



## Notes on mycophagy of *Descomyces albus* (Basidiomycota) in southern Brazil

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### Abstract

*Descomyces albus* is a truffle-like fungus in the Cortinariaceae collected for the first time in *Eucalyptus* plantations in the state of Rio Grande do Sul, Southern Brazil. The morphological features, distribution and taxonomy of the collections are discussed. Additional observations of the external surfaces and gut contents of *Balloniscus sellowii* indicate *D. albus* is consumed by the species and its spores dispersed in the soil organic matter. This is the first published record of mycophagy involving *B. sellowii* in Brazil. This information contributes to the knowledge on mycophagy and truffle-like fungi.

**Key words** – *Balloniscus sellowii* – fungivory – hypogeous – Oniscidae – sequestrate

### Introduction

Extensive plantations of *Eucalyptus* spp. were established in southern Brazil early in the 20<sup>th</sup> century (Lima 1993). Currently *Eucalyptus* plantations in Brazil surpasses 3.75 million hectares (SBS 2008). Various fungal species, including truffle-like ectomycorrhizal fungi (ECM) were introduced accidentally but fortuitously along with these economically important trees, and became established in the exotic plantations as vital symbionts (Nouhra et al. 2008).

Throughout the years, a few studies on fungal diversity associated with the eucalypt plantation in southern Brazil have been conducted, mostly, if not all, focusing only on mycorrhizal fungi. Putzke (1999), Giachini et al. (2000), Giachini et al. (2004), Cortez et al. (2008, 2011), and Sulzbacher et al. (2010, 2013) report a variety of ectomycorrhizal fungal species associated with *Eucalyptus* plantations in Brazil. Amid the detected fungi, species of the cortinarioid truffle-like genus *Descomyces*.

Based on spore morphology (e.g., basidiospores with smooth rostrum and ornamentation embedded in the perisporium), peridium structure (e.g., two layered and with swollen cells), and mycorrhizal status, Bougher and Castellano (1993) proposed *Descomyces* to accommodate truffle-like fungi related to the epigeous, mushroom genus *Descolea* (Bougher & Castellano 1993, Bougher 2009). A comprehensive discussion about the relationships and limits of *Descomyces* have been previously presented (Smith 1973, Thiers 1984, Lago et al. 2001, Peintner et al. 2001, Francis & Bougher 2002, 2004, Castellano et al. 2004, Cortez et al. 2008). *Descomyces* evolved from stipitate taxa (Peintner et al. 2001, Tedersoo et al. 2010), and has high morphological variability (Beaton et al. 1985, Bougher & Castellano 1993, Lago et al. 2001, Francis & Bougher 2004). Phylogenetically, *Descomyces* is well-supported and closely related to the agaricoid genus *Descolea* and the secotioid genus *Setchelliogaster* (Martín & Moreno 2001, Peintner et al. 2001), recently grouped in the “*Descolea* clade” (Tedersoo et al. 2010).

*Descomyces* species are endemic to Australia, but have been introduced into Argentina, Brazil, Germany, New Zealand, North Africa, Spain, the UK and the USA (Bougher & Castellano 1993, Keane et al. 2000, Giachini et al. 2000, Francis & Bougher 2004, Moreno-Arroyo et al. 2005, Nouhra et al. 2008). In Brazil, *Descomyces* has been rarely reported, with references for *D. albellus* (Masse & Rodway) Bougher & Castellano, *D. albus* (Klotzsch) Bougher & Castellano, and *D. giachinii* Trappe, V.L. Oliveira, Castellano & Claridge (Giachini et al. 2000), all associated with eucalypt plantations in the state of Santa Catarina. In the State of Rio Grande do Sul, the only species recorded was *D. albellus* (Masse & Rodway) Bougher & Castellano, also from eucalypt plantations (Cortez et al. 2008).

