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Zygomycetes from "Reserva Biológica de Mogi Guaçu", São Paulo State, Brazil

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Abstract — The taxonomic composition of zygomycetes from a reserve of Brazilian Cerrado was analyzed. Soil and leaf litter samples were collected at five sampling dates. Cultures of *Absidia, Backusella, Circinella, Rhizopus* and *Conidiobolus* were obtained from canopy- and soil-plates. Due to the scarcity of detailed taxonomic information for Brazilian zygomycetes, additional information such as descriptions, standard colony colour codes, illustrations, geographical coordinates and vouchers are provided for *Absidia spinosa* var. *spinosa, Backusella lamprospora, Circinella simplex, Rhizopus stolonifer* var. *stolonifer* and *Conidiobolus coronatus.*

Key words — *Entomophthorales, Mucorales,* taxonomy

Introduction

Zygomycetes are an ecologically heterogeneous assemblage of fungi that are generally saprobes but may also act as pathogens or parasites of plants, other fungi, invertebrates, and vertebrates, including humans (Alexopoulos et al. 1996). They have commonly been isolated from soil, herbivore dung, almost all plant materials, mushrooms and insects (O'Donnell 1979), and also from diverse types of habitats and substrates such as bee brood chamber (Hesseltine et al. 1990), cave crickets (Zalar et al. 1997) and from Antarctic mosses (Tosi et al. 2004). Although they are not the most numerous and combative fungi in soil (Dix & Webster 1995), they are morphologically and ecologically one of the most diversified groups of fungi with highly successful survival strategies, although still little studied (Benny et al. 2001).

The "Reserva Biológica de Mogi Guaçu" (RBMG), constitutes one of the few remnants of Cerrado vegetation (Brazilian savanna) in São Paulo

State and is being conserved by the Instituto de Botânica since 1970. Due to the high diversity and endemism of species, the Cerrado is listed among the world's 25 hotspots (Durigan et al. 2004). Originally it covered ca. 23% of Brazil (including ca. 14% of São Paulo State), but the intense demand of agricultural areas for sugar cane, *Pinus, Eucalyptus, Citrus,* and pastures led to destruction and fragmentation of this vegetation and decreased it to less than 1% of its natural distribution in São Paulo State (Durigan et al. 2003). In RBMG taxonomic studies on *Glomeromycota* (Bononi & Trufem 1983, Carrenho et al. 1997, Carrenho & Trufem 2001), terrestrial and aquatic anamorphic fungi (Grandi 1985, Schoenlein-Crusius 2002, Grandi & Silva 2006), *Agaricomycetes* (Bononi 1984, Gugliotta 1997, Baseia & Milanez 2001, 2002a, b, Baseia 2005), and lichens (Jungbluth 2006, Marcelli et al. 2007) have been already carried out, but no detailed studies on zygomycetes.

In order to study the taxonomy and ecology of zygomycetes from leaf litter and soil and of zoosporic organisms from soil and water (Nascimento et al. 2011), a research project is being carried out in RBMG. The aim of this paper is to provide descriptions, including standard colony colour codes, and illustrations for *Absidia spinosa* var. *spinosa, Backusella lamprospora, Circinella simplex, Rhizopus stolonifer* var. *stolonifer,* and *Conidiobolus coronatus* obtained from leaf litter and soil samples of RBMG. These taxa generally have only been cited in lists of fungi from Brazil, with little or no detailed taxonomic information provided.

Material & methods

Study area – The RBMG is composed by two remnants of Cerrado, named areas A (343 ha) and B (127 ha), totalling 470 ha, which are located in a region of savannah-like tropical moist climate (Aw) with dry winter (Sparovek et al. 2007). Area A has 585–635 m of altitude, its vegetation types were characterized as wet field, gallery forest, Cerrado sensu stricto, rapanean Cerrado, transition zone, Cerrado field and Cerrado burnt field, surrounded by deforested areas, *Pinus* spp. plantations and annual crops (Mantovani & Martins 1993), but there is no recent information about alterations on its vegetation. Area B is located ca. 5 km from area A has similar altitude and is mainly covered by cerradão (forested savanna).

