and nutrient mineralization (Moseley & Proctor, 2016).

In general, springtails are bioindicators of soil quality, precisely because of their sensitivity to environmental changes (Janssens & Christiansen, 2011). Therefore, its presence in riparian zones helps in understanding and classifying the quality and preservation levels of the area. Together with fungi, springtails form part of the group of microorganisms responsible for formation and enrichment of availability of macronutrients and micronutrients in soil, through the process of nutritional cycling from organic matter (Hernández-Santiago et al., 2020).

The fungi ability to attract predators may be related to its morphology and physiology, where nutritional preference appears to correspond to the conditioning of each species involved in mycophagy (Costa et al., 2023). Also, the substrate to which the fungi is attached influences this interaction (Del Cid et al., 2023). In studies related to the mycophagy behavior of springtails, it is generally observed that dark-colored fungi, such as the brownish tones of apothecial are more enticing to mycophagy (Hernández-Santiago et al., 2020).

Thus, this study aimed to provide an unprecedented description of the interaction between mycophagy and the use of *Peziza arvernensis* apothecium within a colony of *Anurida granaria* found in a riparian zone in sourthem Brazil.

The sample area was a riparian zone in Vale do Rio Pardo, Vera Cruz city, Rio Grande do Sul state, Brazil (-29°42'53"S and -52°30'20"W). The apothecial fungi and springtails were collected under license SISBIO n° 78538-2. To identify fungi,

we used the key by Larsen (2023), and to identify springtails the key by Jarssens & Lebeaux (2023). For both species the macrostructures were observed, microstructures were analyzed using an Olympus DP53 optical microscope.

To investigate the use of apothecium as source, the colony and fungi were placed in a sterile enclosure air-ventilated for observation. Due to the need to rehydrate the fungi since it is no longer associated with the substrate, the apothecium was rehydrated with distilled water twice daily during the observation period. All the analyses were conducted at Universidade Federal do Pampa, in São Gabriel city, Rio Grande do Sul state, Brazil (-30°33'61"S and -54°35'43"W).

Apothecium weight measurements and springtail counts were conducted weekly using a precision scale and photographic images, respectively. The data were summarized using Microsoft Excel version 2010 (Microsoft, 2010), and population estimates for each week were calculated using Schumacher and Eschmeyer method (Edwards & Eberhardt, 1967). Graphs were generated in BioEstat version 5.3 software (Santos, 2007).

The analyzed colony contained 515 individuals of *A. granaria* in association with one apothecium *P. arvernensis* that was collected together with the colony. Observations of colony occurred during 5 weeks, period that apothecium served as a food resource, and subsequently the colony was released (Figure 1). The initial weight of the apothecium ranged from 0.250 g to 0.012 g during the observation period (Figure 1 A-B).

