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Original article

Myxomycetes associated with pecan walnut leaf litter, *Carya illinoinensis* (Wangenh.) K. Koch (Juglandaceae)

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ABSTRACT: The pecan tree (*Carya illinoinensis*, Juglandaceae) was introduced in Brazil at the beginning of the 20th century by American immigrants and its cultivation currently extends from Rio Grande do Sul to Minas Gerais states, with approximately 2,400 hectares planted. We present the first report of litter-associated myxomycetes of *C. illinoinensis*, directly observed in the field, sporulating on dry leaves (*Physarum cinereum*, *P. melleum*, *P. polycephalum*), twigs and dry leaves (*Diachea leucopodia*) and twigs (*Fuligo cinerea*, *F. septica*, *Hemitrichia serpula* var. *serpula*). The most constant species were *P. melleum* and *D. leucopodia*. *Fuligo cinerea* is recorded for the first time for Rio Grande do Sul; *D. leucopodia* and *P. cinereum* are cited for the first time for the Brazilian portion of the Pampa, bringing the number of species known for the biome to 61.
Keywords: Amebozoa, Microhabitat, Phytopathology, Protist.

RESUMO (*Mixomicetos associados* à serapilheira da nogueira-pecan, *Carya illinoinensis* (Wangenh.) K. Koch (Juglandaceae)): A nogueira-pecan (*Carya illinoinensis*, Juglandaceae) foi introduzida no Brasil no início do século XX por imigrantes americanos e seu cultivo atualmente se estende desde o Rio Grande do Sul até Minas Gerais, com aproximadamente 2,4 mil hectares plantados. Apresenta-se o primeiro relato de mixomicetos associados à serapilheira de *C. illinoinensis*, diretamente observados no campo, esporulados sobre folhas secas (*Physarum cinereum*, *P. melleum*, *P. polycephalum*), gravetos e folhas secas (*Diachea leucopodia*) e galhos (*Fuligo cinerea*, *F. septica*, *Hemitrichia serpula* var. *serpula*). As espécies mais constantes foram *P. melleum* e *D. leucopodia*. *Fuligo cinerea* é registrado pela primeira vez para o Rio Grande do Sul; *D. leucopodia* e *P. cinereum* são citados pela primeira vez para a porção brasileira do Pampa, elevando para 61 o número de espécies conhecidas para o bioma.
Palavras-chave: Amebozoa, Fitopatologia, Microhabitat, Protista.

INTRODUCTION

Carya illinoinensis (Wangenh.) K. Koch, the pecan tree is originally from North America and about 120 years ago it was introduced in Brazil by North American immigrants, in the state of São Paulo (Backes & Irgang 2004). In Rio Grande do Sul, cultivation began in the 1940s, in the municipality of Anta Gorda, with the planting of seedlings from the United States, considered the ancestors of most pecan trees in the region (Fronza *et al.* 2013). Currently, its cultivation is increasing in Brazil (ca. 2.4 thousand ha), especially in the South and Southeast, with greater extensions of planted area in the regions of Vale do Taquari, Rio Pardo and Central Rio Grande do Sul state (Poletto *et al.* 2016).

The class Myxomycetes (Amoebozoa) encompasses about 1.000 species, found in different climates and ecosystems, occupying microhabitats where there is availability of decomposing organic matter, such as dead logs and leaf litter, both in

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natural and altered environments by human activity (Lado 2005-2022, Keller *et al.* 2022; Schnittler *et al.* 2022). Although they are not harmful to plants, the frequent coexistence with arthropods and fungi can have negative consequences, in the possibility of being vectors of pathogens. The genera *Stemonitis* Roth, *Arcyria* Hill, *Lycogala* Pers. and *Tubifera* J.F. Gmel. have characteristics that favor the attraction of insects, especially Coleoptera and Diptera, which feed on their spores and use them as nurseries for oviposition, such as: long-lasting and abundant sporophores, development in woody substrate and during almost the entire year (Dudka & Romanenko, 2006, Lemos *et al.* 2010, Laaksonen *et al.* 2010, Kirschner *et al.* 2017, Sá *et al.* 2019).

