

## ZYGOMYCETES FROM HERBIVORE DUNG IN THE ECOLOGICAL RESERVE OF DOIS IRMÃOS, NORTHEAST BRAZIL

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### ABSTRACT

Thirty-eight taxa of Zygomycetes distributed in 15 genera were recorded from tapir (*Tapirus terrestris*), camel (*Camelus bactrianus*), horse (*Equus caballus*), deer (*Cervus elaphus*), agouti (*Dasyprocta aguti*), donkey (*Equus asinus*), llama (*Llama glama*) and waterbuck (*Kobus ellipsiprymnus*) dung collected at the Reserva Ecológica de Dois Irmãos located in Recife, State of Pernambuco, Northeast Brazil. The samples were collected on a monthly basis from June 2005 to May 2006, taken to the laboratory and incubated in moist chambers. Higher number of taxa was observed in the excrements of tapir, followed by deer and donkey. The highest number of species was detected for *Mucor*, followed by *Pilobolus*. Statistical analyses showed significant differences in richness of Zygomycetes taxa between the herbivore dung types. Differences of species composition, however, were weak. Seasonality influenced the Zygomycetes species composition but not its richness. Variations in taxa composition between ruminants and non-ruminants dung were non significant.

**Key words:** Coprophilous, taxonomy, ecology, ruminants.

### INTRODUCTION

Coprophilous fungi are important components of ecosystems, actively participating in the cycling of nutrients in animal excrements (28). These organisms often grow and reproduce under a narrow range of conditions, occurring at different temperatures, pH and moisture levels, and are important biological control agents, source of enzymes and antibiotics. Their fruiting bodies often occur in succession. Zygomycetes are often the first group to appear, followed by Ascomycetes and Basidiomycetes (11). Among the

Zygomycetes commonly found in dung, some are obligate coprophilous, requiring culture media added of dung extract or hemin for their growth, while some are facultative coprophilous, developing in common culture media (18).

A number of studies on the diversity and succession of coprophilous fungi have been carried out using different animal dung as substrate (10, 13, 20, 23, 25, 26, 27, 28). However, there are few records exclusively regarding the coprophilous Zygomycetes. According to Krug *et al.* (18), the majority of the published records of this group from dung comes from Europe and North America, rarely from Africa and South

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America. Mirza *et al.* (22) reported 78 species in 26 genera in Pakistan. In Brazil, a small number of Mucorales and Zoopagales have already been reported from herbivore dung, most of them from studies carried out in São Paulo State (34, 35, 36, 37, 38). In the State of Pernambuco, there are only a few reports registering the isolation of Mucorales from herbivore dung (1).

Considering the few studies of coprophilous Zygomycetes in Brazil, especially in Northeastern Region, the aims of this study were to report the occurrence of Zygomycetes on dung from herbivores kept in captivity in Recife city zoo, and to compare species composition and number of occurrence in this substrate.

## MATERIAL AND METHODS

Dung samples from tapir (*Tapirus terrestris*), camel (*Camelus bactrianus*), agouti (*Dasyprocta aguti*), donkey (*Equus asinus*), llama (*Llama glama*), horse (*Equus caballus*), deer (*Cervus elaphus*) and waterbuck (*Kobus ellipsiprymnus*) were collected at the Ecological Reserve of Dois Irmãos (8°7'30"S, 34°52'30"W), that includes a Zoological Park, located in Recife, Pernambuco State, Northeast Brazil. The area is an Atlantic Forest Ecological Reserve with 387 ha.

The samples were collected monthly, from June 2005 to May 2006 with a sterilized spatula, placed in plastic bags, taken to the laboratory and incubated in moist chamber at 28°C ± 2°C for 15 days under alternating light and dark periods, during which mycelial growth was observed.

