





https://doi.org/10.11646/phytotaxa.295.2.4

Coprophilous ascomycetes with passive ascospore liberation from Brazil

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Abstract

Ascomycetes with passive ascospore liberation fruiting on herbivore dung are discussed. A total of 270 samples of cattle, goat and horse dung were collected for 20 months along an edaphic and climatic gradient from the Atlantic Forest complex to the semi-arid Caatinga complex in Pernambuco, northeastern Brazil. Thirteen species were identified and described. *Lophotrichus bartlettii* and *Kernia nitida* were the most frequently recorded species. *Corynascus sepedonium, Leuconeurospora pulcherrima, Melanospora damnosa, M. zamiae, Mycoarachis inversa, Zopfiella erostrata* and *Zopfiella longicaudata* are reported for the first time in Brazil. Descriptions, a photographic plate and an identification key to the studied species, along with a table with key characters of the most common genera of coprophilous ascomycetes with passive ascospore liberation are provided.

Key words: Ascomycota, coprophilous fungi, Microascales, non-ostiolate ascomycetes

Introduction

Coprophilous fungi form a collective group of saprobes able to live, feed and reproduce in dung, especially from herbivores (Webster 1970, Krug *et al.* 2004, Kirk *et al.* 2008). These fungi are associated with various animals (most notably mammals), domesticated or wild (Richardson 2001), presenting an array of morphologic and physiologic life strategies to efficiently exploit their substrate (Ingold 1961, Dix & Webster 1995, Kirschner *et al.* 2015).

The majority of the known genera of coprophilous fungi belong to Ascomycota Caval.-Sm. (Krug *et al.* 2004), and passive ascospore liberation can be present in several ascomycetes fruiting on dung (Doveri 2004). Asci that passively liberate mature ascospores inside the ascomatal cavity have evanescent cell walls. These asci can develop either in ostiolate ascomata (perithecia), with profuse production of ascospores that eventually ooze through the ostiole, or in non-ostiolate ascomata (cleistothecia), closed fruit bodies lacking an innate opening. While some notably known coprophilous fungi have elaborate active ascospore liberation mechanisms (e.g. *Podospora* Ces., *Ascobolus* Pers., *Sporormiella* Ellis & Everh.), ascospores of coprophilous ascomycetes with passive ascospore liberation are usually dispersed by insects or mites visiting dung to feed or lay eggs. Entomophilous dispersion in these fungi can include adherent mucilaginous droplets containing spores formed at the apex of some perithecia (e.g. *Sphaeronaemella* P. Karst.), a spore cirrus that oozes through the ostiole and can get caught in tufts of hairs (e.g. *Chaetomium* Kunze, *Lophotrichus* R.K. Benj.), or cleistothecia with hooked hairs that are weakly attached to the substrate (e.g. *Kernia* Nieuwl.), among other strategies.

The importance of a closed fruit body in ascomycetes taxonomy was reassessed by Stchigel & Guarro (2007). Ascomycetes with closed ascomata delimiting a single cavity in a fruit body that lacks active ascospore liberation (i.e. cleistothecial ascomycetes) occurred more than once in evolution. The most well known example can be found in Eurotiomycetes, characterized by the formation of mainly spherical, non-ostiolate ascomata with a peridium varying from a loose hyphal reticulum to a thick pseudoparenchyma, lacking interascal elements, completely enclosing globose to subglobose, evanescent asci, which are formed throughout many "layers" in the ascomatal cavity (Geiser *et al.* 2006). In this case, asci are produced in chains due to the fact that karyogamy and meiosis take place in many cells along the ascogenous hypha rather than in a single apical mother-ascus cell in a crozier (Fennel 1973, Reynolds 1981), resulting in

an ascoma without any sign of an organized hymenial layer. Alternatively, in Sordariomycetes, non-ostiolate ascomata present saccate to clavate asci weakly attached to the ascomatal cavity base at early stages of development, resembling a poorly organized hymenium (Zhang & Wang 2015). In Dothideomycetes, Pleosporomycetidae are characterized by species with uniloculated pseudothecia (Schoch *et al.* 2006). Some genera, such as *Faurelina* Locq. -Lin., present globose to elongated stromata completely enclosing evanescent asci in a single locule (Reblová *et al.* 2011), which can be considered, despite the lack of an organized peridium, analogous with the non-ostiolate ascomata found in the Eurotiomycetes and Sordariomycetes.

Considering both recent and previous works on coprophilous fungi, cleistothecial ascomycetes have received little attention. This work presents a comprehensive treatment on dung ascomycetes with passive ascospore liberation, both with ostiolate and non-ostiolate ascomata, sampled during surveys in Northeastern Brazil, including new records.

Material and Methods

Two hundred and seventy dung samples from goat, cattle and horse were collected in equal proportions over three years from animal precincts in three different municipalities: Recife (8°00'54" S, 34°56'59" W), Caruaru (8°01'59" S, 36°06'59" W) and Serra Talhada (7°54'59" S, 38°17'00" W), located in Pernambuco, Northeastern Brazil. Samples were collected in clean plastic bags, taken to the laboratory and incubated in moist chambers at room temperature (28 $^{\circ}C \pm 2 ^{\circ}C$) for at least 75 days under alternating natural light and dark periods. The material was observed directly from substrata with the aid of a stereomicroscope, and ascomata (with or without their asexual morphs) were mounted in tap water, Indian ink, Congo red, lactophenol or lactophenol with cotton blue for identification under light microscopy. Species were identified based on macroscopic and microscopic structures according to Ames (1961), von Arx (1970, 1973, 1975), von Arx et al. (1988), Bell (1983, 2005), Benny & Kimbrough (1980), Cannon & Hawksworth (1982), Doveri (2004, 2010), Guarro et al. (1991), Hawksworth & Pitt (1983), Lundqvist (1972), Malloch & Cain (1970, 1971, 1973), Richardson & Watling (1997), Rossman et al. (1999), Seth (1971), Stchigel et al. (2004a), Udagawa & Furuya (1973) and Vakili (1984). A careful literature revision and a survey of the main national mycological herbarium (URM - Pe. Camille Torrend Herbarium, Universidade Federal de Pernambuco, Recife, Brazil) were performed to access information regarding older records of coprophilous ascomycetes without active ascospore liberation in Brazil. High resolution images showing key morphological characters were taken with an Olympus QColor 3 digital camera mounted on an Olympus BX51 microscope equipped with bright-field and Nomarski interference optics. Permanent slides were mounted with Polyvinyl-Lacto-Glycerol and deposited in the URM herbarium. For a full list of species synonyms, see Index Fungorum (http:// www.indexfungorum.org). Additional information regarding all records and deposited specimens, along with high quality micrographs, are available at the "INCT - Herbário Virtual da Flora e dos Fungos" database website (http://inct.florabrasil.net).