Truffle-like fungi produce fruiting bodies underground (hypogeous) which impedes detection by humans. Truffle-like fungi are dependent on mycophagy for spore dispersal (Castellano et al. 2004). Animals excavate and consume sporocarps, later dispersing the spores when they defecate (Johnson 1996, Maser et al. 2008, Trappe et al. 2009). The relationship between truffle-like fungi and some animal groups is well-documented in North America (Maser et al. 2008, Trappe et al. 2009), Europe (Montecchi & Sarasini 2000, Moreno-Arroyo et al. 2005) and Australia (Claridge 2002, Jumpponen et al. 2004, Maser et al. 2008). In South America, one of the few studies involving truffle-like fungi and mycophagy was presented by Nouhra et al. (2005), where *Alpova austroalnicola* was confirmed as part of the diet of an armadillo species (*Dasypus novemcinctus novemcinctus*). A second study by Perez Calvo et al. (1989), studied the relationships of small mammals and the mycophagy from Patagonia. Recently, non-native mammals have been identified as important dispersers of non-native ECM fungi in Argentina (Nuñez et al. 2013). In the Guyana forests, the relationship between fungi and arthropods was briefly investigated by Henkel et al. (2010). The means of spore dispersal is unknown for many truffle-like fungi (Lilleskov & Bruns 2005), and studies in tropical and subtropical regions, like Brazil, where mycophagy related studies are lacking, are needed to clarify these interactions.

The consumption and spore dispersal of basidiomata, including truffle-like fungi, by invertebrates have been studied by numerous scientists outside South America (Fogel & Peck 1975, Pacioni et al. 1991, Claridge 2002, Lilleskov & Bruns 2005). Among the invertebrates that can be involved in this relationship is *Balloniscus* (Oniscidae, Balloniscidae), an isopod genus with two representative species in Rio Grande do Sul (Buckup & Buckup 1999, Kern et al. 2012). *Balloniscus sellowii* (Brandt, 1833) is common in Southern Brazil, Uruguay, and the region surrounding Buenos Aires in Argentina (Schmalzfuss 2003). This species has a wide ecological range, revealing the possibility to use it in biomonitoring programs (Wood et al. 2012, Kern & Köhler 2012).

Information on the relationship between fungus and isopod species may be important to elucidate the biogeography and distribution of truffle-like fungi around the world. The objective of this study was to investigate the association of *Balloniscus sellowii* encountered within fresh basidiomes of *Descomyces albus*, in relation to potential mycophagous status and prospective dispersal of spores.

## Materials & Methods

### Basidiome sampling and morphological studies

Fresh basidiomes were collected in plantations of 20–30 year old *Eucalyptus* spp. in the state of Rio Grande do Sul, in the municipalities of Santa Maria and Vila Nova do Sul. Climate in the region is subtropical of Cfa/Cfb type (wet all year with mild to warm summers). All collections obtained are preserved either at the SMDB (Department of Biology of the Federal University of Santa Maria), or at the UFRN-fungos (Herbarium of the Federal University of Rio Grande do Norte) herbaria. Fungal specimens were collected at random 1000 m<sup>2</sup> plots in the plantations. Basidiomes were located by raking the top soil litter (Claridge et al. 2000). Basidiomes were photographed in the field, then studied macro- and microscopically following previously described methodology (Montecchi & Sarasini 2000). Taxonomically relevant microscopic characteristics were photographed with the aid of optical microscopes (Eclipse-Ni Nikon), and microscopy digital camera (DS-Ri1 Nikon) used for microscopic photos. Line drawings of the microstructures were made with the aid of a drawing tube attached to the microscope (BX41 Olympus), with 100× magnification. Color codes followed Kornerup and Wanscher (1978). Prior to storage, basidiomes were dried with the aid of a fruit-drier (forced air, not exceeding 40°C). Basidiospore data follow the methodology proposed by Tulloss et al. (1992). Measurements and statistics are based on 30 mature spores. Abbreviations include L(W) = average basidiospore length (width), Q = the length:width ratio range as determined from all measured basidiospores, and Qm = the Q value averaged from all basidiospores measured. Basidiospore measurements do not include the ornamentation. For scanning electron microscopy (SEM), dried material (from herbarium) was mounted directly on aluminum stubs and coated with a 5 nm thick layer of gold using a Balzers SCD 050 Sputter. Stubs were examined in a Philips - XL30 microscope at the Federal University of Rio Grande do Norte. Herbaria acronyms follow Thiers (2015).