Fragments of mycelia were transferred to the surface of the M agar (24) and V8 juice agar (6) media added of chloramphenicol (Neo Fenicol – Neo Química) (100mg/L) for isolation. Identification was based on macroscopic (color, aspect and diameter of the colonies) and microscopic (microstructures) aspects according to Benny (4), Benny and Benjamin (6), Domsch *et al.* (12), Hesseltine and Fennel (15) and Schipper (33). Species of *Dispira*, *Dimargaris*, *Pilaira*, *Pilobolus*, *Piptocephalis* and *Syncephalis* were studied directly from the substrate under stereomicroscope (Carl Zeiss Axioscope 40) and light microscope (Leika EZ4) according to

Benjamin (3), Hu *et al.* (16), Indoh (17) and Mehrotra and Prasad (21).

Differences in the associations of Zygomycetes species occurring between different herbivores dung and between the different months of the year were analyzed using similarity analysis (ANOSIM Primer 5.2.4), according to Clarke and Warwick (9). Differences on Zygomycetes species richness in herbivores dung and between months were tested by Friedman analysis, species richness differences between ruminants and non-ruminants were tested using the Mann-Whitney test (39). For all analysis the significance level was 0.05. However, for the ANOSIM analysis, only values of  $R_{Global}$  greater than 0.5 were considered an indication of important Zygomycetes association changes.

## RESULTS

Ninety six dung samples were collected during the study. Altogether, 38 taxa of Zygomycetes, belonging to 15 genera and distributed over Mucorales (33 taxa), Dimargaritales (2) and Zoopagales (3) were identified. The highest number of taxa was found in excrement of tapir (23 taxa), followed by deer (18), donkey (16), llama (15), agouti and camel (14), waterbuck (12), and the lowest number of taxa was found in horse feces (6). Among the genera of Zygomycetes, *Mucor* presented the highest number of taxa (9 species and 6 forms), followed by *Pilobolus*, with 4 species and 7 varieties (Table 1). *Thamnostylum piriforme* occurred all over the year, showing a frequency of occurrence of 100% (Table 2). The frequency of occurrence was above 49% for twelve other taxa, while the remaining 25 taxa presented frequency varying from 8.3% to 41.7%.

Most of the species occurred in excrement of both, ruminants and non-ruminants, except *Lichtheimia blakesleeana*, *Circinella rigida*, *M. circinelloides* f. *griseocyanus*, *M. fuscus* and *Syncephalis cornu* that were found only in ruminants dung, and *Backusella lamprospora*, *Cunninghamella phaeospora*, *Dimargaris bacillispora*, *M. circinelloides* f. *lusitanicus*, *M. guilliermondii*, *P. roridus* var.

*roridus*, *P. roridus* var. *umbonatus*, *Piptocephalis lepidula* and *Rhizopus arrhizus* var. *arrhizus* that occurred exclusively in non-ruminant excrements (Table 1). The highest numbers of taxa occurred in August/2005 (16 taxa), September/2005 (20) and March/2006 (18) (Table 2).

The ANOSIM analysis showed significant differences though without important modification of Zygomycetes composition among the herbivorous excrements ( $R_{Global} = 0.269$ ,  $p < 0.001$ , number of permutations = 10,000). The Friedman test indicated significant differences in the number of taxa ( $Fr = 23.38$ ,  $GL = 7$ ,  $p = 0.0015$ ) of Zygomycetes between

the herbivorous excrements. Changes in taxa composition in the excrement of each animal during the months were not important ( $R_{Global} = 0.102$ , number of permutations = 10,000,  $p < 0.001$ ). Differences were also not found either for the number of taxa present in each animal dung over the studied months ( $Fr = 17.54$ ,  $GL = 10$ ,  $p = 0.0632$ ) or for the composition of Zygomycetes taxa between non-ruminants and ruminant animals ( $R_{Global} = 0.126$ , number of permutations = 10,000,  $p < 0.001$ ). The Mann-Whitney test indicates that no differences occurred in the number of taxa between the excrement of ruminants and non-ruminants [ $Z (U) = 1.064$ ,  $p = 0.2873$ ].