Results

Thirteen taxa were identified from the 93 occurrences on the 270 substrate samples studied. These represented six orders in two classes: Hypocreales Lindau, Melanosporales N. Zhang & M. Blackw., Microascales Luttr. ex Benny & R.K. Benj., Sordariales Chadef. ex D. Hawksw. & O.E. Erikss. (Sordariomycetes O.E. Erikss. & Winka), Eurotiales G.W. Martin ex Benny & Kimbr. and Onygenales Cif. ex Benny & Kimbr. (Eurotiomycetes O.E. Erikss. & Winka) (Plate 1). Most recorded taxa (10) were cleistothecial, fruiting superficially on the dung. Perithecial species were more common by the end of the first month of incubation, mostly presenting hairy semi-immersed ascomata. Both typical coprophilous species, such as representatives of *Kernia* and *Zopfiella* G. Winter, as well as ubiquous species commonly recorded on other substrates, such as representatives of *Monascus* Tiegh. and *Thielavia* Zopf, were sampled during this survey. *Kernia nitida* (Sacc.) Nieuwland (1916: 379) and *Lophotrichus bartlettii* (Massee & E.S. Salmon) Malloch & Cain (1971: 866) were the most frequently recorded species, with 27 and 30 occurrences, respectively. *Corynascus sepedonium* (C.W. Emmons) Arx (1973: 292), *Leuconeurospora pulcherrima* (G. Winter) Malloch & Cain (1970: 1820), *Melanospora damnosa* (Sacc.) Lindau (1897: 353), *M. zamiae* Corda (1837: 24), *Mycoarachis inversa* Malloch & Cain (1970: 1822), *Zopfiella erostrata* (Griffiths) Udagawa & Furuya (1974: 208) and *Z. longicaudata* (Cain) Arx (1973: 291) are reported for the first time in Brazil. Among the exsiccata revised in URM, the only material with reference to coprophilous fungi with passive ascospore liberation were labeled as *Sporormia* De Not. After

examination, all material was determined as species of *Sporormiella*, ascomycetes with active ascospore liberation, and thus, were not described. Table 1 presents a comparison of key morphological characters used to distinguish the most common genera of fungi with passive spore dispersal on herbivore dung, with additional data from Greif *et al.* (2009), Sandoval-denis *et al.* (2016) and Stchigel *et al.* (2004b).

Species	Ascomata	Peridium	Ascomatal hairs	Ascospores
Chaetomidium	Non-ostiolate	Pseudoparenchymatous or cephalothecoid	Long and hyphoid, evenly distributed	Limoniform to fusiform, with a single germ pore
Chaetomium	Ostiolate, without a neck	Pseudoparenchymatous, textura angularis	Terminal (long, by the ostiole) usually coiled and lateral (short, evenly distributed)	Limoniform to subglobose, lacking conspicuous germ pores
Corynascus	Non-ostiolate	Pseudoparenchymatous, textura angularis	Glabrous, occasionally setose, evenly distributed	Ellipsoidal to fusiform, with two germ pores
Faurelina	Non-ostiolate	Stromatic, with cells varying in shape	Glabrous	Ellipsoidal-navicular, with longitudinal striae, lacking conspicuous germ pores
Kernia	Non-ostiolate	Pseudoparenchymatous, textura angularis	Circinate, in tufts of few to several, at opposite parts of the ascoma	Obovate to reniform, with a single germ pore
Leuconeurospora	Non-ostiolate	Cephalothecoid	Glabrous	Ellipsoidal, with anastomosing ridges, lacking germ pores
Lophotrichus	Ostiolate, with short to long neck	Pseudoparenchymatous, textura angularis	Terminal (long, by the ostiole) usually straight and lateral (short, evenly distributed)	Ellipsoidal to fusiform, with two germ pores
Melanospora	Ostiolate	Pseudoparenchymatous	Glabrous to tomentose, evenly distributed	Ellipsoidal to limoniform, with two germ pores
Microascus	Ostiolate, with short to long neck	Pseudoparenchymatous, textura angularis	Glabrous or in loose tufts by the ostiole	Reniform to triangular, with a single germ pore
Monascus	Non-ostiolate	Prosenchymatous, textura epidermoidea	Glabrous	Ellipsoidal, lacking conspicuous germ pores
Mycoarachis	Non-ostiolate	Pseudoparenchymatous, with a hyaline outer layer in one species	Glabrous	2-celled, arachiform, lacking conspicuous germ pores
Pseudoallescheria	Non-ostiolate	Pseudoparenchymatous, textura epidermoidea	Hyphoid, flexuous, evenly distributed	Ellipsoidal to fusiform, with two germ pores
Thielavia	Non-ostiolate	Pseudoparenchymatous, textura epidermoidea	Glabrous to tomentose, evenly distributed	Ellipsoidal to clavate, with a single germ pore
Tripterosporella	Non-ostiolate	Pseudoparenchymatous, textura angularis	Glabrous to tomentose, evenly distributed	2-celled, with a dark head cell and a pedicel, bearing gelatinous caudae
Zopfiella	Non-ostiolate	Pseudoparenchymatous, textura angularis	Glabrous or with tufts of setose hairs, evenly distributed	2-celled, with a dark head cell and a pedicel, lacking gelatinous caudae

TABLE 1. Diagnostic characteristics of	common coprophilous ascomvcetes	genera with passive ascospore liberation

Key to the studied species of coprophilous ascomycetes with passive ascospore liberation

