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Notes and Comments

First report of *Zygothrica candens* Burla, 1956 (Diptera, Drosophilidae) in mycophagic association with the mushroom *Oudemansiella cubensis* (Berk. and M.A. Curtis) R.H. Petersen, 2010 (Agaricales, Physalacriaceae) in southern Brazil

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Mycophagy associations in Diptera can be divided into four distinct groups based on the lifestyle of each species. Primary mycophagists feed exclusively on fungi (basidiome or mycelium). Secondary mycophagists are called saprobes and feed on decaying hosts. Detritivores may feed on much decaying biological material, in this case they may oviposit on basidiome. Predators occur in association with all fungal structures, and their larvae are mainly predatory in the last stage (Santa-Brígida et al. 2012). For a better understanding of mycophagous flies, the selection of habit depends of fungi occurrence (Bunyard, 2018). In addition, basidiomata only form with humidity and temperature specific to each species (Putzke and Putzke, 2017). An important point is that the larval phase of Diptera is relatively short (Yamashita and Hijii, 2007), which may well coincide with the period of basidiome occurrence.

Gottschalk et al. (2009) and Bunyard (2018) infer in their studies that mycophagous flies of Drosophilidae are associated with more than 31 species of mushrooms, some toxic such as *Amanita muscaria* (L.) Lam (Agaricaceae), including *Drosophila fallen* (Fallen, 1823), *Leucophenga varia* (Walker, 1849), *Mycodrosophila dimidiate* (Loew, 1862), and *Zygothrica hypandriata* (Burla, 1956). In addition, Bunyard and Foote (1990) reported that the life cycle of *Drosophila putrida* (Sturtevant, 1916) was associated with the mushroom *Oudemansiella radicata* (Relhan) Singer (Physalacriaceae).

Oudemansiella cubensis (Berk. & MA Curtis) RH Petersen (2010) has a fleshy pileus and is described in Brazil, Costa Rica, Cuba, Dominican Republic, Ecuador, Colombia, and Argentina (Petersen et al., 2008; Wartchow et al., 2010). The species is mentioned as native in South America (Putzke and Putzke, 2017). There are no known cases of mycophagous flies associated with this mushroom in the literature. There are no reports of mycophagous of feeding habits to *Zygothrica candens* (Burla, 1956), and this is the first report involving *O. cubensis*. Also, the fly is distributed only in Brazil and Ecuador, considered native to South America (Tidon et al., 2022). Thus, the present study aimed to describe the first record of mycophagy and the association of *Z. candens* with the mushroom *O. cubensis* in southern Brazil.

The study was conducted in September 2021 in the National Forest (FLONA) in São Francisco de Paula, Rio Grande do Sul, Brazil (-29°25'22"S and -50°23'11"W), a conservation unit of approximately 1,606.00 hectares of native Atlantic Forest, with fragments of dense ombrophilous forest and mixed ombrophilous forest. Samples were collected in the Centenarian Araucaria Trail. Four basidiomata of mushroom with signs of mycophagy growing on the substrate of *Araucaria angustifolia* Bertol. (Kuntze), and five adult flies in the basidiomata were collected to identification. The mushrooms and flies were collected under license SISBIO n° 78538-1.

Basidiomata were dried at 40°C and stored in paper bags for taxonomic identification using the key of Putzke and Putzke (2017). Drosophilids were preserved in tubes containing 70% ethyl alcohol and identified based on external morphology and genitalia (male and female) according to Burla (1956) and Grimaldi (1987). Both samples were examined with optical microscope Olympus DP53 and Zeiss Discovery V20. Subsequently, the samples were deposited at the Laboratório de Taxonomia de Fungos in Universidade Federal do Pampa, Campus São Gabriel, Rio Grande do Sul, Brazil.

As taxonomic characters, *O. cubensis* has a pileus 30-120 mm in diameter, grayish white. Surface of the pileus with scales and glutinous. Fleshy context with grayish-white lamellae of 1.5 mm, adnate. Stipe white, fibrillose-squamous with a base of 30-140 × 2-20 mm (Figure 1A). White or cream spores with 10-16 µm, globose to subglobose, smooth and thick-walled, inamyloid, hyaline. Basidia with 50-120 × 18-30 µm, clavate and tetrasporic.