In Brazil, 255 species are recorded, of which 110 have a known distribution for Rio Grande do Sul state and 58 are present in the geographical area of the Pampa biome (Xavier de Lima & Cavalcanti 2017, Velloso *et al.* 2020, BFG 2022). In different ecosystems and in anthropic areas, they have been recorded in litter, aerial litter, dead trunks, bark of living trees and inflorescences of native or introduced species, with the families Physaraceae, Trichiaceae and Stemonitaceae being the most common (Cavalcanti & Mobin 2004, Maimoni-Rodella & Cavalcanti 2006, Bezerra & Cavalcanti 2007, Silva & Cavalcanti 2010, Parente & Cavalcanti 2013, Araújo *et al.* 2014, Cavalcanti *et al.* 2014, 2016, Sá *et al.* 2022).

Litter in natural environments and agricultural areas are good microhabitats for myxomycetes, as evidenced by Bezerra *et al.* (2011) who, in field collections and cultivation in a moist chamber, recorded 15 species in the litter and aerial litter of *Cecropia adenopus* Mart. ex Miq., in a fragment of Atlantic Forest, in Rio Grande do Norte, a northeast Brazilian state; Physarales (47%) and Trichiales (33%) were predominat, with most species represented by a single specimen.

In areas cultivated with sugarcane (*Sacharum officinarum* L.), in northeastern Brazil, using only field collections, Santos & Cavalcanti (1991) found five species in leaf litter and two in aerial litter. In the Philippines, Alfaro *et al.* (2014) compared communities of myxomycetes in the litter of sugarcane fields with those found in humid forests, where the substrate supply was more heterogeneous; in cultivated areas, no species was observed in the field and only *Arcyria cinerea* (Bull.) Pers. and *Tubifera ferruginosa* (Batsch) J. F. Gmel. were obtained in 18% of the moist chambers. The authors commented that their results differed from the study carried out in Thailand by Tran *et al.* (2008) who, using the same techniques, found higher richness in plantations of *Musa* sp. (banana), *Mangifera indica* L. (mango) and *Zea mays* L. (corn) than in forests and attributed the difference to the type of leaf, considering that sugarcane would capture spores less easily. Comparing myxomycete communities in the litter of plantations of *Camellia sinensis* (L.) Kuntze. (tea), *Dimocarpus longan* Lour. (longan) and *Psidium guajava* L. (guava) in Vietnam, Redeña-Santos *et al.* (2017) concluded that leathery leaves, such as those of *P. guajava*, are good traps for spores dispersed in the environment. However, differences can be found with the same plant species, as evidenced in research carried out in the Philippines by Buisan *et al.* (2020) in organic and conventional rice fields, where only three species were associated with litter and aerial litter, with the substrate coming from conventional rice fields being more productive.

In pecan walnut crops, litter increases in winter, when there is a large deposition of leaves, as they are deciduous, in addition to twigs and fruits, creating a favorable environment for the development of myxomycetes, but no study was found in the literature. Aiming to expand the knowledge about myxomycetes occurring in the litter of cultivated plants and about the myxobiota of the Pampa biome, this work aimed to record species associated with pecan walnut in a crop in the municipality of São Gabriel, Rio Grande do Sul state, Brazil.

MATERIAL AND METHODS

The pecan walnut tree can reach a maximum height of 55 m and 210 cm in diameter, with a straight trunk and smooth grayish bark in young individuals, becoming ridged, rough and reddish brown over the years (Peterson 1990), with leaves alternate and pinnate, with 9 to 17 lanceolate leaflets, 5-10 cm long (Reed & Davidson 1954), chartaceous in consistency, glabrous on the adaxial surface, lighter and sparsely pubescent on the abaxial surface (Marchiori 1997). It is a monoecious plant, with separate male and female flowers, with flowering occurring between October and November, and fruit maturation between March and May (Duarte & Ortiz 2001). It is deciduous, losing its leaves between April and June, during the winter (Backes & Irgang 2004).