Sampling, isolation and slides preparation – On five sampling dates, leaf litter and soil samples were collected from 12 sites (Table 1), established either in gallery forest (GF) ≤ 10 m from watercourses (streams) or in Cerrado (CE, savanna, and forested savanna) ≥ 10 m from watercourses. Samples of moist and decomposing leaf litter and soil were collected with trowels and plastic bags. Soil samples were collected at ≤ 10 cm in depth immediately below the sites of leaf litter collection. Canopy- and soil-plates were prepared with CMYA (cornmeal agar 10 g, malt extract 10 g, yeast extract 2 g, agar 10 g in 1 L distilled water). Chloramphenicol (CP) 500 mg and rose Bengal (RB) 50 mg were added in order to avoid bacterial growth but also because RB restricts

fungal colony sizes (Bills & Foster 2004). The pH of CMYA plus RB ranged from 5.6–5.9. Leaf litter from each site was fragmented in a Petri dish with two scalpels, mixed with water agar medium (0.7%, w v^{-1}) and spread over ca. 60% of the internal surfaces of three CMYA plates supplemented with CP and RB (modified canopy-plate method, Drechsler 1952). Small aliquots of soil (0.11 ± 0.07 g, n = 22) were sprinkled over three plates of the same medium (modified soil-plate method, Warcup 1950). Dishes were incubated in the dark at 25°C. Colonies were isolated after 5–30 days from canopy-plates and after 3–7 days from soil-plates, transferred to SMA plates (Hesseltine 1954), incubated and re-isolated until obtaining pure cultures, which were identified at generic level and preserved by the Castellani method (Smith & Onions 1994). Cultures from water storage were re-incubated on SMA during 5–16 days while its mycelial fragments were removed for slide preparations with distilled water plus glycerol or glycerin (ca. 10 drops in 30 mL). Twenty measurements of fungal structures were taken from each specimen. Colour names more appropriate as possible were attributed to the colonies whereas its respective standard colour codes, in percentage combinations of black ($N_{\#}$), cyan ($C_{\#}$), magenta ($M_{\#}$) and yellow ($Y_{\#}$), were adopted according to Küppers (2002). Cultures were deposited at CCIBt culture collection, "Coleção de Culturas de Algas, Cianobactérias e Fungos do Instituto de Botânica", at São Paulo City, São Paulo State, Brazil.

Table 1. Soil and leaf litter collection sites in RBMG, Mogi Guaçu Municipality, São Paulo State, Brazil.

Sites	Coordinates	Vegetation	Areas
S1	22°14′49″S 47°10′21″W	GF	A
S2	22°15′35″S 47°11′37″W		
S3	22°15′35″S 47°11′35″W		
S4	22°15′02″S 47°08′54″W	CE	
S5	22°14′59″S 47°09′57″W		
S6	22°15′01″S 47°10′24″W		
S7	22°11′58″S 47°08′40″W	GF	B
S8	22°11′24″S 47°08′53″W		
S9	22°11′27″S 47°08′55″W		
S10	22°11′58″S 47°08′41″W	CE	
S11	22°11′27″S 47°08′54″W		
S12	22°11′26″S 47°08′54″W		

Vegetation types: GF = gallery forest; CE = Cerrado

Results & discussion

Cultures of *Absidia, Backusella, Circinella, Conidiobolus, Cunninghamella, Mucor, Piptocephalis, Rhizopus,* and *Zygorhynchus* were obtained from canopy- and soil-plates. Five taxa were selected for description and illustration here, because previous reports of Brazilian material have been incomplete and vouchers of them are scarce or nonexistent. Vouchers of different specimens and provenances will be valuable for subsiding molecular taxonomic studies.

Absidia spinosa Lendn., Bull. Herb. Boissier, Sér. 2, 7: 250. 1907.
var. ***spinosa***. FIGS 1–6

SPECIMEN EXAMINED: **BRAZIL. SÃO PAULO STATE**, Mogi Guaçu, RBMG, S5, from soil-plate; *J.I. de Souza,* 12.IX.2007; CCIBt 2307.

COLONIES 6–15 days old on SMA at 25°C, obverse reddish chestnut (N_{60} Y_{40} M_{20}) at 15th day, reverse greyish beige milky, 87 × 71 mm in size, 12 mm high touching Petri dish lid. SPORANGIOPHORES single or 2–6 in each verticil, greenish hyaline to pale chestnut, 22–225 µm long, 2.7–7.7(–8.9) µm in basal and (2.4–)3.1–6.5 µm in apical diameter. SPORANGIA pyriform, with deliquescent walls, greenish chestnut, 9.0–41.6 × 10.6–44 µm. COLUMELLAE hemispheric with or without 3.3–10.6 µm erect projections, or almost spherical lacking projections, some with granular content and/or collar, greenish hyaline to grayish, (4.0–)5.0–43 × 6.1–43 µm. SPORANGIOSPORES cylindrical, smooth, pale olive green, (2.6–)3.0–5.5 × 2.0–3.5 µm. CHLAMYDOSPORES rare in feeding mycelium. YEAST CELLS absent. ZYGOSPORANGIA globose to applanate, smooth, hyaline to greyish when young, pale chestnut to dark brown when mature, 42–69(–81) × 37–69(–80) µm, scarse; suspensors opposite, unequal, majority of major suspensors with pale brown granular content whereas the minor not having it, finger-like appendages arising from both or only one suspensor.