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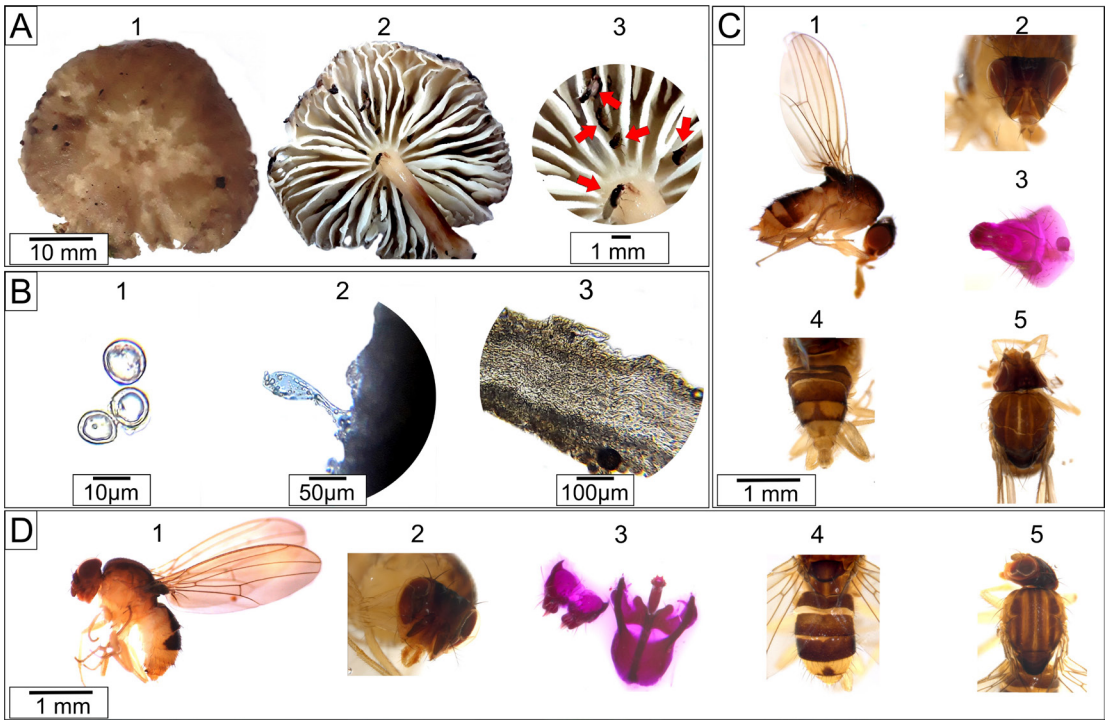

Figure 1. Macro and microstructures of the species analyzed in this study. *Oudemansiella cubensis*: pileus top view 1-A, bottom view 2-A, arrows indicating adult flies found in the basidiome 3-A, spores 1-B, basidium 2-B, lamellar trama 3-B. *Zygothrica candens* female: lateral view 1-C, frontal view head 2-C, genitalia (oviscapt valve + spermathecal capsule) 3-C, dorsal view abdomen 4-C, dorsal view thorax 5-C. *Zygothrica candens* male: lateral view 1-D, frontal view head 2-D, genitalia (hypandrium in V-shaped and pregonites + phallus and phallapodeme) 3-D, dorsal view abdomen 4-D, dorsal view thorax 5-D.

Pleurocystidia and cheilocystidia numerous with 70-200 × 15-50 µm, versiform (Figure 1B). Pileipellis formed by piriform or subglobose elements. Context formed by broad filamentous hyphae. Cortical layer of the stipe formed by thin filamentous hyphae. Grows on wood. Distribution in Brazil in Porto Alegre city - Rio Grande do Sul state (Putzke and Pereira, 1988), and São Paulo city - São Paulo state (Singer, 1989). In São Francisco de Paula city - Rio Grande do Sul state this is the first record of the species.

During the collection of material, individuals in holometabolous development stages (embryos, larvae, pupae and adults) of *Z. candens* were found in the lamellar region of *O. cubensis*. According to Burla (1956) as taxonomic characters, *Z. candens* has head brown, eyes red, pedicel and scape brown, total length 18-25 mm. Orbital and bristles plates are brown. Front and face ochre with ocellar tringle occupying approximately 1/2 of the frontal length. Thorax with dark and light bands brownish-brown, scutellum brown and slighter in the margins, legs yellow. Wings hyaline, without distinct spots with veins straight. Abdomen yellow with brown bands on tergites. Terminalia in U-shaped form with frontal lobes small. Hypandrium in V-shaped form containing many growth lines. Phallapodeme short in lateral view (Figure 1C and D). Distribution in Brazil in Boracéia city - São Paulo state (Val and Kaneshiro, 1988) and Itatiaia city - Rio de Janeiro state (Burla 1956; Wheeler, 1970). There is already a case for the São Francisco de Paula city - Rio Grande do Sul state (Gauterio et al., 2020).