The litter of a domestic pecan plantation (ca 0.35 ha), located in the municipality of São Gabriel (Figure 1), in the Midwest region of Rio Grande do Sul, was analyzed biweekly during the winter of 2020 (July - September), when the monthly temperature averages are from 14.1°C to 15°C and the rainfall averages vary between 380 mm and 400 mm (Wrege *et al.* 2011). The climate in the region is humid and temperate subtropical, with well-defined seasons, classifying it as a steppe (IBGE 2020). Six field visits were carried out, inspecting for three hours branches, twigs, fruits and leaves on each occasion, looking for the presence of myxomycetes. For each collection, abiotic data were also collected, such as average temperature and sum of rainfall for the previous 10 days. The number of specimens collected in each expedition was also noted (Table 1). The material found was collected while still attached to the substrate, dehydrated in a laboratory environment and stored for later identification (Silva & Cavalcanti 2012).

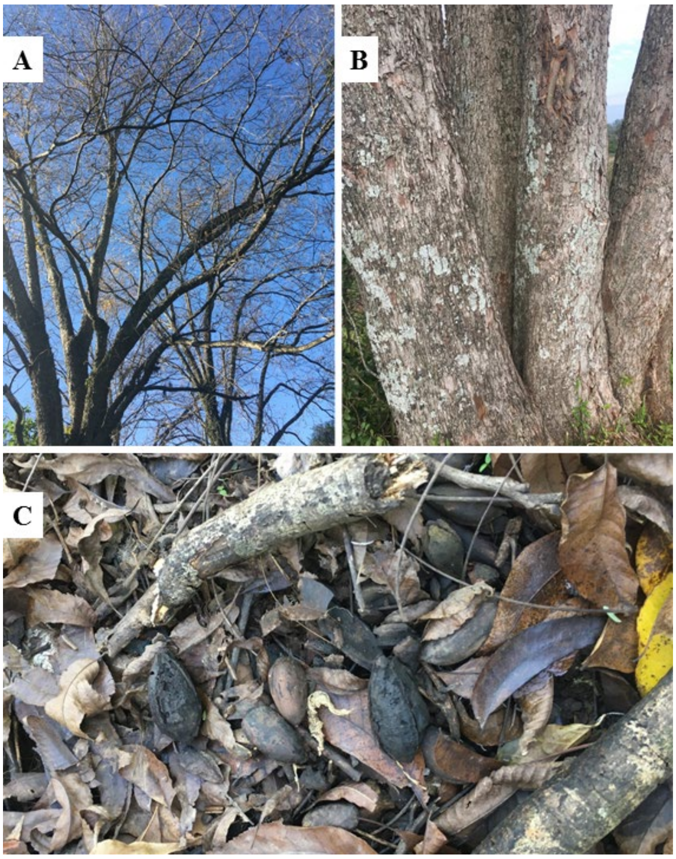

Figure 1. Study area, in São Gabriel, RS, southern Brazil. A. Trees of *Carya illinoinensis* (Wangenh.) K. Koch (pecan walnut) during winter, completely leafless. B. Detail of the rugose bark. C. Elements of leaf litter: fruits, twigs, twigs and leaves.

Macromorphological and micromorphological analyzes were carried out at the Laboratory of Taxonomy of Fungi (LATAF), at the Universidade Federal do Pampa, São Gabriel campus, Rio Grande do Sul state, Brazil. Identifications were performed according to Martin & Alexopoulos (1969), Farr (1976), Poulain *et al.* (2011) and the taxonomic nomenclature and abbreviations of the authors' names follow Lado (2005-2022). Representative specimens of the material studied were deposited in the collection of the Herbarium Bruno Edgar Irgang (HBEI).