### Invertebrate collection

During fieldwork, specimens of *Balloniscus sellowii* were collected from some basidiomes of *Descomyces albus*, at an *Eucalyptus saligna* Sm. plantation, at the Experimental Forestry Station (FEPAGRO), in the municipality of Santa Maria, central region of Rio Grande do Sul, in Southern Brazil (29°45'S, 53°43'W). Only one collection in 22<sup>nd</sup> May 2013 was made. The invertebrates were collected using forceps on the peridial surface and within basidiomes following Lilleskov & Bruns (2005). No fecal material was collected. All invertebrates were collected and placed in an eppendorf tube with 70% alcohol and deposited in the CESC of University of Santa Cruz do Sul.

### Microscopic examination

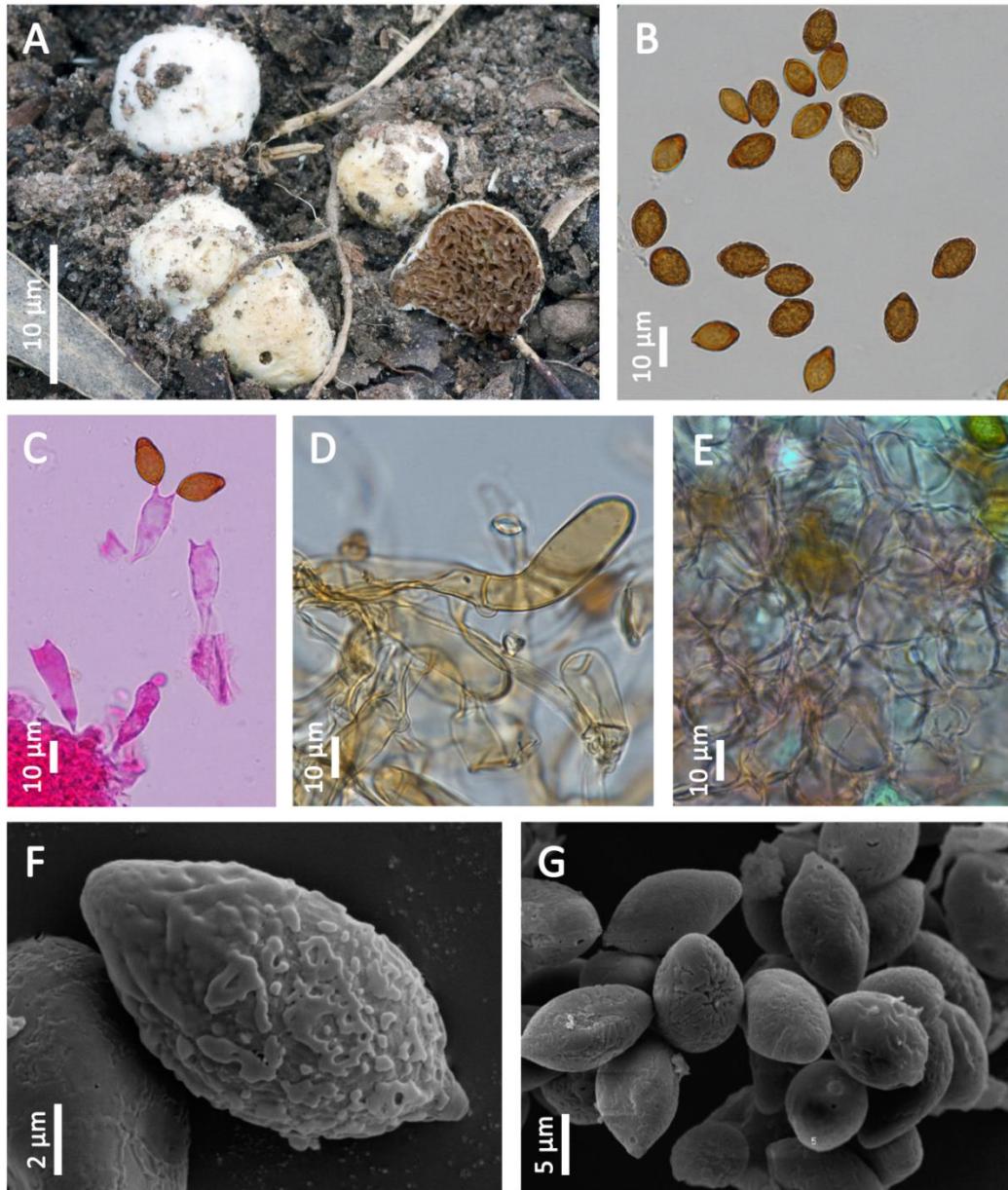
External surfaces and gut contents of animals were examined using a light microscope to detect *Descomyces albus* structures, e.g. basidiospores, basidia and hyphae. Structures of *D. albus* are identified by their distinctive shape, color and spore ornamentation (Bougher & Castellano 1993). A stereomicroscope (EZ4 Leica) was used to examine and photograph isopoda.