NOTES. Identified using Hesseltine & Ellis (1964). This is the first description of *A. spinosa* var. *spinosa* from Brazil, but *A. spinosa* var. *biappendiculata* was already reported by Trufem (1981a). Other named varieties are *A. spinosa* var. *madecassensis* and *A. spinosa* var. *azygospora* (Mycobank 2010).

Backusella lamprospora (Lendn.) Benny & R.K. Benj., Aliso 8(3): 320. 1975.
FIGS 7–11

SPECIMEN EXAMINED: **BRAZIL. SÃO PAULO STATE**, Mogi Guaçu, RBMG, S1–S3, S5–S7, from leaf litter canopy-plates; *J.I. de Souza, J.F. Santos & J.P. Costa*, 12.IX.2007, 28.I.2008, 20.X.2008; CCIBt 2308 and 2334.

COLONIES 5–15 days old on SMA at 25°C, obverse beige (N_{10} Y_{40} M_{10}) at 15th day, reverse beige with pale orange centre, 90 mm in size, 14 mm high touching Petri dish lid. SPORANGIOPHORES straight or slightly curved, recurved near apex when young, mono- or sympodially branched, some inflated, some constricted at apex, septate near branches and/or sporangia and columellae, hyaline to greyish, with chestnut granular content and olive green droplets, (38–)50–2473 µm long, 4.9–20.3 µm in basal and 3.9–15.4 µm in apical diameter. SPORANGIA globose, with deliquescent walls, olive green to greenish/dark chestnut, 25.2–82 × 25.2–85 µm. COLUMELLAE globose, cylindrical, ellipsoidal, cuneiform, conical and hemispherical, collar present or absent, hyaline to greyish, with or without yellowish chestnut to chestnut granular content, 7.3–46 × 8.2–44 µm.

Sporangiolar pedicels slightly curved to curved, some lightly constricted at apex, single or having 1–3 lateral branches, coenocytic, hyaline, greenish to greyish, with or without olive green granular content, 11–190 µm long, 2.2–13.2(–17.3) µm in basal and 1.6–8.6(–11.4) µm in apical diameter. Unispored sporangiola globose, lightly spiny, pale chestnut to yellowish chestnut, 8.4–33 × 8–33 µm, rare. Multispored sporangiola globose, with persistent and undulate walls, olive green to yellowish, 11–50 × 13.5–53 µm, abundant, containing 2–38 sporangiospores. Sporangiospores ovoid, rounded, some ellipsoidal, or irregular, greyish, pale olive green to greyish chestnut, with homogeneous or granular content, 4.0–24.6(–29) × 3.5–21.2 µm. Feeding mycelium with inflated regions, pale chestnut to olive green content, ending in thin rhizoid-like filaments; having oidia-like cells, rounded, ellipsoidal or irregular, hyaline, olive green to dark chestnut, abundant in whitish-dense spots in colony regions that can be seen at naked-eye. Chlamydospores and zygosporangia absent.

Notes. Identified using Benny & Benjamin (1975). Some multispored sporangiola may be detached and remain intact in slide preparations. This description complements the information of *B. lamprospora* from Brazil; Trufem (1978) reported *Mucor lamprosporus* (= *B. lamprospora*) but the specimen was destitute of the typical unispored and multispored sporangiola.

Circinella simplex Tiegh., Annls Sci. Nat., Bot., Sér. 6, 1: 92. 1875. Figs 12–16

Specimen examined: **BRAZIL. São Paulo State**, Mogi Guaçu, RBMG, S3, S9, from soil-plates; *J.I. de Souza, J.F. Santos & J.P. Costa*, 28-VII-2008; CCIBt 2312.

Colonies 7–15 days old on SMA at 25°C, producing baker's yeast odour, obverse yellowish (N_{10} C_{00} Y_{10}) to reddish beige (N_{30} Y_{50} M_{20}) with white to ivory margin at 15th day, reverse pale beige to greyish white milky, 83 × 81 mm in size, up to 4 mm high. Vegetative mycelium hyaline, containing yellowish green granules and septa along hyphae. Sporangiophores sympodially branched, lightly to very circinate, septate near sporangia and columellae, hyaline to greyish containing olive green to pale chestnut droplets, (3.6–)6.0–17.5 µm in basal and 3.0–14 µm in apical diameter. Sporangia globose, with smooth and deliquescent walls, or having undulated surface when minor, yellowish chestnut to dark chestnut, 14.5–82 × 14.5–84 µm. Columellae globose, ovoid, conical and obpyriform, generally with collars, hyaline to greyish, sometimes with olive green content, 7.7–39(–51) × 8.9–34(–43) µm. Sporangiospores irregular-shaped, rounded or elongated (minority), greyish to dark grey, 2.6–15.4(–19.8) × 2.4–6.7 µm. Chlamydospores, yeast cells and zygosporangia absent.