In relation to the characteristics of the interaction of mycophagy between fly and mushroom, the mushroom has shown signs of predation in the lamellar portions of the basidiome. In the analyzes of internal structures of digestive system of the fly, spores mushroom were found in the hindgut. External structures, such as wings, legs, thorax and abdomen also showed attached spores.

The study reports the first observation of mycophagy association of *Z. candens* with *O. cubensis* in southern Brazil. The natural population of *Zygothrica* has already been described in Rio Grande do Sul state (Valer et al., 2016; Gauterio et al., 2020). However, the occurrence of *Z. candens* on *O. cubensis* is new. This fact can be associated with fungi species whose known distribution from southern region of the country (Petersen et al., 2008; Wartchow et al., 2010).

Neotropical mycophagous flies are also represented in the Drosophilidae. Bunyard (2018) studied three mycophagous flies feeding on *Amanita* sp., which is considered a toxic species. Nevertheless, drosophilids are not only associated with toxic mushrooms. Other Agaricomycetes, belonging to the families Agaricaceae and Boletaceae (Bunyard, 2007), as well as *Oudemansiella* sp. (Physalacriaceae) have already been described in the literature in mycophagy associations with drosophilids (Bunyard and Foote, 1990). The latter are classified as non-toxic mushrooms (Putzke and Putzke, 2017). In the

study by Gauterio et al. (2020) with analyzes of the phylogenetic relationships of *Zygothrica* and other genera of drosophilids associated with fungi, it was inferred that the neotropical group has a lineage close with neotropical fungi of Auriculariales and Agaricales. In these two cited orders, the use of the fly as a breeding site and the presence of its larvae are reported in the fungi, but the taxonomic information on the fungi was not mentioned. However, this factor could be an of the most important indicators of this association.

In studies conducted in the Brazilian Amazon involving arthropods associated with edible mushrooms, Amaringo-Cortegano et al. (2013) found a relationship among *O. cubensis* and individuals of Poduromorpha and Siphonophorida. The latter order was identified in 87% of the collected samples containing mycophagous individuals. However, the authors did not report individuals of drosophilids. Doge et al. (2015) reported that resource variability is the main factor for the Drosophilidae population size. In addition, fleshy basidiomata such as *O. cubensis* are reported to be preferred by the mycophagous flies (Santa-Brígida et al. 2012). According to Gauterio et al. (2020), mycophagous flies and fungi coevolved. Moreover, most flies already analyzed are polyphagous and complete their life cycle in basidiomata (Bunyard, 2018). Another important factor is the presence of soft tissues in Agaricales, macro and micronutrients, and the tolerance of mycophagous flies to fungal toxins, as concluded by Yamashita and Hijii (2007). Thus, even if *Z. candens* have occurrence in this region, it is more feasible to infer that the fly uses the mushroom as a temporal resource. In this sense, the mushroom is not the main responsible for the maintenance of its guild richness. As a benefit, the fly disperses the spores of this mushroom to distances reachable by its flight.

The adaptation of the fly in using a spatially unpredictable temporal resource such as the basidiome may be the key that determines the success of species presence in the region. Also, the attractiveness of the mushroom resulting both from the quality of the substrate, as in the case of *A. angustifolia* trees, and the natural influence of the FLONA are intrinsically involved in this association. However, *O. cubensis* is considered an arboreal host, with wood as its main substrate (Putzke and Putzke, 2017), so the occurrence of the fungi is not restricted to the distribution of *A. angustifolia*. According to Bunyard (2007, 2018), it is unpredictability that drives the mechanisms of speciation in drosophilids. The occurrence of the fly and the mycophagy associated with the entire life cycle of the studied individuals, which includes oviposition, growth phases and return of the adult individual, are possibly interconnected. Therefore, further analysis should be conducted, considering that the description of this mycophagy association is unprecedented for both species and has only been described in southern Brazil.

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