## Results

*Descomyces albus* (Berk.) Bougher & Castellano, Mycologia 85: 280. 1993.

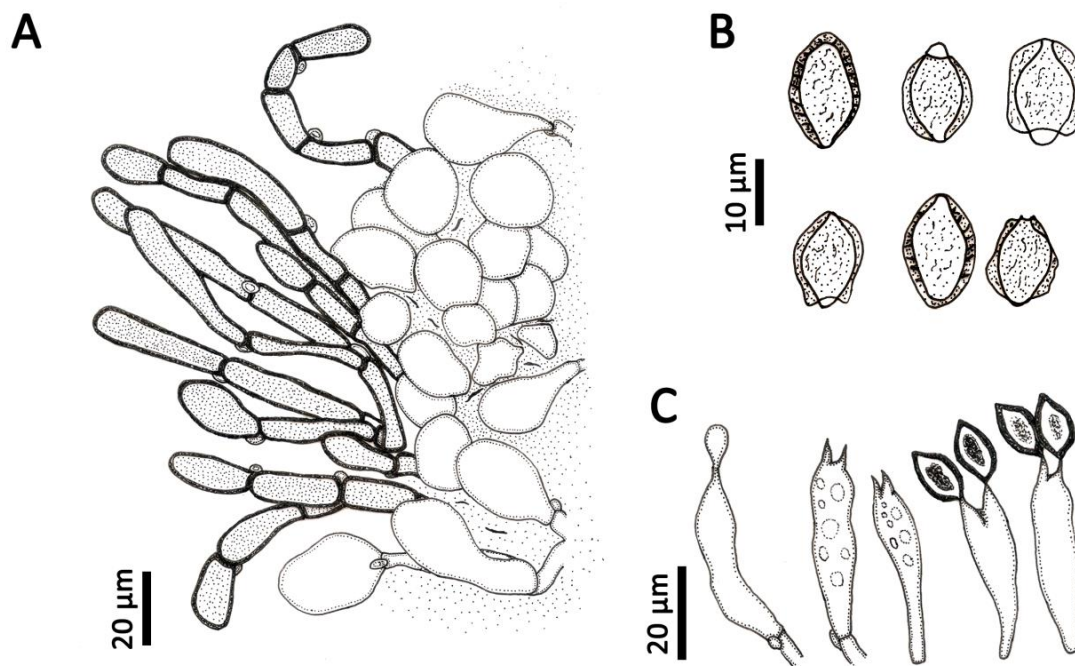
Figs. 1–2, 4.