Notes. Identified using Hesseltine & Fennell (1955). Trufem (1981b) reported two specimens isolated from soil in Brazil.

Rhizopus stolonifer (Ehrenb.) Vuill., Revue mycol. Toulouse, 24: 54. 1902.
var. ***stolonifer*** Figs 17–21

Specimen examined: **BRAZIL. São Paulo State**, Mogi Guaçu, RBMG, S10, from soil-plate; *J.I. Souza, J.F. Santos & J.P. Costa,* 28.VII.2008; CCIBt 2329.

Colonies 7–13 days old on SMA at 25°C, with scarce mycelium, obverse greenish chestnut (N_{60} Y_{50} M_{20}) dotted with great black sporangia at 13th day, reverse greyish white, 90 mm in diameter, 7 mm high. Sporangiophores short to long, monopodially branched, coenocytic, hyaline to chestnut (amber), with olive green granular content, 108–2228 µm long, 3.7–28 µm in basal and 4.9–24.6 µm in apical diameter. Sporangia globose, with deliquescent walls, chestnut to black, 45–333 × 45–345 µm. Columellae globose, subglobose and ellipsoidal (majors), hemispheric or applanate (minors), with or without collar, hyaline, with olive green content, 11.2–160 × 18.4–166 µm. Sporangiospores ovoid to rounded, striate (hazelnut-like), pale chestnut, with homogeneous content, (6.5–)8.2–16.7 × 6.1–16.3 µm. Chlamydospores, yeast cells and zygosporangia absent.

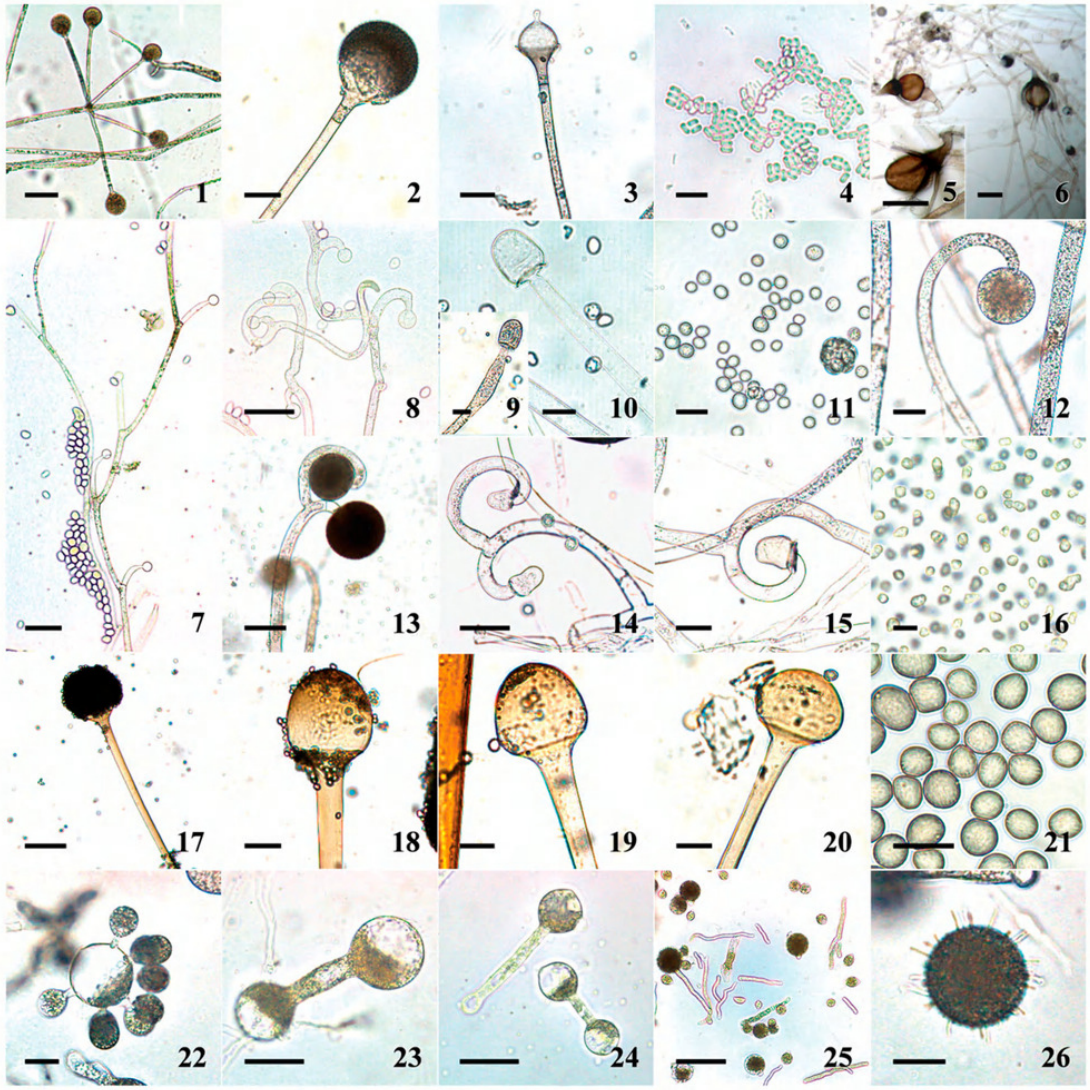
Notes. Identified using Schipper (1984). This description complements the information of *R. stolonifer* var. *stolonifer* from Brazil; Trufem (1981a) reported *R. nigricans* (= *R. stolonifer* var. *stolonifer*). Other named varieties are *R. stolonifer* var. *luxurians* and *R. stolonifer* var. *lyococcus* (Mycobank 2010).

