BLACK MILDEW FUNGI IN POLYCULTURES FROM AGROECOLOGICAL TRANSITION AREAS

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Abstract: Five species of *Meliola* and one of *Prillieuxina* (black mildews) were recorded in agroecological transitional areas in polyculture crop (consortium of plants – COPs – and agroforestry systems – AFSs) located in Pernambuco State, Brazilian northeast. *Meliola anacardii* on *Anacardium occidentale*, *M. annonacearum* on *Annona montana*, *M. mangiferae* on *Mangifera indica*, *M. rhoina* on *Schinus terebinthifolius*, *M. trichostroma* on *Psidium guajava* and *Prillieuxina winteriana* on *A. muricata* and *A. montana*. These species differed for host relationship and taxonomic structures. Illustrations of *P. winteriana*, *Meliola* spp. and its epiparasites are provided.
Index terms: *Asterina winteriana*, *Meliolaceae,* Mycoparasitic Fungi, *Spiropes* sp., *Trichothyrium reptans*.

MÍLDIOS NEGROS EM POLICULTIVOS DE ÁREAS DE TRANSIÇÃO AGROECOLÓGICA

Resumo: Cinco espécies de *Meliola* e uma de *Prillieuxina* (Míldios negros) foram registradas em policultivos de áreas de transição agroecológica (consórcio de plantas – COPs – e sistemas agroflorestais – AFSs) localizados no estado de Pernambuco, nordeste do Brasil. *Meliola anacardii* em *Anacardium occidentale*, *M. annonacearum* em *Annona montana*, *M. mangiferae* em *Mangifera indica*, *M. rhoina* em *Schinus terebinthifolius*, *M. trichostroma* em *Psidium guajava* e *Prillieuxina winteriana* em *A. muricata* e *A. montana*. Estas espécies diferiram pelo hospedeiro a quem estão relacionadas e estruturas taxonômicas. Ilustrações de *P. winteriana*, *Meliola* spp. e seus epiparasitas são apresentadas.
Termos para indexação: *Asterina winteriana*, Fungos micoparasitas, *Meliolaceae, Spiropes* sp., *Trichothyrium reptans*.

INTRODUCTION

Meliola Fries (*Meliolales*, *Sordariomycetes*) and *Prillieuxina* Arnaud (*Asterinales*, *Dothideomycetes*) include parasitc biotrophic species with a distinct distribution pattern in tropical and subtropical forests (Du *et al.* 2012). Due to

the black coloring which results of the strong melanization of their colonies, these fungi are commonly called black mildew (Gautam 2015; Macedo *et al.* 2010). *Meliola*, with the genus *Amazonia* Theiss., *Appendiculella* Höhn., *Asteridiella* McAlpine and *Irenopsis* F. Stevens belong *Meliolaceae* (*Sordariomycetes*, *Ascomycota*), and *Meliola* is the genus with the highest number of species has about 3000 species (Hansford 1961; Wijayawardene *et al*. 2018). *Prillieuxina winteriana* (Pazschke) G. Arnaud belongs to *Asterinaceae* (*Dothideomycetes*, *Ascomycota*), having *Annona* species as its main hosts (Santos *et al*. 2018). Despite being biotrophic, *P. winteriana* can act as a necrotrophic, causing the host killing it upon or shortly after infection and acquiring nutrients from dead or dying tissues (Giraldo and Valent, 2013, Caliman *et al.* 2021). These fungi are mandatory biotrophics and must to interact with living plant cells for growth and reproduction, without causing the kill of its host. They are usually host specific or have a very narrow host range (Pinho *et al.* 2012). It cannot be cultured in artificial media and their identification is based on morphological characteristics and, when possible, amplification of genes regions (Pinho *et al.* 2012, Zeng *et al*. 2020).

Agroecological polycultures are diversified systems of agricultural production, organized in a standard of area and time that care about sustainable management, adapting to the conditions of farmer's interests (Hecht 2018). The consortium of plants (COPs) and agroforestry systems (AFSs) are examples of agroecological crop polycultures. The differences between them are in the composition of plants. The AFS has annual crops and/or pastures and woody trees and the COP has annual crops and/or pastures (Alves *et al.* 2021).