**Table 1.** Zygomycetes from herbivore dung in Ecological Reserve of Dois Irmãos in Recife, Pernambuco, Brazil.

Zygomycetes	Excrement							
	Tapir (NR)	Agouti (NR)	Donkey (NR)	Horse (NR)	Camel (NR)	Deer (R)	Waterbuck (R)	Llama (R)
<i>Backusella lamprospora</i> (Lendn.) Benny & R.K. Benj.	-	+	-	-	-	-	-	-
<i>Circinella muscae</i> (Sorokĭn) Berl. & De Toni	+	+	+	-	-	-	-	+
<i>C. rigida</i> G. Sm.	-	-	-	-	-	-	-	+
<i>C. umbellata</i> Tiegh. & G. Le Monn.	+	+	+	-	-	-	+	+
<i>Cunninghamella blakesleeana</i> Lendn.	-	+	-	-	-	-	-	-
<i>C. echinulata</i> (Thaxt.) Thaxt. ex Blakeslee	+	+	-	-	-	+	-	-
<i>C. phaeospora</i> Boedijn	-	+	-	-	-	-	-	-
<i>Dimargaris bacillispora</i> R. K. Benj.	+	-	-	-	-	-	-	-
<i>Dispira cornuta</i> Tiegh.	+	-	-	-	-	+	+	-
<i>Gilbertella persicaria</i> (E.D. Eddy) Hesselt.	+	-	+	-	-	+	-	-
<i>Lichtheimia blakesleeana</i> (Lendn.) K. Hoffm., Walther & K. Voigt	-	-	-	-	-	-	-	+
<i>Mucor circinelloides</i> f. <i>circinelloides</i> Tiegh.	+	+	+	+	-	+	+	+
<i>M. circinelloides</i> f. <i>griseocyanus</i> (Hagem) Schipper	-	-	-	-	-	-	+	-
<i>M. circinelloides</i> f. <i>janssenii</i> (Lendn.) Schipper	-	+	-	-	+	-	-	+
<i>M. circinelloides</i> f. <i>lusitanicus</i> (Bruderl.) Schipper	-	+	-	-	-	-	-	-
<i>M. guilliermondii</i> Nadson & Philippow	+	-	-	-	-	-	-	-
<i>M. hiemalis</i> f. <i>hiemalis</i> Wehmer	+	-	-	-	+	+	+	+
<i>M. mousanensis</i> Baijal & B.S. Mehrotra	+	+	-	-	+	+	+	+
<i>M. mucedo</i> Fresen	+	+	+	-	+	+	+	+
<i>M. racemosus</i> f. <i>chibinensis</i> (Neophyt.) Schipper	+	+	+	+	+	+	+	-
<i>M. ramosissimus</i> Samouts.	+	-	+	-	+	+	-	-
<i>M. subtilissimus</i> Oudem.	+	-	+	-	-	+	+	+
<i>M. fuscus</i> (Berk. & M.A. Curtis) Berl. & De Toni	-	-	-	-	-	-	-	+
<i>Pilaira anomala</i> (Ces.) J. Schröt.	-	-	-	-	+	-	-	-
<i>Pilobolus crystallinus</i> var. <i>crystallinus</i> F.H. (Wigg) Tode	+	-	+	-	+	+	+	-
<i>P. crystallinus</i> var. <i>hyalosporus</i> (Boedijn) F.M Hu & R.Y. Zheng	+	-	+	-	+	-	-	+
<i>P. crystallinus</i> var. <i>kleinii</i> (Tiegh.) R.Y. Zheng & G.Q. Chen	+	-	+	+	+	+	+	+
<i>P. lentiger</i> var. <i>lentiger</i> Corda	+	-	+	-	+	+	-	-
<i>P. lentiger</i> var. <i>minutus</i> (Speg.) R.Y. Zheng & G.Q. Chen	+	+	+	-	+	+	-	+
<i>P. longipes</i> Tiegh.	-	-	+	+	+	-	-	-
<i>P. roridus</i> var. <i>roridus</i> (Bolton) Pers.	-	-	+	+	-	-	-	-
<i>P. roridus</i> var. <i>umbonatus</i> Buller	-	-	-	+	-	-	-	-
<i>Piptocephalis lemonnieriana</i> Vuill.	-	-	-	-	+	+	-	-
<i>P. lepidula</i> (Marchal) P. Syd.	+	-	+	-	-	-	-	-
<i>Rhizopus arrhizus</i> var. <i>arrhizus</i> A. Fisch	+	-	+	-	-	-	-	-
<i>Syncephalastrum racemosum</i> Cohn ex J.Schröt.	+	-	-	-	-	+	-	-
<i>Syncephalis cornu</i> Tiegh. & G. Le Monn.	-	-	-	-	-	+	-	-
<i>Thamnostylum piriforme</i> (Bainier) A.R.X. & H.P. Upadhyay	+	+	-	-	+	+	+	+
Total of taxa	23	14	15	6	14	18	12	15