1.	Ascomata non-ostiolate	2
-	Ascomata ostiolate	11
2		1.

-	Mature ascospores usually one-celled, occasionally with two cells similar in morphology
3.	Young ascospores sigmoid to cylindrical. Pedicels persistent, long, usually bent near the distal end. Apical and basal gelatinous cauda present
-	Young ascospores clavate. Pedicels usually collapsing at maturity, short to long, not bent. Gelatinous caudae absent
4. -	Cleistothecia with long, septate, thick-walled hairs, dark brown to black. Pedicels 6–10 µm in length
5.	Cleistothecia arising singly from a distinct, hyaline, stalk-like hypha. Peridium prosenchymatous, composed of loose interwoven hyphae
-	Cleistothecia sessile on somatic hyphae. Peridium pseudoparenchymatous, cephalothecoid or not
6. -	Ascospores with germ pores
7. -	Ascospores with one germ pore
8. -	Cleistothecia glabrous. Peridial cells elongated, irregular (<i>textura epidermoidea</i>)
9.	Cleistothecia 80–110 μ m diam., glabrous, bearing ridges in a reticulated pattern in the outermost peridial cells. Ascospores 12.5–18 × 8.5–9.5 μ m
-	Cleistothecia 150–250 μ m diam., sparsely adorned by hyphoid hairs. Peridial cells smooth. Ascospores 5–6.5 × 3–4 μ m
10.	Peridium with an outer layer of hyaline, angular cells. Ascospores peanut-shaped, two-celled, markedly constricted at the septum
-	Peridium cephalothecoid, consisting of large, angulated plates of flattened cells. Ascospores ellipsoid to fusoid, one-celled
11.	Perithecial neck short, inconspicuous, up to 40 µm long. Terminal hairs scarce, long, not forming a tuft, up to 1350 µm long. As- cospores sublimoniform, hyaline, golden to pale brown, copper colored en masse
-	Perithecial neck conspicuous, more than 40 µm long. Terminal hairs short, setose, up to 225 µm long. Ascospores limoniform, dark brown to black
12.	Perithecia scarcely tomentose, short-necked, up to 100 μm long. Ascospores rhomboid-ellipsoid, 10–12.5 μm long
-	Perithecia almost to completely glabrous, long-necked, 150–370 μm long. Ascospores ellipsoidal to limoniform, 12.5–15 μm long

Taxonomy

1. *Corynascus sepedonium* (C.W. Emmons) van den Brink & Samson, in van den Brink, Samson, Hagen, Boekhout & de Vries, Fungal Diversity 52(1): 206 (2012) (Plate 1, Figs. 1–4)

Ascomata non-ostiolate, scattered to gregarious, immersed, globose, dark gray to black at reflected light, dark brown at transmitted light, 80–110 μ m diam., glabrous. *Peridium* pseudoparenchymatous, membranaceous, formed by an inner layer of flattened, angulated (*textura angularis*), thin-walled, light brown cells, 5–7.5 μ m diam., and by an outer layer of angulated to elongated, irregular (*textura epidermoidea*), thick-walled, light brown cells, up to 18 μ m diam., adorned with a reticulated pattern. *Asci* 8-spored, globose, evanescent. *Ascospores* 1-celled, ellipsoid to fusoid, smooth, light brown to brown, 12.5–18 × 8.5–9.5 μ m, with two terminal germ pores. *Conidia* globose, with a spinulose ornamentation, hyaline, 9.5–11 μ m diam.

Material examined:—BRAZIL. Pernambuco, Instituto Agronômico de Pernambuco (IPA), Serra Talhada, on goat dung, 03 Jan 2012, R.F.R. Melo (URM86691!).

Habitat:—Soil and herbivore dung.

Distribution:—Africa (Senegal) and Europe (Canary Islands). This is the first record of this species in Brazil. **Notes:**—This species is remarkable for its cleistothecia, which present a typical peridial pattern of ridged cells,

and for the finely spinulose conidia. The material from Pernambuco, analyzed directly from the substrate, showed smaller ascospores and conidia size than the material described from culture.

2. *Kernia nitida* (Sacc.) Nieuwl., Am. Midl. Nat. 4: 379 (1916) (Plate 1, Figs. 5–6)

Ascomata non-ostiolate, gregarious, rarely scattered, superficial, subglobose, ellipsoid, ovoid or polygonal, dark gray to black, 95–290 μ m diam. *Peridium* pseudoparenchymatous, membranaceous, opaque, black, 15–20 μ m thick, consisting of several layers of cells, the outermost dark, globose to angulated (*textura globulosa-angularis*), thick-walled, the innermost brown, clearer, thinner, angular (*textura angularis*). *Terminal hairs* simple, thick-walled, septate, smooth, dark brown, 3–6.5 μ m thick at the broadest part, 95–600 μ m long, arranged in two or three loose tufts of 4–10 hairs, attached at two or three parts of the ascoma, usually inclined upwards to form an angle of approximately 45° in relation to the substrate, with strongly circinate ends, forming hooks in different directions. *Lateral hairs* hyphoid, simple, thinwalled, 1.5–2 μ m thick at the broadest part, usually absent in mature ascomata. *Asci* 8-spored, globose to ovoid, 6–12.5 μ m diam., evanescent. *Ascospores* 1-celled, ellipsoid, smooth, light brown to ochraceous, copper colored "en masse", 3.5–6 × 2.5–4.5 μ m, guttulate, with one terminal germ pore.

Material examined:—BRAZIL. Pernambuco, Instituto Agronômico de Pernambuco (IPA), Caruaru, on goat dung, 27 Dec 2011, R.F.R. Melo (URM86679!, 86680!); Instituto Agronômico de Pernambuco (IPA), Serra Talhada, on goat dung, 27 Feb 2012, R.F.R. Melo (URM86681!).

Habitat:-Soil, plant material and dung of many herbivores.

Distribution:—Worldwide.

Notes:—Ascomata of *Kernia nitida* were commonly recorded in the studied areas, mainly from goat dung. This species can be distinguished by the peculiar shape of the cleistothecia (subglobose, ellipsoid, ovoid or polygonal) and the long, circinate hairs, originating from two or three opposite points of the ascoma. The material examined from goat dung presented many ascomata covering entire pellets, interspersed with white, cottony tufts forming a hairy surface on the substrate.

3. *Leuconeurospora pulcherrima* (G. Winter) Malloch & Cain, Can. J. Bot. 48(10): 1820 (1970) (Plate 1, Figs. 7–9)

Ascomata non-ostiolate, scattered, immersed to superficial, globose, black at reflected light, dark red at transmitted light, 160–210 μ m diam., glabrous. *Peridium* cephalothecoid, coriaceous, opaque, composed of large, polygonal plates consisting of flattened, angular (*textura angularis*), thick-walled, reddish-brown cells, 7.5–22.5 μ m wide at the larger axis, separated by well-defined dehiscence lines. *Asci* 4-spored, globose to slightly clavate, 9–11.5 μ m diam., evanescent. *Ascospores* 1-celled, mainly ellipsoid, but considerably variable in morphology, rhomboid to doliiform, ornamented by conspicuous, anastomosing ridges, forming an incomplete reticulum, hyaline, 6–10 × 3–5 μ m, lacking germ pores.