In an agroecological transition process, some organisms can occur as agricultural pests and pathogens in their hosts. However, the plant diversification and sustainable management of the system favor the control population of these pathogens and pests (Alves *et al.* 2021). Through plant diversification, agroecological polycultures systems incorporate ecological processes with regulation of pests and pathogenic populations and nutrient cycling that give them greater resistance and resilience (Schroth and do Socorro 2014). The known of fungi on agroecological polycultures systems is important to assess the effects of the sustainable management under populations phytopathological importance. Here, we reported five species of *Meliola* and their epiparasites and *P. winteriana* and also, based on morphologic taxonomy and compared with specific literature, two species of *Meliola* like newly recorded from the country and Brazilian northeast (*M. annonacearum* and *M. rhoina*),. We provide illustrations and notes on the hosts and microscopic characteristics for *Meliola* spp. (Colonies, hyphae, appressoria, conidiogenous cells, mycelial setae, ascome and ascospores), *P. winteriana* and *T. reptans* (Colonies, ascome and ascospores) and *Spiropes* sp. (Colonies, conidiophore and conidium) and we provide a map of distribution of *M. annonaceaurm* and *M. rhoina*.

MATERIALS AND METHODS

Our study was conduce at the Chico Mendes III settlement (ACM III) in an AFS (7°57'28.9"S, 35°06'26.5"W) and a COP (7°57'30.0"S, 35°06'28.6"W) in municipality of Paudalho and in an AFS (7°58'51.7"S, 35°05'34.4"W) and in a COP (7°58'10.9"S, 35°05'31.5"W) in municipality of São Lourenço da Mata, located in Pernambuco State, Brazilian northeast.

We collected samples of leaves of arboreal plants with signals of fungal infection from August 2014 to October 2015 at the four properties. The leaves were packaged in plastic bags and transported to the laboratory. For to observe structures of taxonomic value fungal structures were analyzed under a stereomicroscope *Olympus SZ2 – ILST* and an optical microscope *Nikon Eclipse Ni* equipped with photo camera *Nikon Digital Sight DSFi2*, with slides colored with Amann blue or Melzer reagents (Alves et al. 2021). The designation of structures, concepts, and nomenclature were based on specific literature (Batista 1964, 1967; Hansford 1961; Hofmann 2011), *Index Fungorum* (http://www.indexfungorum.org/) and *GBIF* (http://www.gbif.org/). The leaves were dried and reserved frozen for seven days, and the specimens were deposited at the Herbarium Padre Camille Torrend (URM). Some black mildew fungi were also identified based according to the extraction protocol DNA of Pinho et al. (2012). The electrospherograms were analyzed using the software Sequencer 4.7 (Gene Codes, Ann Arbor, Michigan, USA), from which the consensus nucleotide sequences were obtained and exported as a FASTA file. Using these sequences, BLAST searches were conducted in the National Center for Biotechnology Information (NCBI) database, in order to determine the more similar sequences and species complexes. The sequences generated were deposited in the NCBI (Table 1).

RESULTS

We identified *Meliola anacardii* Zimm. (Figure 1A, Figure 2ABC) on leaves of *Anacardium occidentale* L. (cashew tree), *M. annonacearum* Stev. (Figure 1B, Figure 2DEF) on leaves of *Annona montana* Macfad (araticum tree), *M. mangiferae* Earle (Figure 1C, Figure 2GHI) on leaves of *Mangifera indica* L. (mango tree), *M. rhoina* Doidge (Figure 1D, Figure 2JKL) on leaves of *Schinus terebinthifolius* Raddi (brazilian pepper-tree) and *M. trichostroma* (Kunze) Toro (Figure 1E, Figure 2MNO) on leaves of *Psidium guajava* L. (guava tree), *P. winteriana* (Figure 1F, Figure 2PQ) on leaves of *A. muricata* L. (soursop tree) and leaves of araticum tree, *Trichothyrium reptans* (Berk. & M.A.Curtis) S. Hughes (Figure 1G, Figure 2RS) on *M. annonacearum* Stev. and *Spiropes* sp. Ciferri (Figure 1H, Figure 2TU) on *M. mangiferae* Earle. We obtained DNA sequences of *M. anacardii*, *M. mangiferae, M. trichostroma* and *P. winteriana* according DNA extraction protocol of Pinho et al. (2012) and these sequences were deposited in NCBI (Table 1).