(R) ruminant; (NR) non-ruminant; + presence; - absence.

**Table 2.** Frequency of occurrence of Zygomycetes in herbivores dung in Ecological Reserve of Dois Irmão in Recife, Pernambuco, Brazil from June/05 to May/06.

Zygomycetes	Month/year											Frequency occurrence	
	Jun/05	Jul/05	Aug/05	Sept/05	Oct/05	Nov/05	Dec/05	Jan/06	Feb/06	Mar/06	Apr/06		May/06
<i>Backusella lamprospora</i>	-	-	-	-	-	-	-	-	+	+	+	+	33.3%
<i>Circinella muscae</i>	+	+	-	+	-	-	+	+	-	+	+	+	66.7%
<i>C. rigida</i>	-	-	-	-	-	-	-	-	+	+	-	-	16.7%
<i>C. umbellata</i>	+	+	+	+	+	-	+	+	-	-	+	+	75.0%
<i>Cunninghamella blakesleeana</i>	-	-	-	-	-	-	-	-	-	+	-	-	8.3%
<i>C. echinulata</i>	-	-	-	+	+	-	-	-	-	+	-	-	25.0%
<i>C. phaeospora</i>	-	-	-	+	-	-	-	-	-	-	-	-	8.3%
<i>Dimargaris bacillispora</i>	-	-	-	-	-	-	-	-	-	-	-	+	8.3%
<i>Dispira cornuta</i>	-	+	+	+	+	+	+	+	+	+	-	-	75.0%
<i>Gilbertella persicaria</i>	-	-	+	-	-	-	-	-	+	-	-	-	16.7%
<i>Lichtheimia blakesleeana</i>	+	-	-	-	-	-	-	+	+	+	+	+	33.3%
<i>Mucor circinelloides</i> f. <i>circinelloides</i>	+	+	-	+	+	+	+	-	+	+	+	+	83.3%
<i>M. circinelloides</i> f. <i>griseocyanus</i>	-	-	-	-	-	-	-	-	-	+	+	-	16.7%
<i>M. circinelloides</i> f. <i>janssenii</i>	-	-	+	-	-	-	-	-	+	-	-	+	25.0%
<i>M. circinelloides</i> f. <i>lusitanicus</i>	-	-	+	-	-	-	-	-	-	-	-	-	8.3%
<i>M. guilliermondii</i>	+	-	-	-	-	-	-	-	-	-	-	-	8.3%
<i>M. hiemalis</i> f. <i>hiemalis</i>	-	+	+	+	-	+	-	-	-	-	-	-	33.3%
<i>M. mousanensis</i>	+	+	+	+	-	-	+	+	-	+	+	+	75.0%
<i>M. mucedo</i>	+	+	-	-	+	-	+	-	-	+	+	+	58.3%
<i>M. racemosus</i> f. <i>chibinensis</i>	+	-	-	+	+	+	+	-	-	+	+	-	58.3%
<i>M. ramosissimus</i>	+	+	-	+	-	-	-	-	-	+	+	+	50.0%
<i>M. subtilissimus</i>	+	+	+	+	-	+	+	+	-	+	-	+	75.0%
<i>M. fuscus</i>	-	-	+	-	-	-	-	-	-	-	-	-	8.3%
<i>Pilaira anomala</i>	-	+	-	+	-	-	-	-	-	-	-	-	16.7%
<i>Pilobolus crystallinus</i> var. <i>crystallinus</i>	+	-	+	+	+	+	+	-	-	-	-	-	50.0%
<i>P. crystallinus</i> var. <i>hyalosporus</i>	-	+	+	-	+	-	+	-	-	+	-	-	41.7%
<i>P. crystallinus</i> var. <i>kleinii</i>	-	-	+	+	+	+	+	+	+	+	-	-	66.7%
<i>P. lentiger</i> var. <i>lentiger</i>	-	-	+	+	+	-	-	+	+	+	-	-	50.0%
<i>P. lentiger</i> var. <i>minutus</i>	-	-	+	+	+	+	-	-	+	-	-	-	41.7%
<i>P. longipes</i>	+	-	+	-	-	+	+	+	-	-	-	-	41.7%
<i>P. roridus</i> var. <i>roridus</i>	-	-	-	+	+	-	-	-	-	-	-	-	16.7%
<i>P. roridus</i> var. <i>umbonatus</i>	-	-	-	-	+	-	-	-	-	-	-	-	8.3%
<i>Piptocephalis lemonnieriana</i>	-	+	-	+	-	-	-	-	-	-	-	+	25.0%
<i>P. lepidula</i>	-	+	-	+	-	-	-	-	-	-	-	-	16.7%
<i>Rhizopus arrhizus</i> var. <i>arrhizus</i>	+	-	-	-	-	-	+	-	+	-	-	-	25.0%
<i>Syncephalastrum racemosum</i>	+	-	-	-	-	-	-	-	-	-	-	-	8.3%
<i>Syncephalis cornu</i>	-	+	-	-	-	-	+	-	-	-	-	+	25.0%
<i>Thamnostylum piriforme</i>	+	+	+	+	+	+	+	+	+	+	+	+	100.0%
Total of taxa	15	15	16	20	14	10	15	10	12	18	11	14	