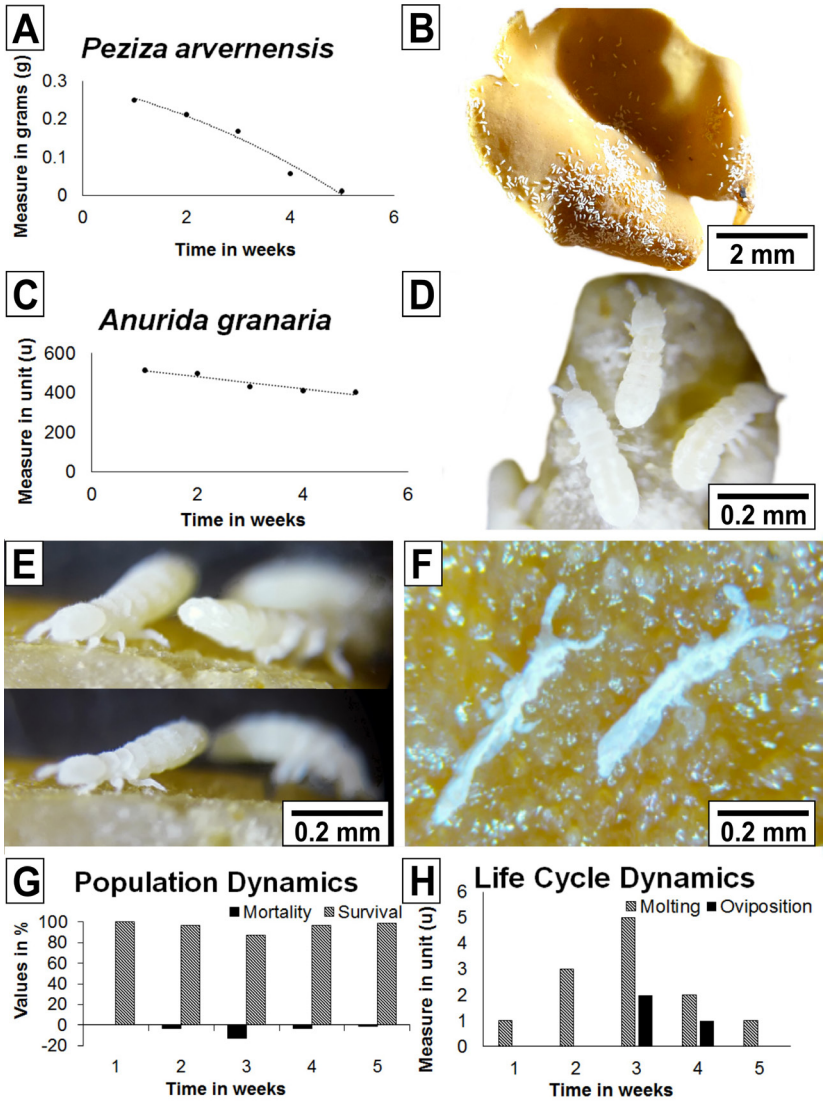

Figure 1. *Peziza arvernensis* and *Anurida granaria.* Weight of the apothecium (A). Apothecium contained the colony of Collembola (B). Springtails number during the observation (C). Feeding events in the apothecium (D). Springtails effectuated cycle movements (E). Molting (F). Mortality and survival rate (G). Molting and oviposition rate (H).

Regarding the Collembola colony, the number of individuals ranged from 515 to 403 during the same period (Figure 1-C). Feeding events and possible courtship behavior without copulation due to circular movements, as well as molting were observed (Figure 1 D-F). The survival and mortality rate of the colony exhibited fluctuations over the observed time period. Third week experienced highest mortality rates, with stability in survival rates observed during weeks 1, 2, 4, and 5 (Figure 1-G). The molting process occurred over the course of 5 weeks, with events involving 1 individual in week 1 and 5, 2 individuals in week 4, 3 individuals in week 2, and 5 individuals in week 3. However, egg-laying process occurred only between weeks 3 and 4, with 2 individuals in week 3 and 1 individual in week 4 engaging in the process without performing parental care (Figure 1-H).

The association described in the study is the first report involving *P. arvernensis* and *A. granaria*. Apothecium used as a nutritional resource by springtails has highlighted the significance of dynamics in riparian zones. According to Del Cid et al. (2023), beneficial associations between microfauna and fungi not only contribute to the maintenance of these areas but also to their regeneration.

In general, the edibility and nutritional potential of *P. arvernensis* have been little studied, but the genus includes edible species (Pfister et al., 2022). For springtails, the interaction proved to be beneficial, as the colony survived in confinement for 5 weeks only with the apothecium as a food source. Additionally, *Anurida* sp. has been cited for its fungivorous feeding habits (Deharveng & Bedos, 2019), demonstrating that mycophagy may be more common in this group than previously thought.

During the 5 weeks of observation of Collembola colony, events involving feeding, possible courtship behavior, molting and oviposition occurred, demonstrating the potential for interaction between the apothecium and springtails. Both taxonomic groups there are benefits from this association. For springtails, it provides nutritional supply, and for the apothecium, it facilitates the dispersion of spores. According to Hernández-Santiago et al. (2020) the potential for the dispersion of spores by springtails is broad, especially in preserved areas, due to the group's susceptibility to environmental changes. Fungi in these areas also contribute to the decomposition of organic matter. Consequently, in conjunction with their presence, the process of nutritional mineralization occurs, enhancing the quality of the soil (Moseley & Proctor, 2016).

Edaphic microfauna is the base of the food chain, during observations carried out with the colony in confinement, individuals spent most of their time in the apothecium. In general, the morphology of the apothecium serves as a protective structure for springtails, as they are often targets of predation (Hernández-Santiago et al., 2020). Once the colony has completed stages of the life cycle in the apothecium, indications of its use as not only a source of food but also as a living space can be inferred. In the study by Costa et al. (2023), when analyzing the mycophagy behavior of a Diptera, in addition to predation by adults and larvae on the mushroom, and adults returned for egg laying. This dynamic partly coincides with the findings regarding springtails in our study.

Through the data obtained in this study, it was possible to provide an unprecedented description of the mycophagy behavior and its use during the development and various stages of the life cycle of *A. granaria* with *P. arvernensis*. In this interaction observed in a specific area, positive factors such as molting, courtship behavior, egg laying, and its use as a nutritional resource could be observed. In this form, our findings serve as a basis for further studies in the area.

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