Basidiomes subglobose to globose, 7–17 mm broad, 8–12 mm tall (fig. 1a, 4a). peridium 0.2 mm thick, white (1a1), light yellow (1a4) to dark yellow (4c8), smooth and dry or slightly moist when fresh, covered by soil, opening by irregular dehiscence at the top of the basidiomes, rhizomorphs absent. stipe absent. Columella usually absent or reduced to a basal disc. Gleba dry, composed of irregular, empty locules, yellowish brown (5e7–5e8) at maturity (Fig. 1a).



**Fig. 1A–G** – *Descomyces albus*. A. Fresh mature basidioma. B. Basidiospores mounted in 5% KOH. C. Basidia. D. External peridiopellis elements, mounted in KOH. E. Inner peridiopellis elements, mounted in 5% KOH with Congo Red. F–G. SEM image of basidiospores. Photos: M.A. Sulzbacher.

BASIDIOSPORES [30/1/1] (11–) 13–17 × 7–9 µm, (L = 15 µm, W = 8.5 µm, Q = 1.5–2), Qm = 1.8, excluding ornamentation (UFRN-fungos 2214), ovoid to limoniform in frontal view, ovoid to fusiform in profile, color rusty in 5% KOH, walls <1 µm broad, ornamentation a sub-reticulate to verrucose perisporium, 0.5–2.5 µm thick (Fig. 1B, 2B). Under SEM basidiospores are limoniform and ornamentation appears verrucose with isolated, irregular warts (Fig. 1F–G). BASIDIA 21–48.5 × 7.5–11 µm, clavate, mainly bisporic, some tetrasporic (Fig. 1C, 2C). BASIDIOLES 12–28 × 5–8 µm, clavate, wall hyaline and thin, basal clamp connection present. PERIDIUM two layered: the external layer formed by non-gelatinized, ascendant to prostrate hyphae, 4–13.5 µm diam., with thickened, golden yellow walls, smooth, all septa with clamp connections; internal peridiopellis elements composed of a hymeniform layer, thin-walled, hyaline hyphae, end-cells clavate or pyriform 19–56 × 10–26 µm (Fig. 1D–E, 2A). HYMENOPHORAL TRAMA regular to subregular, of thin-walled, hyaline, smooth hyphae, 3–6 µm broad. CLAMP CONNECTIONS present at most septa. RHIZOMORPHS not observe.



**Fig. 2A–C** – *Descomyces albus*. A. Peridiopellis elements showing two layers; outer layer with thick-walled, clamped hyphae; inner layer of hyaline, inflated hyphae. B. Basidiospores. C. Basidia.

Habitat – hypogeous, under soil and dead *Eucalyptus* leaves, ectomycorrhizal (Agerer et al. 2001).

Host species – *Eucalyptus saligna*, *Eucalyptus* spp.

Known distribution – widespread in eucalypt plantations throughout the world (Bougher & Castellano 1993, Lago & Castro 2004, Sulzbacher et al. 2013). Probably indigenous to Australia (Bougher & Castellano 1993). In Brazil, the only records of this species outside Rio Grande do Sul are for the state of Santa Catarina (Giachini et al. 2000).

Material examined – BRAZIL. Rio Grande do Sul: Santa Maria, Boca do Monte District, Estação Experimental de Silvicultura-FEPAGRO, 29°30'27.2"S 53°54'49.8"W, 19 Oct 2012, leg. Grebenc T DESALB/191012-001E (UFRN-fungos 1751); *ibid.*, 22 May 2013, leg. Coelho G & Sulzbacher MA (UFRN-fungos 2214); Vila Nova do Sul, 30°20'23.8"S, 53°51'58.4"W, near to BR 290, 09 Nov 2009, leg. Sulzbacher MA 203 (SMDDB).