Table 1. Abiotic data and number of specimens in each collection.

Collect	Month	Temp (°C)	Pluviosity (mm)	Specimens (Nº)
1	July	12.4	68	6
2	July	14.1	85	4
3	August	10.2	54	9
4	August	12.2	72	6
5	September	14.7	82	14
6	September	15.4	65	9

Species constancy was calculated from the ratio between the number of field trips in which the species was collected and the total performed (5), expressed as a percentage (Cavalcanti & Mobin 2004); according to these percentage data, the species were considered constant (>50%), accessory (25%-50%) or accidental (<25%) in the myxobiota. The distribution of species in Brazilian states, regions and biomes was based on Xavier de Lima & Cavalcanti (2017), Velloso *et al.* (2020) and The Brazilian Flora Group (BFG 2022).

RESULTS

Using only the opportunistic collection technique in the field, a total of 48 specimens of myxomycetes were obtained, sporulated in the different components analyzed in the litter of *C. illinoinensis*, representing two orders, three families, four genera and seven species (Table 2; Figure 2). *Diachea leucopodia*, with 22 specimens, and *Physarum melleum*, with 20, were constant species, the former collected in all expeditions and the latter in four of the six expeditions carried out. *Diachea leucopodia, Physarum cinereum* and *P. polycephalum* are cited for the first time for the Brazilian Pampa biome, and *Fuligo cinerea* is cited for the first time for Rio Grande do Sul (Table 2).

DISCUSSION

No research had documented the presence of myxomycetes in the litter of *C. illinoinensis* and, although the inventory was based on collections carried out in only one season, seven species were found, of which 43% correspond to new records for the state of Rio Grande do Sul and to the Pampa biome.

Diachea leucopodia is the most common and abundant representative of the genus in the Neotropics, with records in different regions and vegetation environments in Brazil (Cavalcanti *et al.* 2009, BFG 2022), but its presence in the Pampa biome had not yet been reported. In the present study, it was the most characteristic species of the myxobiota, found in all field visits, in extensive sporulation, covering large areas, on leaves and twigs, even colonizing freshly fallen leaves, still green. In Brazil and in other countries, such as South Korea, the occurrence of large sporulations of *D. leucopodia* on leaves, stolons and petioles of strawberry plants and plant remains in the soil has been reported; although not considered phytopathogenic, its presence can cause a decrease in the photosynthetic rate of the plant or decreases in fruit quality (Lee *et al.* 2008, Domingues *et al.* 2012, Ribeiro & Brioso 2019).

Physarum melleum is reported for all regions of Brazil, present in leaf litter, aerial litter and inflorescences of live plants, in different biomes, including the Pampa (Xavier de Lima & Cavalcanti 2017, BFG 2022). In the litter of the pecan tree, the species was classified as constant, present in four of the six collection expeditions, always on dry leaves (foliicolous) and on all occasions it presented abundant sporocarps. It was reported by Mckenzie (1992) and Mendes *et al.* (1998) as a phytopathogen to palm trees of economic interest, such as *Cocos nucifera* L. and *Rhopalostylis baueri* (Hook.f.) H.Wendl. & Drude and was present in the myxobiota of the two coconut groves studied by Sá *et al.* (2019, 2022). In litter, in addition to the record in *Mangifera indica* in the Brazillian Midwest, where it was the most abundant species in collections done in spring (Araújo *et al.* 2014), there are also records of occurrence in *Dimocarpus longan* Lour. (longan) and *Psidium guajava* L. (guava) trees grown in the Philippines (Redeña-Santos *et al.* 2017).