Material examined:—BRAZIL. Pernambuco, Universidade Federal Rural de Pernambuco (UFRPE), Recife, on goat dung, 04 Apr 2012, R.F.R. Melo (URM86786!).

Habitat:—Dung of dog, fox, hedgehog, rabbit, rat and squirrel.

Distribution:—Europe (Denmark, Greece) and North America (Canada). This is the first record of this species in Brazil.

Notes:— *Leuconeurospora pulcherrima* can be distinguished from other cleistothecial, hyaline-spored coprophilous fungi by the dark red ascomata with cephalothecoid wall and by the reticulated ornamentation pattern on the ascospores. Numerous obovoid to pyriform chlamydospores were observed in this material.

4. *Lophotrichus bartlettii* (Massee & E.S. Salmon) Malloch & Cain, Can. J. Bot. 49(6): 866 (1971) (Plate 1, Figs. 10–12)

Ascomata ostiolate, scattered to gregarious, immersed to superficial, globose, dark grey to black, 290–315 µm diam. *Neck* papillate, inconspicuous, 30–40 µm diam. *Peridium* pseudoparenchymatous, membranaceous, opaque, with thick-walled (*textura angularis*) brown cells. *Neck hairs* simple, thick-walled, septate, smooth, dark brown to black, 3.5–4.5 µm thick at the broadest part, narrowing towards the apex, 1.25–1.35 mm long, arranged in a single tuft. *Lateral hairs* hyphoid, simple, thin-walled, hyaline, septate, 135–147.5 µm long, 2–3 µm diam. at the broadest part,

narrowing towards the apex. *Asci* 8-spored, clavate to subglobose, with a short stipe, $20-35 \times 9.5-15 \mu m$, evanescent. *Ascospores* 1-celled, sublimoniform, non-apiculated, smooth, hyaline when young, then golden to pale brown, copper colored "en masse", $6.5-8 \times 5-6 \mu m$, with two terminal germ pores, early dispersed to form a cirrhus.

Material examined:—BRAZIL. Pernambuco, Instituto Agronômico de Pernambuco (IPA), Caruaru, on goat dung, 03 Jan 2012, R.F.R. Melo (URM8668!), 08 May 2013 (URM86683!); Universidade Federal Rural de Pernambuco (UFRPE), Recife, on goat dung, 05 May 2012, R.F.R. Melo (URM86684!).

Habitat:—On plant material and dung of many herbivores.

Distribution:—Africa (Egypt, Iraq, Nigeria), Asia (India, Japan), Europe (Spain), North America (United States of America), Oceania (Australia) and South America (Brazil, Venezuela). Possibly worldwide.

Notes:—*Lophotrichus bartlettii*, although underrepresented in literature, has been frequently recorded on herbivore dung in Brazil. It was first described by Ames (1961) from a pure culture sent by T. E. Brooks, isolated from rat dung in Kansas, USA. It can be easily identified among representatives of the genus by the presence of short-necked perithecia bearing a tuft of unbranched terminal hairs.

5. *Melanospora damnosa* (Sacc.) Lindau, Nat. Pflanzenfam., Teil. I (Leipzig) 1 (1): 353 (1897) (Plate 1, Figs. 13–14)

Ascomata ostiolate, scattered, immersed to superficial, obpyriform, globose to subglobose, tapering slightly towards the neck, light brown to golden, $220-250 \times 160-185 \ \mu\text{m}$. *Neck* conical, $75-100 \times 50-60 \ \mu\text{m}$, usually with a mucilaginous mass at the apex composed of mature ascospores. *Terminal hairs* setose, straight to slightly flexuous, simple, thick-walled, septate, smooth, hyaline to faintly yellowish, $2.5-5 \ \mu\text{m}$ in diameter at the broadest part, $60-130 \ \mu\text{m}$ long, forming a crown at the neck apex. *Lateral hairs* sparse, hyphoid, flexuous, simple, thin-walled, hyaline. *Peridium* pseudoparenchymatous, membranaceous, translucent, composed mostly of polygonal, (*textura angularis*), thin-walled, light yellow to yellowish brown cells, $12.5-17.5 \ \mu\text{m}$ thick, becoming more elongated towards the neck. *Asci* 8-spored, clavate, with rounded apex and a short stipe, $35.5-40 \times 22.5-28 \ \mu\text{m}$, evanescent, observable only in young perithecia. *Ascospores* 1-celled, rhomboid-ellipsoid to sublimoniform, non-apiculated, smooth, hyaline when young, then dark brown to black, $15-22 \times 10-12.5 \ \mu\text{m}$, with two terminal germ pores, early dispersed to form a dark mass at the neck apex.

Material examined:—BRAZIL. Pernambuco, Instituto Agronômico de Pernambuco (IPA), Caruaru, on goat dung, 30 Sep 2011, R.F.R. Melo (URM86669!).

Habitat:—Herbivore dung, twigs and plant material.

Distribution:—Africa (Tunisia), Asia (Bangladesh), Europe (well distributed), North America (United States), Oceania (Australia, New Zealand) and South America (Argentina). Apparently worldwide.

Notes:—*Melanospora damnosa* resembles *M. brevirostris* (Fuckel) Höhnel (1914: 94), but in additions to its longer necks, they differs by ascospore shape and size: shorter, ellipsoid to limoniform in the latter and longer, rhomboid-ellipsoid in the former.

6. *Melanospora zamiae* Corda, Icon. fung. (Prague) 1: 24 (1837) (Plate 1, Figs. 15–16)

Ascomata ostiolate, usually scattered, semi immersed to superficial, globose to subglobose with a long, cylindrical neck, light brown to golden, $650-750 \times 290-350 \ \mu\text{m}$. *Neck* long, straight to slightly curved, $150-370 \times 70-85 \ \mu\text{m}$, with a mucilaginous mass of mature ascospores at the apex. *Terminal hairs* setose, straight to slightly flexuous, simple, thick-walled, septate, smooth, hyaline to faintly yellowish, $2.5-5(-7.5) \ \mu\text{m}$ in diameter at the broadest part, up to $225 \ \mu\text{m}$ long, crowning the neck. *Lateral hairs* sparse, hyphoid, simple, thin-walled, hyaline. *Peridium* pseudoparenchymatous, membranaceous, translucent, fragile, polygonal a *textura angularis* of thin-walled, light yellow to yellowish brown cells, $15-17.5 \ \mu\text{m}$ diam., becoming more elongated towards the neck. *Asci* 8-spored, clavate, with rounded apex and a short stipe, $35.5-50 \times 20-25 \ \mu\text{m}$, evanescent. *Ascospores* 1-celled, ellipsoidal to limoniform, smooth, hyaline when young, then clear brown and finally dark brown, $15-20(-22.5) \times 12.5-15 \ \mu\text{m}$, with two terminal germ pores early dispersed to form a dark mass at the neck apex.