+ presence; - absence

## DISCUSSION

The results indicated high diversity of Zygomycetes in the herbivores dung examined. The occurrence of Zygomycetes in animal dung in Brazil has been mentioned by Trufem (34), who reported the occurrence of *Mucor hiemalis*, *M. mucedo*,

*M. plumbeus*, *Pilaira anomala*, *Pilobolus crystallinus*, *P. kleinii*, *Piptocephalis repens*, *Syncephalis cornu* and *S. tengi* in excrements of nine herbivores in São Paulo. Viriato and Trufem (37) and Trufem and Viriato (35) described 23 species on excrement of 10 herbivores species in São Paulo. From these taxa, 13 are reported in this paper, although only horse

and camel feces were common to both studies, indicating that several species of Zygomycetes are not specific to a given animal dung. These results are corroborated by reports of Alves *et al.* (1), Caretta *et al.* (8), Delgado Ávila *et al.* (10), Ebersohn and Eicker (13), McCarthy (20), Nyberg and Persson (23) and Richardson (28), who observed the occurrence of different species of Zygomycetes in dung of different animals, mainly herbivores, in different countries.

*Mucor* and *Pilobolus* were the most representative in number of species. According to Krug *et al.* (18), only species of *Pilobolus* are obligate coprophilous, while *Mucor* includes facultative coprophilous species. Mucoraceous fungi are common in dung and can also occur in other types of decomposing organic matter (16). Considering that *Mucor* is also commonly found in soil (12), it is possible that species of *Mucor* isolated in this study are soil contaminants. Alves *et al.* (1) described 12 taxa of *Mucor* from feces of 10 herbivores kept in captivity in the Dois Irmãos Ecological Reserve and seven of these taxa (*M. circinelloides* f. *circinelloides*, *M. circinelloides* f. *griseocyanus*, *M. circinelloides* f. *janssenii*, *M. circinelloides* f. *lusitanicus*, *M. hiemalis* f. *hiemalis*, *M. racemosus* f. *chibinensis* and *M. subtilissimus*) were also isolated in this study. *Pilobolus*, an obligate coprophilous fungi, is one of the first genus, in fungal succession, that occur in excrement (11), and have been reported by Foos and Royer (14), Hu *et al.* (16), Richardson (28) and Santiago *et al.* (31).

Most of the isolated species were previously reported in animal excrement: *M. mucedo*, *M. hiemalis* f. *hiemalis*, *M. mousanensis*, *M. circinelloides* and *M. racemosus* (38). However, *Cunninghamella phaeospora*, *C. echinulata* and *Lichtheimia blakesleeana* are reported for the first time on dung. *Dimargaris bacillispora* (30), *Mucor guilliermondii* (32), *Gilbertella persicaria* (29), *P. crystallinus* var. *hyalosporus*, *P. roridus* var. *roridus* and *P. longipes* (31) are the first records for Brazil. According to Krug *et al.* (18), *Absidia*, *Circinella*, *Mucor*, *Rhizopus*, *Piptocephalis* and *Syncephalis* have many coprophilous and non-coprophilous species; *Dimargaris*, *Dispira*, *Pilaira* and *Thamnostylum* have predominantly coprophilous species, although few non-coprophilous species