Table 2. Species of myxomycetes occurring in litter components of *Carya illinoinensis* (Wangenh.) K. Koch (pecan walnut) in a cultivated area in the municipality of São Gabriel, Rio Grande do Sul state, southern Brazil. Constancy: accidental (<25%), accessory (25% - 50%), constant (>50%). *First occurrence for the Brazilian portion of the Pampa biome. **First occurrence in Rio Grande do Sul state.

Family/Species	Constancy (%)	Substrate
Didymiaceae		
*Diachea leucopodia** (Bull.) Rostaf.	Constant (100)	Twigs, dry leaf
Physaraceae		
*Fuligo cinerea*** (Schwein.) Morgan	Accidental (20)	Dry twigs
Fuligo septica (L.) F.H.Wigg.	Accidental (20)	Dry twigs
*Physarum cinereum** (Batsch) Pers.	Accidental (20)	Dry leaf
Physarum melleum (Berk. & Broome) Massee	Constant (100)	Dry leaf
*Physarum polycephalum**Schwein.	Accessory (40)	Dry leaf
Trichiaceae		
Hemitrichia serpula var. *serpula* (Scop.) Rostaf. ex Lister	Accidental (20)	Dry twigs

Physarum polycephalum occurs in the Northeast (BA, PE), Southeast (SP), Midwest (GO, MS) and South (RS, SC) of Brazil, with records of collections on dead trunks in the Atlantic Forest, Cerrado and Pantanal biomes (BFG 2022). It was mentioned to Rio Grande do Sul by Rodrigues & Guerrero (1990), sporulating on decomposing wood and living grass leaves in a fragment of the Atlantic Forest, being this the first reference for the Brazilian portion of the Pampa biome. It was the only species classified as accessory in the pecan litter, with two specimens collected in July and August, sporulating on dead leaves (foliicolous), after heavy rains, both with few sporocarps.

Physarum cinereum has a wide distribution in Brazil, being found in all regions, in different vegetation environments, with records in Rio Grande do Sul (BFG 2022, Velloso *et al.* 2020), but this is its first confirmed occurrence for the Brazilian portion of the Pampa biome. The sporocarps were collected only in the first excursion, still in July, on dry leaves, being classified as foliicolous and accidental in the litter of the pecan tree plantation.

For a long time, this species was confused with fungi of the genera *Ustilago* and *Microbotryum*, due to the dark aspect of its spore mass, and Agra *et al.* (2018) identified it as the causative agent of false bean smut in soybean [*Glycine max* (L.) Merr.] and common bean (*Phaseolus vulgaris* L.) plantations in different regions of Brazil. In European countries, such as Italy, it caused 5 to 20% of incidence and losses in crops of *Lactuca sativa* L. (lettuce), *Eruca sativa* L. (arugula), *Cichorium endiva* L. (chicory) and *Apium graveolens* L. (celery), in late winter and early spring (Crescenzi *et al.* 2015). In South America, Cabrera-de-Alvarez *et al.* (1993) reported losses causing by the species in tomato (*Lycopersicon esculentum* Mill.) in Argentina and Brazil, causing damage to forage grasses of the genus *Paspalum* in Minas Gerais (Muchovej & Muchovej 1987) and was included in the list of phytopathogenic fungi in the Atlas of the Instituto de Micologia, published in 1960, sporulating on *Carica papaya* L. (papaya tree), in Pernambuco (Mendes & Urben 2022).