Material examined:—BRAZIL. Pernambuco, Universidade Federal Rural de Pernambuco (UFRPE), Recife, on goat dung, 9 Jul 2011, R.F.R. Melo (URM86670!).

Habitat:—Plant material, paper and deer dung. Parasitic on some fungi.

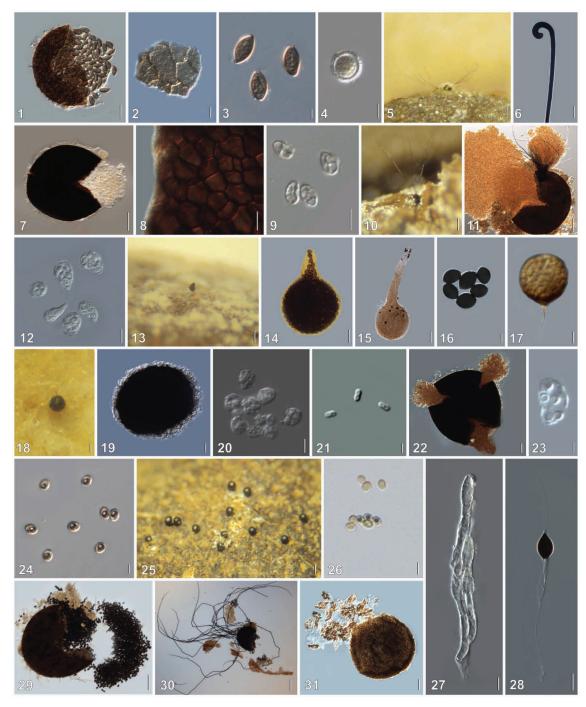


PLATE 1. Coprophilous ascomycetes with passive ascospore liberation recorded in Brazil. *Corynascus sepedonium* 1. Ascoma, 2. peridium, 3. ascospores and 4. conidium. *Kernia nitida* 5. Ascoma on dung and 6. hair tip. *Leuconeurospora pulcherrima* 7. Ascoma, 8. peridium and 9. ascospores. *Lophotrichus bartlettii* 10. Ascoma on dung, 11. mounted and 12. asci. *Melanospora damnosa* 13. Ascoma on dung and 14. mounted. *M. zamiae* 15. Ascoma in mounting and 16. ascospores. *Monascus ruber* 17. Ascoma in mounting. *Mycoarachis inversa* 18. Ascoma on incubation paper and 19. mounted, 20. asci and 21. ascospores. *Pseudoallescheria boydii* 22. Ascoma in mounting, 23. ascus and 24. mature ascospores. *Thielavia terrestris* 25. Ascomata on dung. 26. mature ascospores. *Tripterosporella pakistani* 27. Ascoma in mounting, 28. ascus and 29. mature ascospore. *Zopfiella erostrata* 30. Cleistothecium in mounting. *Z. longicaudata* 31. Cleistothecium in mounting. Scale bars: 1, 6, 9, 16, 17=10 µm. 2, 21= 2.5 µm. 3, 4, 20, 22, 23, 24, 26= 5 µm. 5=200 µm. 7, 11, 14=50 µm. 8, 12, 13= 20 µm. 15, 18=100 µm. 19=25 µm. 10, 25= 300 µm. Figure: R.F.R. Melo.

Distribution:—Africa (Egypt, Libya, Sierra Leone, Zambia), Asia (India, Israel), Europe (Germany, United Kingdom), North America (Canada, Martinique and United States), Oceania (Australia and New Zealand) and South America (Argentina).

Notes:—This species can be identified by its long neck (150–370 µm long), glabrous to slightly pilose perithecia and predominantly limoniform ascospores, not flattened.

7. *Monascus ruber* Tiegh., Bull. Soc. bot. Fr. 31: 226 (1884) (Plate 1, Fig. 17)

Colonies in CYA25 with fast growth, 25–35 mm diam. in seven days, appressed, velutinous to floccose, with fimbriated margin, initially white, then light brown, progressively darker with cleistothecia maturation, occasionally reddish. *Reverse* brown to dark brown, smooth. *Mycelium* abundant, consisting of hyaline, slender, branched, smooth-walled hyphae, 4–5.5 μ m diam. *Ascomata* non-ostiolate, gregarious, superficial, usually globose, occasionally subglobose, flattened horizontally, light brown to amber, 25–45(–57.5) μ m diam., each supported by a stalk-like hypha. *Ascomatal wall* two-layered, prosenchymatous, translucent, composed of an outer layer with often merging hyphae, covering an inner semitransparent vesicle, thick-walled, with light brown to amber cells, major axis 2.5–5 μ m, collapsing or not when mounted. *Asci* 8-spored, globose, strongly evanescent. *Ascospores* 1-celled, ellipsoid, smooth, hyaline, 5–7.5 × 3.5–4.5 μ m. *Conidia* single or in short chains, globose to pyriform thick-walled, hyaline, smooth, 9.5–15.5 μ m diam. or 10–17.5 × 8.5–15 μ m.

Material examined:—BRAZIL. Pernambuco, Universidade Federal Rural de Pernambuco (UFRPE), Recife, on cattle dung, 15 Mar 2013, R.F.R. Melo (URM86638a!, 86638b!, 86638c!, 86638d!).

Habitat:—Recorded on plant material, in rumen contents of herbivores, industrial waste, . It is uncommon on herbivore dung.

Distribution:—Worldwide.

Notes:—*Monascus ruber* is a common cleistothecial ascomycete, primarily known for its application in food industry, particularly in China and Japan. It differs from *M. sanguineus* P.F. Cannon, Abdullah & B.A. Abbas (1995: 661) mainly by the soluble pigment production, usually absent, brown when present.