Figure 1 - Colonies (yellow arrow) of *Meliola anacardii* Zimm URM 90240 (A), *Meliola annonacearum* Stev. URM 90238 (B), *Meliola mangiferae* Earle URM 90236 (C), *Meliola rhoina* Doidge URM 90234 (D), *Meliola trichostroma* (Kunze) Toro URM 90235 (E), *Prillieuxina winteriana* G. Arnaud URM 90233 (F), *Trichothyrium reptans* (Berk. & M.A. Curtis) S. Hughes (G) and *Spiropes* sp. Ciferri URM 90237 (H) on agroecological transition areas at Paudalho and São Lourenço da Mata, Pernambuco.

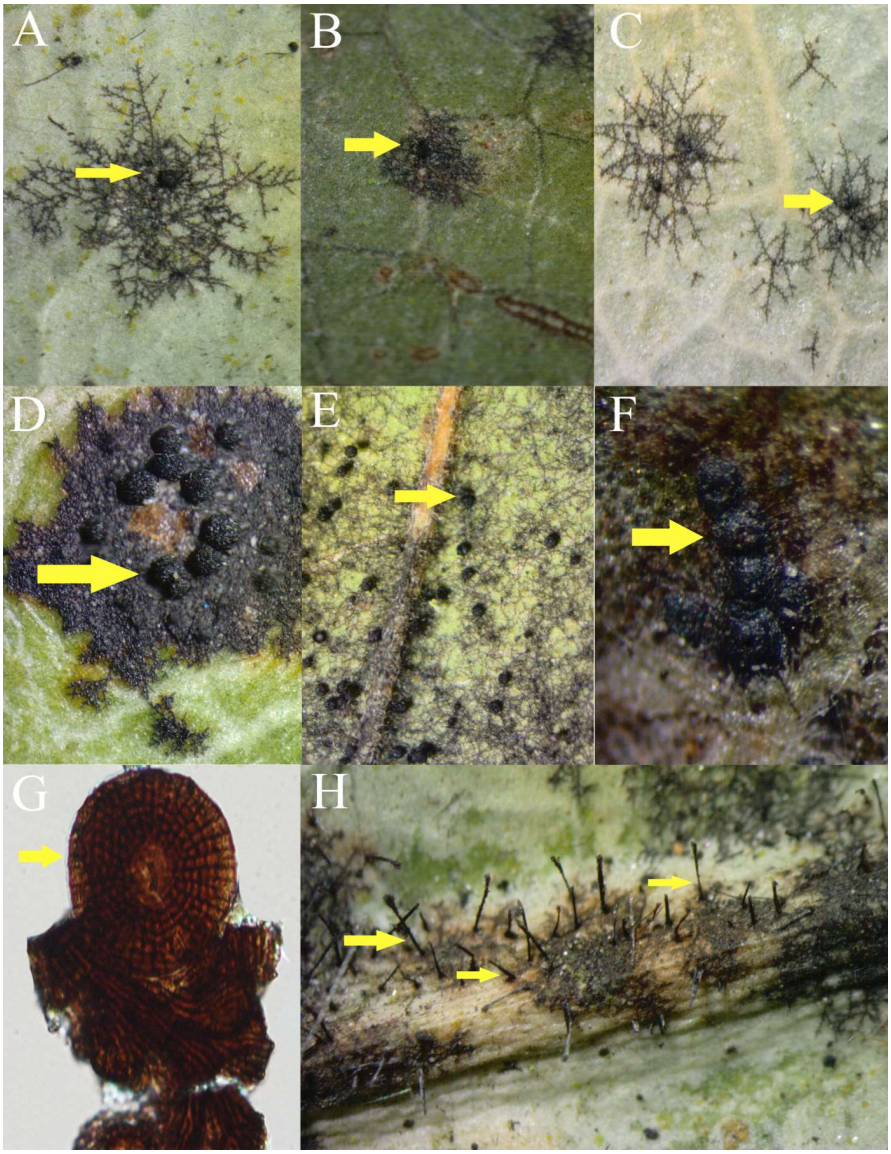


Figure 2 - Microstructures from *Meliola anacardii* Zimm URM 90240 (A. Mycelial setae with apex attenuated - yellow arrow; B. Apressoria - red arrow - alternate or opposite; C. Ascospore brown, oblong, with 4 strong septation constrict, extremities obtuse, 50 × 20 µm), *Meliola annonacearum* Stev. URM 90238 (D. Mycelial setae with apex obtuse - yellow arrow; E. Hyphopodia phialidic - yellow arrow - and apressoria - red arrow; F. Ascospore brown, oblong, with 4 strong septation constrict, extremities obtuse, 32 × 20 µm), *Meliola mangiferae* Earle URM 90236 (G. Mycelial setae with apex attenuated - yellow arrow; H. Hyphopodia cells - yellow arrow; I. Ascospore brown, oblong, with 4 strong septation constrict, extremities obtuse, 52 × 25 µm), *Meliola rhoina* Doidge URM 