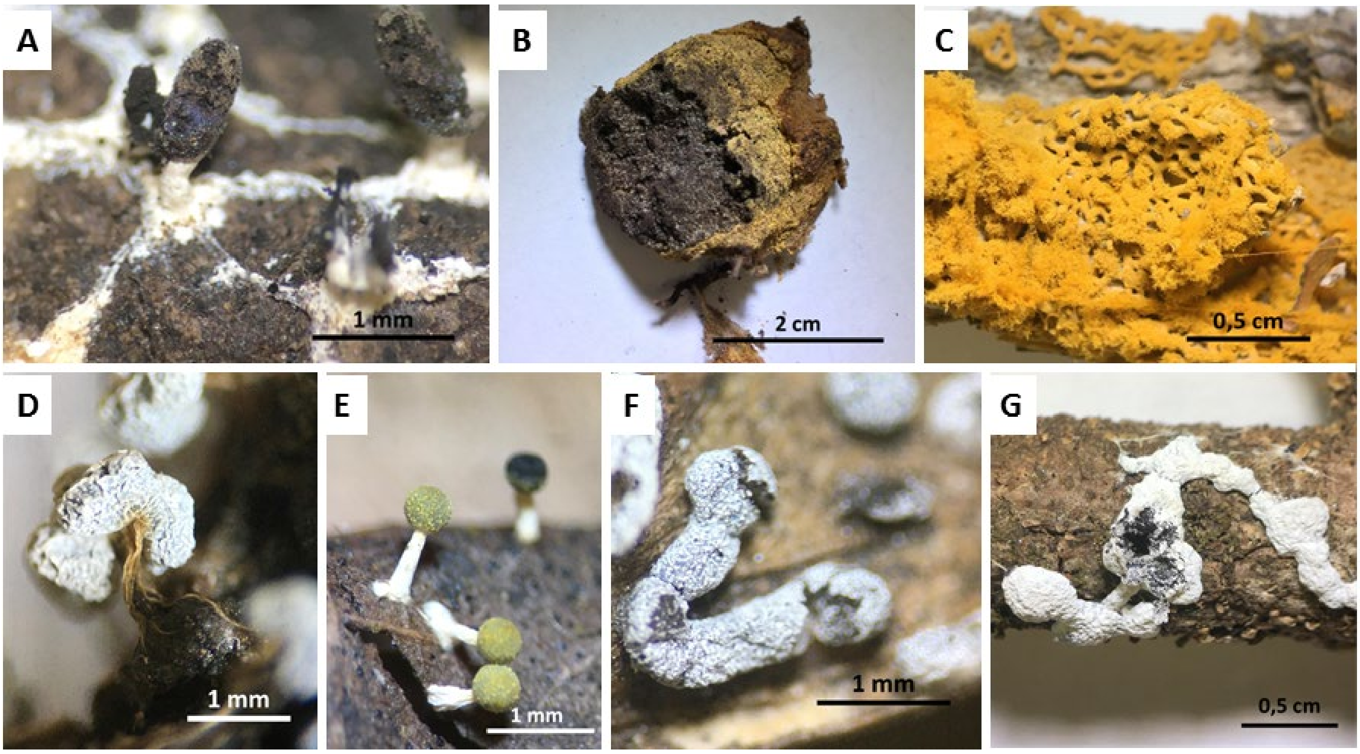

Figure 2. Myxomycetes recorded in the litter of *Carya illinoinensis* (Wangenh.) K. Koch (pecan walnut) cultivated in São Gabriel, RS, southern Brazil. A. *Diachea leucopodia*. B. *Fuligo septica*. C. *Hemitrichia serpula* var. *serpula* D. *Physarum polycephalum*. E. *Physarum melleum*. F. *Physarum cinereum*. G. *Fuligo cinerea*.

The two species of *Fuligo* were classified as accidental in the litter of *C. illinoinensis*, recorded only in the last field trip, at the end of winter, sporulating on a decomposing branch of the pecan tree (lignicolous). Both have a wide distribution in Brazil, but this is the first report of *F. cinerea* for Rio Grande do Sul and the Pampa biome, while *F. septica* has a known occurrence in the state, found in different phytophysiognomies of the Atlantic Forest and in riparian forest in the Pampa biome (Rodrigues & Guerrero 1990, Xavier de Lima & Cavalcanti 2017, BFG 2022).