8. *Mycoarachis inversa* Malloch & Cain, Can. J. Bot. 48(10): 1822 (1970)

(Plate 1, Figs. 18–21)

Ascomata non-ostiolate, scattered, semi immersed to superficial, globose to subglobose, dark green to black, 110–215 μ m diam., glabrous, with a metallic appearance at reflected light, formed directly on dung or on wet paper in incubation. *Peridium* pseudoparenchymatous, membranaceous, opaque, with two distinct layers, the outermost composed of one to four inflated globose, angular or elongated, thin-walled, hyaline cells, 5–20 μ m diam., the innermost composed of flattened, angular (*textura angularis*), dark brown , thick-walled cells, 5–17.5 μ m diam. *Asci* 8-spored, globose to subglobose, 5–10 μ m diam., evanescent. *Ascospores* 2-celled, peanut-shaped, with a marked constriction at the central septum dividing them into two globose cells, smooth, hyaline, 3–5 × 2–2.5 μ m, lacking conspicuous germ pores.

Material examined:—BRAZIL. Pernambuco, Instituto Agronômico de Pernambuco (IPA), Caruaru, on cattle dung, 12 Dec 2012, R.F.R. Melo (URM86655a!, 86655b!); Serra Talhada, on cattle dung, 30. May 2012, R.F.R. Melo (URM86656!), 15 Oct, R.F.R. Melo 2012 (URM86657a!, 86657b!).

Habitat:—Elephant and cattle dung, as well as on dung of unknown origin.

Distribution:—Africa (Tanzania, Uganda) and North America (United States). This is the first record for Brazil. Notes:—*Mycoarachis inversa*, which until 1988 was the only representative of *Mycoarachis*, can be easily recognized by the small cleistothecia presenting a thick layer of hyaline cells in the outer peridium, as if the peridium were turned inside out, "reverse", as suggested by the epithet's etymology, and by the shape of ascospores. It differs from *M. tetraspora* Valldosera & Guarro (1988:231) mainly by having 8-spored asci.

9. *Pseudallescheria boydii* (Shear) McGinnis, A.A. Padhye & Ajello, Mycotaxon 14(1): 97 (1982) (Plate 1, Figs. 22–24)

Ascomata non-ostiolate, gregarious, superficial, globose, black or dark brown, 150–250 μm diam., glabrous to sparsely hairy. *Hairs* hyphoid, simple to rarely branched, thin-walled, septate, smooth to finely roughened, hyaline, 2–2.5 μm thick at the broadest part, narrowing towards the apex. *Peridium* pseudoparenchymatous, membranaceous, 33–35 μm thick, formed by angular to elongated (*textura epidermoidea*), light brown to golden cells, 5–15 μm, thin-walled. *Asci* 8-spored, saccate, 15–16 μm long, "pars sporifera" 7–8.5 μm, evanescent. *Ascospores* 1-celled, ellipsoid to slightly

fusoid, smooth, light yellow to pale golden, copper colored "en masse", $5-6.5 \times 3-4 \mu m$, with two terminal germ pores.

Material examined:—BRAZIL. Pernambuco, Instituto Agronômico de Pernambuco (IPA), Caruaru, on goat dung, 03 Jan 2013, R.F.R. Melo (URM86685a!, 86685b!, 86685c!, URM86688a!, 86 688b!, 86688c!). Universidade Federal Rural de Pernambuco (UFRPE), Recife, on cattle dung, 20. Mar 2012, R.F.R. Melo (URM86686!), 26 Mar 2012, R.F.R. Melo (URM86687!).

Habitat:—Occasionally recorded in clinical samples and commonly on soil and dung of various animals, especially domestic herbivores.

Distribution:—Worldwide.

Notes:—*Pseudallescheria boydii* has similar morphology to *P. fusoidea* (Arx) McGinnis, A.A. Padhye & Ajello (1973: 98), which differs by having broader ascospores ($3-4 \mu m$) and to *P. africana* (Arx & G. Franz) McGinnis, A.A. Padhye & Ajello (1982: 97), which differs mainly by its smaller ascomata. Isolates identified as *P. boydii* were agents of mycosis in humans, associated with some degree of immunosuppression. The material analyzed here has shorter ascospores than the average for this species. However, the slightly fusoid shape differs from *P. minutispora* Gilgado, Gené, Cano & Guarro (2005: 4938), with ellipsoidal ascospores, with rounded ends (Guarro *et al.* 2012).

10. *Thielavia terrestris* (Apinis) Malloch & Cain, Can. J. Bot.50(1): 66 (1972) (Plate 1, Fig. 25–26)

Ascomata non-ostiolate, gregarious, rarely scattered, semi immersed to superficial, subglobose to globose, dark brown to black, 175–200 μ m diam., glabrous. *Peridium* pseudoparenchymatous, membranaceous, opaque, composed of large, elongated (*textura epidermoidea*), thin-walled, dark brown cells. *Asci* 8-spored, ovoid to irregularly clavate, 15–22.5 \times 2–4 μ m, evanescent. *Ascospores* 1-celled, ovoid, non-apiculated, smooth, hyaline to brown, copper colored "en masse", 3.5–4.5 \times 3–4 μ m, with a single apical germ pore.

Material examined:—BRAZIL. Pernambuco, Instituto Agronômico de Pernambuco (IPA), Caruaru, on cattle dung, 27 Oct 2012, R.F.R. Melo (URM86717!). Universidade Federal Rural de Pernambuco (UFRPE), Recife, on cattle dung, 27 Jun 2012, R.F.R. Melo (URM86718a!, 86718b!).

Habitat:—Recorded on soil, plant material and herbivore dung.

Distribution:—Worldwide.

Notes:—Representatives of *Thielavia* can be found forming dark-colored, glabrous to hairy cleistothecia, usually with pigmented ascospores, commonly found on soil and plant material. *Thielavia terrestris* can be easily recognized by its glabrous cleistothecia and uncommonly small ascospores $(3.5-4.5 \times 3-4 \ \mu\text{m})$ that are ovoid and hyaline to weakly pigmented.