Invertebrate analyses – Five samples of *Balloniscus sellowii* were collected (Fig. 3A–C). Invertebrate samples had exposed spores in gut contents (Fig. 4B). A total of 10 basidiospores were observed under light microscopy from two isopoda samples (Fig. 4E–F). The gut contents revealed a significant proportion of *D. albus* structures, e.g., spores and hyphal fragments with clamp connections (Fig. 4C–D). These hyphae are probably from the external peridiopellis layer, since they are characterized by the non-gelatinized, wide, thick-walled (<1 µm diam.), hyphae, hyaline to golden yellow, smooth, septa with clamps, 5–9.5 µm diam., with rounded terminal hyphae. The basidiospores examined were 11.5–16 × 6.5–9.5 µm, excluding ornamentation, n = 10, ovoid to limoniform, rusty in 5% KOH, thick-walled (<1 µm diam.) and ornamented with a sub-reticulate to verrucose perisporium, 1–2.5 µm diam.

## Discussion

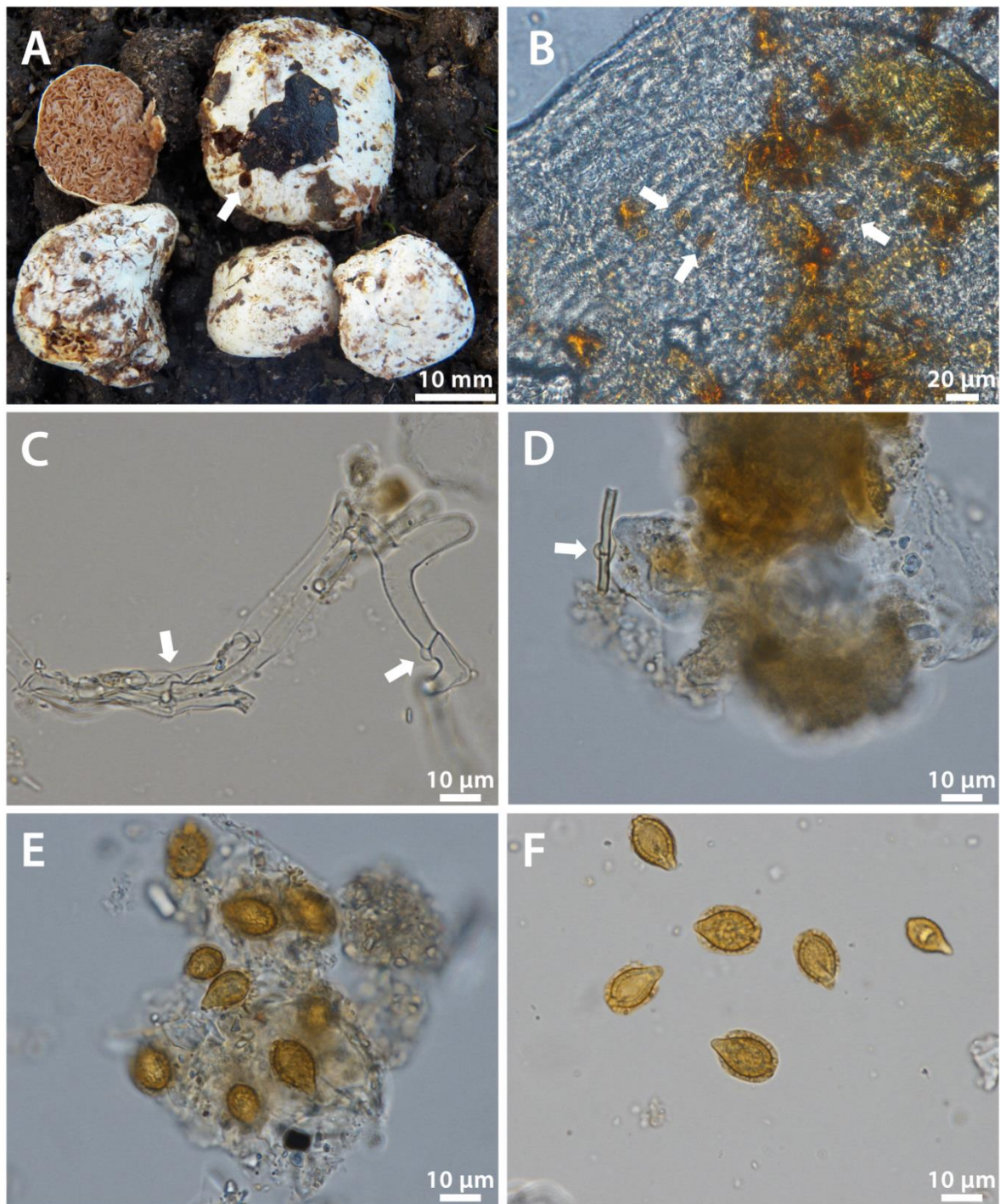
*Descomyces albus* is characterized by its inner peridiopellis layer that is composed of a polycystoderm of inflated elements (Bougher & Castellano 1993, Francis & Bougher 2004). Three species of *Descomyces*, *D. albus*, *D. albellus* and *D. giachinii*, are similar in morphology and ecological traits. The only significant difference among them is related to the structure of the