F. cinerea was included by Wordell Filho & Boff (2006) among the harmful parasites for onion cultivation, known as felt, and they report that, in Santa Catarina state, one of the authors observed the death of up to 90% of the seedlings in the beds where the plasmodium managed to spread quickly on the surface of the soil.

Fuligo septica can also occasionally be harmful to cultivated plants, forming extensive aethalia in urban grasslands and in herbaceous crops such as sweet potato [*Ipomoea batatas* (L.) Lam.] and strawberry (Berestetskaya 1998, Kim *et al.* 2007). In

Brazil, it caused damage in cultivated areas in Maranhão state, when its large aethalia were formed on live plants of long coriander (*Eryngium foetidum* L.), watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], cabbage (*Brassica* sp.), lettuce (*L. sativa*) and the weed *Alternanthera* sp., also occurring in the litter of *Eucalyptus* sp. (Silva & Bezerra 2005, Silva *et al.* 2008).

Hemitrichia serpula var. *serpula*, the only representative of Trichiales recorded in the present study, is the most common species of the genus in Brazil (Bezerra *et al.* 2009), with records as lignicolous to Rio Grande do Sul, occurring in the riparian forest environment in the Pampa biome and Mixed Ombrophilous Forest in the Atlantic Forest (Xavier de Lima & Cavalcanti 2017, Velloso *et al.* 2020). In studies carried out with aerial litter of *C. nucifera* in Northeastern Brazil, *H. serpula* var. *serpula* was the most abundant and frequent species (Sá *et al.* 2019, 2022), but in the litter of pecan walnut it was classified as very rare, collected only on the last excursion, on the same fallen branch where the *F. septica* specimens were found.

Some species of myxomycetes are included in lists of phytopathogens, such as those by Mendes *et al.* (1998), Wordell Filho & Boff (2006) and Mendes & Urben (2022), observing a predominance of Physarales, as found in the pecan tree litter, where 86% of the species belonged to the order. The nine species found in the litter of *Mangifera indica* L. by Araújo *et al.* (2014) also belonged to the order Physarales, with *D. leucopodia* and *P. melleum* also standing out as the most frequent. In the aerial leaflet and stipe of living individuals of *C. nucifera* investigated by Sá *et al.* (2019, 2022) in coconut groves in Pernambuco, Physarales where also predominant (60% in Goiana; 78% in Bonito), represented by Didymiaceae (*Diderma*, *Didymium*) and Physaraceae (*Physarella*, *Physarum*).

Despite the fact that 71% of the registered species have reports of damage to cultivated plants, no indications were observed that could be harmful to the pekan walnut. Considering the distribution in the litter components analyzed, it was observed that there was a balance between lignicolous and foliicolous species and only *D. leucopodia* occupied both types of microhabitats. Although myxomycetes are predominantly bacterivorous, Fukasawa *et al.* (2018) suggest that fungi that decompose wood may be an important part of the diet of lignicolous species and that their foraging associations would not be specific; thus, it is possible that the presence of *D. leucopodia, F. cinerea, F. septica* and *H. serpula* var. *serpula* in the litter of pecan walnut is contributing to the balance of the mycobiota.

CONCLUSION

Knowledge about the distribution of myxomycetes in Brazilian biomes, particularly Pampa and Pantanal, is still precarious and one of the contributions of the present work was to add the record of *Diachea leucopodia*, *Fuligo cinerea* and *Physarum cinereum* to the Brazilian portion of the Pampa and of *F. cinerea* for Rio Grande do Sul, bringing to 110 and 61 the number of species with known occurrence for the state and biome, respectively.

In addition to contributing to the knowledge of the distribution of myxomycetes species in Brazil, the results obtained in the present work can be considered as a reference for future studies on the myxobiota associated with the pecan tree. New records can be obtained by directly investigating the occurrence of lignicolous, foliicolous and corticicolous myxomycetes in the field during all seasons and associating the cultivation technique in moist chambers mounted with the different components of the litter, aerial leaf litter and bark of the living tree.

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