11. *Tripterosporella pakistani* (J.H. Mirza) Malloch & Cain, Can. J. Bot. 50(1): 67 (1972) (Plate 1, Figs. 27–29)

Ascomata non-ostiolate, scattered, semi immersed to superficial, globose, dark gray to black in stereomicroscopy, light brown to dark brown in mounting, 350–400 µm diam., hairy. *Hairs* sparse, hyphoid, simple or branched, thick-walled, septate, smooth, light brown to golden, becoming less pigmented towards the tip, with a bulbous base and rounded to tapered tip, 2.5–3 µm diam. at the broadest part, narrowing towards the apex, very scarce in some ascomata. *Peridium* pseudoparenchymatous, membranaceous, opaque, composed of angular to slightly subglobose (*textura angularis*), thin-walled, light brown cells, 5–10 µm diam. at the inner layers, becoming more thick-walled and less angular at the outermost layer. *Asci* 8-spored, cylindrical-clavate, with a long stipe and a tapered apex, $215-255 \times 20-25$ µm, persistent, with subapical globule smooth to finely verrucose, globose to subglobose, 3–5 µm diam. ($5.5-6 \times 3-5$ µm when subglobose), irregularly biseriate or triseriate. *Ascospores* 1-celled, cylindrical to sigmoid and hyaline when young, with rows of oil droplets, usually geniculate at the base, smooth, $55-60 \times 5-7.5$ µm, becoming swollen at the apical part to finally two-celled with the formation of a transverse septum. *Head cell* ellipsoid, apex umbonate, base strongly tapered, smooth, brown, $19.5-22.5 \times 10-12.5$ µm, with a subapical germ pore, guttulate. *Pedicel* cylindrical, hyaline, occasionally geniculate near the base, occasionally collapsing, $35-39 \times 5-7.5$ µm. *Apical caudae* lashlike, hyaline, $10-20(-25) \times 1-2.5$ µm, usually collapsing in mounting. *Basal caudae* similar to the apical caudae in morphology, usually collapsing in mounting.

Material examined:—BRAZIL. Pernambuco, Universidade Federal Rural de Pernambuco (UFRPE), Recife, on cattle dung, 25 Apr 2012, R.F.R. Melo (URM86756a!, 86756b!).

Habitat:—Herbivore dung.

Distribution:—Asia (Pakistan) and Europe (Italy). This is the first record from Brazil.

Notes:—Representatives of this genus have sparsely pilose cleistothecia, formed superficially on dung, differing from most genera with similar habit by the presence of cylindrical-clavate, unitunicate asci with a distinct apical ring. *Tripterosporella pakistani* was named after the place where it was first described by Mirza, from horse dung in Pakistan in 1968, from material under the name *Cleistobombardia pakistani* J.H. Mirza. along with *T. coprophila* Subramanian & Lodha (1968: 246), type species of the genus, which was described by Subramanian & Lodha (1968). Both circumscriptions were recombined by Malloch & Cain (1971), under the name *Tripterosporella pakistani* (J.H. Mirza) Malloch & Cain. It differs from *T. coprophila* by the smaller ascospore head cells (19.5–22.5 × 10–12.5 μ m) and shorter apical and basal gelatinous caudae.

12. *Zopfiella erostrata* (Griffiths) Udagawa & Furuya, Trans. Mycol. Soc. Japan 15(3): 208 (1974) (Plate 1, Fig. 30)

Ascomata non-ostiolate, scattered to gregarious, semi immersed to superficial, globose, metallic black in stereomicroscopy, dark brown to black in mounting, 300–450 µm diam. *Hairs* setose, simple, thick-walled, septate, smooth, dark brown to black, becoming less pigmented to near hyaline towards the apex, 4.8-6 µm diam. at the broadest part. *Peridium* pseudoparenchymatous, membranaceous, opaque, composed of angular (*textura angularis*), brown to dark brown, thick-walled cells. *Asci* 8-spored, clavate, evanescent, irregularly biseriate, only observable in young cleistothecia. *Ascospores* 1-celled, lageniform to clavate and hyaline and when young, smooth, becoming swollen and forming a transversal septum to delimit a head and pedicel. *Head cell* subglobose, ovoid or lageniform, with umbonate apex and truncated base, smooth, brown to dark brown, $7.5-12.5 \times 6-8.5$ µm, guttulate, with a subapical germ pore. *Pedicel* cylindrical, thin-walled, occasionally collapsing, with rounded base, $6-10 \times 2.5-5$ µm. *Apical caudae* absent.

Material examined:—BRAZIL. Pernambuco, Instituto Agronômico de Pernambuco (IPA), Caruaru, on cattle dung, 06 Oct 2011, R.F.R. Melo (URM86757!), Serra Talhada, on horse dung, 13. Sep 2011, R.F.R. Melo (URM86758!).

Habitat:—On dung of many herbivores.

Distribution:-Worldwide.

Notes:—*Zopfiella* can be distinguished from other coprophilous lasiosphaeriaceous, which share similar ascospore morphology, due the presence of non-ostiolate ascomata with ascospores forming a head and a tail, occasionally with septate heads (previously allocated to *Tripterospora* Cain). *Zopfiella erostrata* is a common representative of this genus on herbivore dung. The material from Pernambuco had a limited number of mature cleistothecia on the substrates, which were identified based on key characters such as ascomata with long dark brown to black, thick-walled hairs and ascospores with symmetrical head cells, differing from *Z. longicaudata* by the shorter pedicel, $(6-10 \times 2.5-5 \,\mu\text{m}.)$

13. *Zopfiella longicaudata* (Cain) Arx, Proc. K. Ned. Akad. Wet., Ser. C, Biol. Med. Sci. 76(3): 291 (1972) (Plate 1, Fig. 31)

Ascomata non-ostiolate, scattered to gregarious, immersed to superficial, globose, metallic black in stereomicroscopy, dark brown to black in mounting, 300–360 μ m diam. *Hairs* hyphoid, simple, thin-walled, septate, smooth, flexuous. *Peridium* pseudoparenchymatous, membranaceous, opaque, composed angular (*textura angularis*), dark red to dark brown, thick-walled cells. *Asci* 8-spored, clavate, 25–35 × 10–15 μ m, evanescent, irregularly biseriate, only observable in young cleistothecia. *Ascospores* 1-celled, lageniform to clavate and hyaline when young, smooth, becoming swollen at the apical part to forma transversal septum delimiting a head and pedicel. *Head cell* ellipsoid to lageniform, usually symmetrical, with umbonate apex and truncated base, smooth, light brown to chestnut brown, 7.5–12.5 × 6–8.5 μ m, guttulate, with a subapical germ pore. *Pedicel* cylindrical, thin-walled, occasionally collapsing, with rounded base, 6–15 × 2–5 μ m. *Apical caudae* absent.

Material examined:—BRAZIL. Pernambuco, Universidade Federal Rural de Pernambuco (UFRPE), Recife, on horse dung, 09 Nov 2012, R.F.R. Melo (URM86759a!, 86759b!).

Habitat:—Soil and dung of many herbivores.

Distribution:-Worldwide.