may occur; *Backusella*, *Cunninghamella*, *Gilbertella* and *Syncephalastrum* have species that primarily colonize other substrates, however one or more coprophilous species may occur.

Nyberg and Persson (23) and Richardson (25) mentioned that a few species show higher frequency of occurrence while most of them occur less often, as was also found in this study. Our results pointed out that *Thamnostylum piriforme* was the commonest species (100% frequency). According to Benny and Benjamin (5) this taxa occurs most commonly in excrement, but can be found in soil and organic matter undergoing decomposition.

The number of species, and to a lower extent the Zygomycetes community composition, in dung varied according to the animal species. Differences in community composition may result from several abiotic and biotic factors. Types of digestive system and variations in animal nutrition, geographical location, moisture of the excrements, intra and inter-specific competitions and presence of insects and predators can influence the mycota of feces (7, 11, 13). As the dung samples were kept in moist chambers, under laboratory conditions (temperature, light intensity and away from insect predators), some of these variables can be disregarded, and the feeding of the animals probably determines the mycota composition in this study.

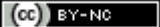
Some taxa were specific to ruminant or non-ruminant feces. The digestive processes of ruminants and non-ruminants are important selective factors for the composition of species in excrement, as the digestive enzymes work longer on the fungal spores in ruminants (11). However, ANOSIM indicates that differences in the composition of Zygomycetes were not significant between these two groups.

The Zygomycetes species composition was little affected by seasonal changes, however, the number of taxa was not. Similar results were reported by Viriato (38) when studying Zygomycetes in dung of eight herbivores. In a three years study on opossum (*Trichosurus vulpecula* Kerr) dung, in New Zealand, Bell (2) reported a higher occurrence of some fungal species during the wet season.

The results pointed out that herbivorous mammals' dung are favorable substrates for growth of Zygomycetes. Several taxa reported in this study are being cited for the first time in excrement of herbivores, contributing to the knowledge of coprophilous species. This study contributes to the knowledge of the diversity of Zygomycetes in dung. As observed for other groups of fungi, the composition of Zygomycetes in herbivores excrement vary depending on the animal species but seems directly dependent on the diet of the herbivores. However, further studies are needed to uncover these facts.

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