90234 (J. Mycelial setae with apex acute - yellow arrow; K. Hyphopodia cells - yellow arrow - and setae - red arrow; L. Ascospore brown, oblong, with 4 strong septation constrict, extremities obtuse, 40 × 18 µm), *Meliola trichostroma* (Kunze) Toro URM 90235 (M. Mycelial setae with apex acute - yellow arrow; N. Apressoria - yellow arrow; O. Ascospore light brown, subellipsoid, with 4 strong septation constrict, extremities cuneate, 45 × 17 µm), *Prillieuxina winteriana* G. Arnaud URM 90233 (P. Pycnothyriothecia mature with dehiscence stellar irregular - yellow arrow - and fringed edges with regular cells - red arrow. **C.** Pycnothyriothecia imature - yellow arrow; Q. Conidium 2-celled, clavate to ellipsoidal, with truncate hilum on lower cell, brown when mature, 40 × 20 µm), *Trichothyrium reptans* (Berk. & M.A.Curtis) S. Hughes (R. Tirothecium - yellow arrow - and hyphae - red arrow - under hyphae of *M. annonacearum*; S. Ascospores matures, clavates, extremities obtuse, 18 × 6 µm) and *Spiropes* sp. Ciferri URM 90237 (T. Conidiophore sinematous with apical dilatation - yellow arrow - until 3 mm; U. Conidium fusoid, 3-septation, base cell truncate and apex cell mucronate, smooth walled, light brown, 28 × 5 µm) on agroecological transition areas at Paudalho and São Lourenço da Mata, Pernambuco.