Conidiobolus coronatus (Costantin) A. Batko, Entomophaga, Mémoires hors série 2: 129. 1964 ['1962']. Figs 22–26

Specimens examined: **BRAZIL. São Paulo State**, Mogi Guaçu, RBMG, S4, S11, from leaf litter canopy-plates; *J.I. de Souza,* 20.X.2008; CCIBt 2335 and 2336.

Colonies 7–16 days old on SMA at 25°C, obverse white (Y_{00} M_{00} C_{00}) to yellowish (Y_{10} M_{00} C_{00}) and reverse yellowish (Y_{10-20} M_{00} C_{00}) at 16th day, 90 mm in diameter, or composed of up to 10 mm colonies occupying almost all the plate surfaces, flat, aerial mycelium absent. Feeding mycelium filamentous, branched, rarely septate, greenish to pale brown containing olive green droplets, 5–20.8 µm in diameter, releasing or not hyphal bodies (segments) of 37–180 µm long. Conidiophores undifferentiated from feeding mycelium. Primary conidia spherical to globose, pale olive green to greenish chestnut, 23.5–65 µm in diameter. Papillae applanate or cone-shaped with blunt or sharp ends, respectively, 4.9–19 × 8.3–20.9 µm. Villose conidia spherical, rarely papillate, similar to primary conidia in size. Replicative conidia rare. Microconidia ovoid, lemon-shaped to rounded 14.5–28 × 14–24 µm (CCIBt 2335) or absent (CCIBt 2336). Chlamydospores and zygosporangia absent.

Notes. Identified using King (1976a,b, 1977). Both specimens showed poor growth on CMYA when compared to growing on SMA. This description



FIGS 1–26. ***Absidia spinosa* var. *spinosa*** (1–6): 1, verticil with five sporangiophores bearing sporangia; 2, sporangium; 3, columella with erect projection; 4, sporangiospores; 5–6, zygosporangia with finger-like appendages from suspensors. ***Backusella lamprospora*** (7–11): 7–8, sympodially branched sporangiophores; 9, 10. sporangiophores bearing columellae; 11, sporangiospores and detached sporangium. ***Circinella simplex*** (12–16): 12–13, circinate sporangiophores bearing sporangia; 14–15, circinate sporangiophores bearing columellae; 16, sporangiospores. ***Rhizopus stolonifer* var. *stolonifer*** (17–21): 17, sporangiophore bearing sporangium; 18–20, columellae; 21, sporangiospores. ***Conidiobolus coronatus*** (22–26): 22, primary conidium bearing microconidia; 23–24, replicative conidia; 25, primary conidia, hyphal bodies and microconidia; 26, villose conidium.

Bars: 1, 5–8, 13 = 50 µm; 2, 3, 9–12, 15, 19–24, 26 = 20 µm; 4, 16 = 10 µm; 14, 18 = 40 µm; 17, 25 = 100 µm.

complements the information on *C. coronatus* from Brazil; Porto et al (1987) isolated *C. coronatus* from soil with and without vegetal detritus.

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