**Fig. 3A–C** – *Balloniscus sellowii*. A–B Mature Isopoda. C. Immature specimen showing dorsal surface with gut contents.

peridiopellis (Lago & Castro 2004), which is trichoderm in *D. albellus* and *D. giachinii*, while epithelial in *D. albus* (Bougher & Castellano 1993, Lago & Castro 2004, Cortez et al. 2008). Nouhra et al. (2008) cite two new species: *D. fusisporus* Trappe & Claridge, characterized by the spores that are fusoid-citriform, lacking a peridial epithelium of inflated cells, and *D. varians* Trappe & Claridge, based on the variable development of the polycystoderm epithelium and the ornamentation of the spores. The latter species is similar to *D. albus*, differing only by a polycystoderm epithelium in *D. albus* (Nouhra et al. 2008). Giachini et al. (2000) reported *D. albus* for the first time in Brazil plantations of *Eucalyptus dunnii* in Santa Catarina. This is the first record of the species in the state of Rio Grande do Sul, and the second for Brazil.

Our observations on the spores within gut material from this isopod confirms mycophagy of this species by *Balloniscus sellowii* and implies that these animals contribute to spore dispersal for this fungal species under these field conditions. It is likely that different mycophagous animals, including insects, collembola, millipedes, mites, small mammals and birds disperse different truffle-like fungi from *Eucalyptus* forests in Brazil, as described for similar forests in Australia (Claridge 2002, Danks et al. 2013).



**Fig. 4A–F.** A. Basidiomata of *Descomyces albus* (UFRN-fungos 2214) in situ showing a channel into the peridium (arrow). B. Isopoda internal gut contents showing *D. albus* spores (arrows). C–D. Gut contents showing hyphal fragments with clamps (arrows). E–F. Close-up of *D. albus* spores in gut contents.

For mycophagy to be an effective mechanism for spore dispersal, spores must be able to survive conditions within animal digestive tracts. AM fungal spores can remain viable after passing through the digestive tracts of earthworms (McIlveen & Cole 1976). This phenomenon has also been observed for spores of *Tomentella sublilacina* (Ellis & Holw.) Wakef. Spores can remain viable after passing through the gut passage of a highly diverse group of invertebrate species (Lilleskov & Bruns 2005). This study is the first to report mycophagy involving truffle-like fungi and isopoda species in Brazil.

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