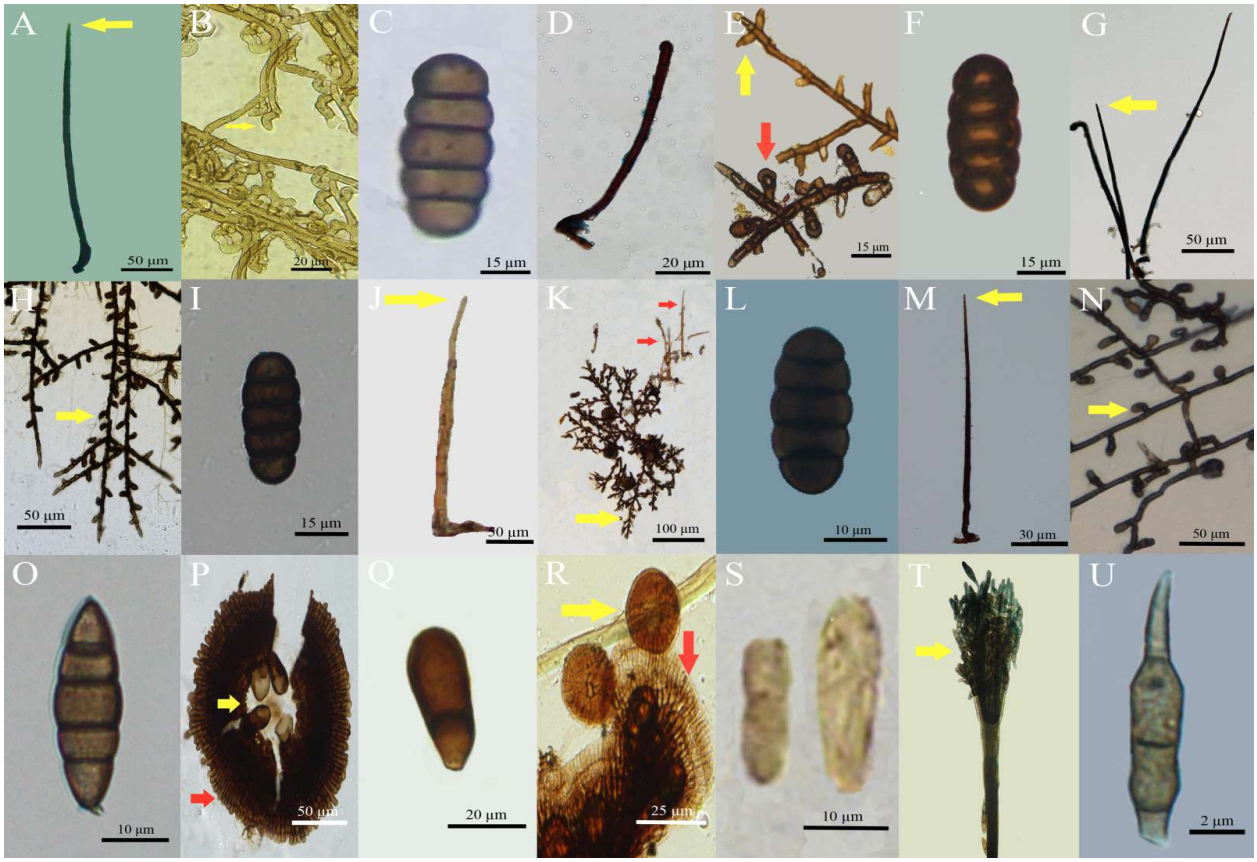


Table 1 - Black mildews *Meliola* spp. (*Meliolales, Sordariomycetes*) and *Prillieuxina winteriana* (Pazschke) G. Arnaud (*Asterinales, Dothideomycetes*) on leaves of host plants in

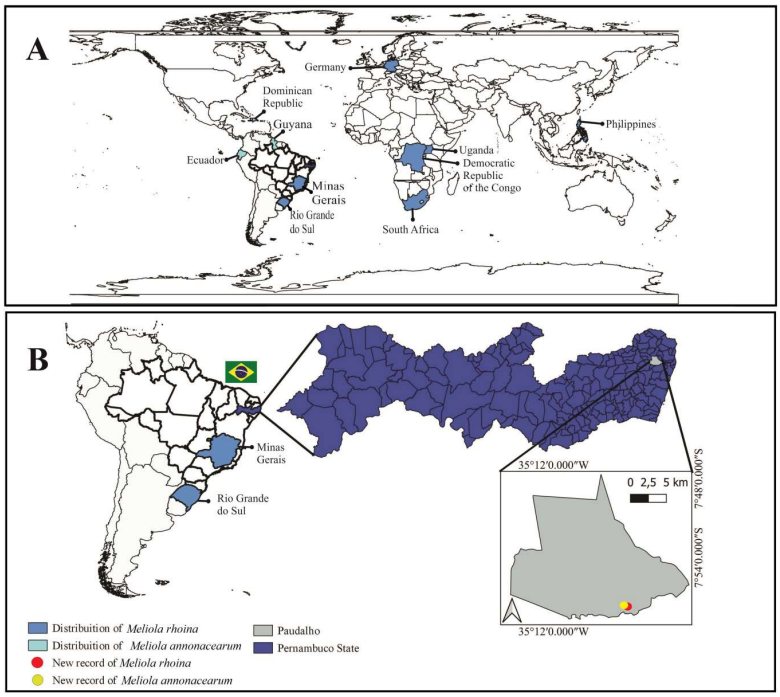
agroecological transition areas (PCOP - consortium of plants at Paudalho; PAFS - agroforestry systems at Paudalho; SCOP - consortium of plants at São Lourenço da Mata; SAFS - agroforestry systems at São Lourenço da Mata) on Chico Mendes III settlement.