Notes:—*Zopfiella longicaudata* has similar morphological features as *Z. marina* Furuya & Udagawa (1975: 249), which can be distinguished by its smaller ascospores. Characteristics features are the non-ostiolate ascomata,

usually glabrous, with clavate asci bearing ascospores composed of an ellipsoid to landform head cell and a long cylindrical pedicel, up to 15 μ m long. The material from Pernambuco presented ascospores with shorter pedicels than the ones described by Guarro *et al.* (1991). However, considering the other characters, this difference did not justify the proposition of a new species or variety.

Discussion

Representants from the two major clades of ascomycetes without active ascospore liberation were sampled in this survey, Sordariomycetes and Eurotiomycetes. Sordariomycetes represents a large clade, including most non-lichenized ascomycetes with perithecial or, less frequently, cleistothecial ascomata and unitunicate or prototunicate asci (Zhang et al. 2006). Among the morphological patterns observed in genera of Sordariomycetes with passive spore liberation, the most common includes non-ostiolate ascomata with asci weakly attached to the bottom of the ascomatal cavity and hairy perithecia with neck varying from absent to very long. Two groups of Sordariomycetes, Sordariales and Microascales, which are considerably different in morphology, were the groups with the most representatives in this survey. Sordariales, despite its great variability, consists of mostly wood and dung inhabiting species with relatively large perithecial ascomata with large-celled, membranaceous or coriaceous ascomatal walls (Zhang & Wang 2015). Members of Lasiosphaeriaceae are common as coprophiles, both with active and passive ascospore liberations strategies. Zopfiella was first established by Winter (1884), monographed by Guarro et al. (1991) and had its phylogenetic relationships studied by Cai et al. (2006). It was a common member of the coprophilous fungal communities studied. Tripterosporella, another lasiosphaeriaceous non-ostiolate genus, is characterized by its elliptical or ovoid dark pigmented head cell, usually asseptate, and by its longer, persistently hyaline, cylindrical to geniculate lower pedicel (Doveri 2010). A few records of *Thielavia terrestris* (Chaetomiaceae) were also obtained in this study. The Microascales are characterized by non-stromatic black perithecia with very long necks or, less frequently, by cleistothecia with globose and evanescent asci, developing singly or in chains (Zhang & Wang 2015). The association with insects can be easily observed in some members of this order. Kernia nitida produces gregarious cleistothecia with circinate appendages, which easily hook onto other surrounding cleistothecia when it is removed from the substrate, usually attached to a visiting arthropod. Lophotrichus bartlettii, formerly a member of Chaetomiaceae, has ostiolate ascomata with long terminal hairs, that probably play a similar role with ascomatal/ascospore dispersion to K. nitida, considering that the hairs are not as coiled and compact as in Chaetomium. These two microascalean taxa associated with entomophilous dispersion were the dominant species among these coprophilous fungi from Brazil. Kernia nitida was recorded in 26 samples, 20 of these from the same area (Serra Talhada), with the records distributed between goat (15) and cattle dung (11). Lophotrichus bartlettii, in turn, was recorded in 30 samples, all records from goat dung and evenly distributed between the three areas of sampling. Pseudallescheria boydii, also commonly found on herbivore dung throughout Pernambuco, is a microascalean species commonly associated with forms of eumycetoma, maduromycosis and pseudallescheriasis, being implicated in the infection of immunocompromised and pneumonia patients (Cumbo-Nacheli et al. 2012). Melanosporales, a member of the Hypocreomycetidae clade, was represented in the sampling by Melanospora damnosa and M. zamiae, both rare. Most Eurotiomycetes were only found in the anamorphic phase of their life cycle with the exception of Corynascus sepedonium (Onygenales), a non-ostiolate species with a peculiar crystalline pattern in its peridium, and *Monascus ruber* (Eurotiales), producing stalked cleistothecia along with its conidial stage on culture media. A single occurrence of a Pseudoeurotiaceae, Leuconeurospora pulcherrima, was recorded on goat dung.

The examination of data related to a single taxonomic group or assemblage may mislead the interpretation of more holistic ecological processes, especially related to community dynamics. However, it may still emphasize differences in occurrences and ecological preferences associated with a specific ecomorphological/ecophysiological strategy, such as the passive ascospore liberation in dung fungi. The knowledge of the biology of representatives of some species/ clades is of great importance in the proposal of hypotheses to explain functional community processes. Coprophilous fungi are known for their high degree of adaptation to their life cycle, which can be observed between distantly related groups. The convergent evolution to that habit can be traced from lower fungi (*Pilobolus*) to higher fungi including basidiomycetes (*Sphaerobolus*) and several ascomycetes (*Ascobolus, Podospora, Sordaria,* etc.), considering that species from these genera presents: (1) phototropic mechanisms; (2) active spore/sporangia liberation; (3) spores resistant enough to withstand the passage through the digestive tract of herbivores; (4) adhesive structures, usually eaten along with the vegetation in grazing, among others. Passive ascospore liberation in coprophilous fungi is less common,

but the strategies are usually associated with (1) spores caught in mucilaginous masses associated with entomophilous dispersion or (2) occasional occupants, species commonly associated with soil and plant material are not often isolated from herbivore dung, but that can exhibit higher stress tolerance than the specialized obligate coprophilous species, taking advantage of their broader niche ranges in situations where the relative importance of competition is lower (Grime 1977). Most records were obtained from goat dung, which included most rare and opportunist species. Of the three types of dung sampled in this survey, goat dung has the smallest pellets, which are usually more scattered, with great area of contact with soil, air currents and insects, which would allow fungi that lacks highly adapted strategies to endocoprophily to germinate, grow and sporulate on the dung, taking advantage of a highly disturbed system, while most of the competitive species are still growing. Factors influencing parameters in coprophilous fungal communities, and with which intensity it influences, have been a constant point of discussion (Richardson et al. 2001, Herrera et al. 2011). Among the most common in literature are: (1) type of animal digestive systems - ruminant or monogastric (Richardson 2001), (2) animal diet (Ebersohn & Eicker 1991, Melo et al. 2012), (3) the predominant vegetation in the area, including its structure (Richardson 2001), among others. Although generalizations should be avoided when studying only one assemblage of coprophilous fungal communities (statismosporic ascomycetes), the study of species/ groups of species with functional and structural similarities in the same substrate will aid the elucidation of ecological strategies trends of these organisms.

Acknowledgements

The authors would like to thank the "*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*" (CAPES) and the "*Conselho Nacional de Desenvolvimento Científico e Tecnológico*" (CNPq–Ciência sem Fronteiras; INCT–Herbário Virtual da Flora e dos Fungos). L.C. Maia acknowledges the research fellowship and grants provided by CNPq (INCT–HVFF, Protax, Sisbiota).

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