Black mildew	Code URM	Access number NCBI	Host plants	Agroecological transition areas
Meliola anacardii	URM 90240	OP221711	*Anacardium occidentale* L. (cashew tree)	PCOP; PAFS; SCOP; SAFS
Meliola annonacearum	URM 90238	-	*Annona montana* Macfad (araticum tree)	PAFS
Meliola mangiferae	URM 90236	OP221712	*Mangifera indica* L. (mango tree)	PCOP; PAFS; SCOP; SAFS
Meliola rhoina	URM 90234	-	*Schinus terebinthifolius* Raddi (brazilian pepper-tree)	PCOP
Meliola trichostroma	URM 90235	OP221713	*Psidium guajava* L. (guava tree)	PCOP; PAFS; SAFS
Prillieuxina winteriana	URM 90233	OP221709	araticum tree	PAFS; SAFS
Prillieuxina winteriana	URM 90232	OP221710	*Annona muricata* L. (soursop tree)	PAFS; SAFS

- = Ausence of information

We consider two species of *Meliola* as new records: *M. annonacearum* for Brazil and *M. rhoina* for Brazilian northeast (Figure 3) and we provided the description of the characteristics of these specimens.

Figure 3 - Distribution of *Meliola annonacearum* and *Meliola rhoina* in world. A. Countries with register of *M. annonacearum* (blue) and *M. rhoina* (light blue). B. Brazilian states with distribution of *M. rhoina* (blue) and the Pernambuco state (dark blue) with the first register of *M. annonacearum* for Brazil and the first register of *M. rhoina* for Brazilian northeast.



Meliola annonacearum Stev., Ann. Mycol. 26: 245 (1928) (Figure 1B, Figure 2DEF)
D e s c r i p t i o n . – *Colonies* brown to black, gregarious on adaxial faces on the leaves, subdense, 1–4 mm diameter. *Hyphae* dark brown, septate, sinuous, with irregular branches forming obtuse angles in relation to the its main axis, slight interlaced to reticulate. *Hyphal cells* 15–25 × 5–7 µm. *Appressoria* alternate or irregularly dispersed, straight to slightly curved, with color and wall thickness similar to the hyphae, 16–22 µm of length; *basal cells* cylindrical, 2–7 µm long; *apical cells* subglobose to obpyriform, with straight apex, 11–15 × 9–13 µm. *Conidiogenous cells* phialidic, light brown to dark brown, occasionally mixed with apressoria, alternate, conical to ampuliform, 12–16 × 6–7 µm. *Mycelial setae* dark brown, numerous, straight to slightly curved, with obtuse apex, up to 220 µm long, 6–9 µm wide at the base. *Perithecia* brown to black, globose, scattered, 130 (–150) µm in diameter. *Ascospores* hyaline when young, becoming dark brown with age, oblong to subellipsoid, smooth-walled, with obtuse ends, 4-septate, strongly constricted at each septum, 32–38 (–40) × 14–16 (–18) µm.
MATERIAL EXAMINED: BRAZIL. **Pernambuco**. col. A. L. Alves. Chico Mendes III settlement, Paudalho, on leaves of *Annona montana* Macfad, 21.IX.2016 (URM 90238).
DISTRIBUITION: Brazil and Mexico.

Meliola rhoina Doidge, Bothalia 2: 454 (1928) (Figure 1D, Figure 2JKL)
D e s c r i p t i o n . – *Colonies* black, gregarious on adaxial face of the leave, velvety, subdense, 3 mm in diameter. *Hyphae* brown to dark brown, septate, slightly sinuous, with alternate or irregular branches forming obtuse angles in relation to its main axis. *Hyphal cells* 17–30 × 7–8 µm. *Appressoria* alternate, slightly antrorse, straight to slightly curved, often formed behind the distal septum of the progenitor cell, with color and wall thickness similar

to hyphae, 18–23 µm long; *basal cells* cylindrical to conical, 3–7 µm long; apical cells ovoid to cylindrical, often hemispherical to sublobate, with apex slightly curved, 10–18 × 6–12 µm. *Conidiogenous cells* phialidic, brown to dark brown, alternate or opposite with apressoria, ampuliform, 15–22 × 6–9 µm. *Mycelial setae* light brown to dark brown, scarce, straight, with acute apex, up to 400 µm long, 7–9 µm of base width. *Perithecia* brown to black, globose, verrucose, 240 µm diameter. *Ascospores* hyaline when young, becoming brown with age, oblong to ellipsoid, smooth-walled, with obtuse ends, 4-septate, slightly constricted at each septum, 33–45 (–48) × 14–18 (–20) µm.
MATERIAL EXAMINED: BRAZIL. **Pernambuco**. col. A. L. Alves. Chico Mendes III settlement, Paudalho, on leaves of *Schinus terebinthifolius* Raddi, 19.IV.2016 (URM 90234).
DISTRIBUITION: Brazil, Germany, South Africa and Uganda.

DISCUSSION

We contributed with the first deposits of DNA sequences of the species *Meliola anacardii*, *M. mangiferae*, *M. trichostroma, Prillieuxina winteriana* in NCBI (Table 1). According to comparisons of our sequences of *M. anacardii* (OP221711), *M. mangiferae* (OP221712), *M. trichostroma* (OP221713), and *Prillieuxina winteriana* (OP221709 and OP221710) against those in the NCBI, the greatest similarities were obtained with *M. brachyodonta*, *Meliola* sp. DSM-2019a, *M. trichostroma,* and *Asterina phenacis,* respectively. The comparison between the DNA sequences allowed to maintain the names identified based on morphology.

For the species *Meliola annonacearum* and *M. rhoina* it was not possible to generate DNA sequences due to a difficulty widely recognized among taxonomists working with obligate biotrophic fungi compared to fungal groups capable of growth in culture medium and cultivation under laboratory conditions. However, considering that these are new occurrences and that studies based on morphology are still usual for the fungal group studied (Example: new species of *Meliola* described based only on morphology by Santos et al. 2021; Gokul and Thomas 2022). In addition, taxonomic keys for identification that include specificity of these fungi to their hosts are still essential in species determination (Zeng *et al*. 2020) and can be useful for studies of taxonomy, biogeography and ecology.

Meliola annonacearum was reported by Stevens (1922) on *Dimorphandra* sp. in Guyana (Demerara-Essequibo) (GBIF) and on *Annona* sp. in Ecuador. Ciferri (1954) also reported *M. annonacearum* on *Oxandra lanceolata* from San Domingo. The morphological characteristics of *M. annonacearum* URM 90238 have close similarity to the original description by Stevens (Hansford 1961), although a few variations in size of perithecia (150 µm) and spores have been observed (40 × 18 µm). We observed that the phialidic conidiogenous cells were not mixed with apressoria and in the description this characteristic was not noted for the authors of species. However, to the best of our knowledge, these differences are not considered enough to propose a new variety. *Meliola rhoina* was reported in 1906 and 1963 in south Brazil (Rio Grande do Sul) and in southeast Brazil, respectively, in 2002 in Minas Gerais (GBIF). *Meliola rhoina* URM 90234 have close similarity to the description by Hansford (1961). A few variations in width and height of spores were observed (48 × 20 µm). We realized the comparison of characters only by observation of the

morphology of these fungi and their respectives hosts.

In this research we identified *M. annonacearum* epiparasited by *T. reptans* (URM 15625) from September 2014 to May 2015, and *Spiropes* sp. on *M. mangiferae* in all collections. *Trichothyrium reptans* has epiparasited other fungi species obtained from different plants hosts (Farr 1985; Wu *et al.* 2014). It has ramificated hifae of smooth walled with continuous growth under hyphae, appressoria, and haustoria of *Meliola* spp. Strains of *T. reptans* exhibit discoidal thiriothecia colored with black and brown, bright and formed by angular cells; clavate, hyaline and uniseptate ascospores (Hughes 1953). *Spiropes* sp. Ciferri has fusoid conidia, symmetrical, smooth walled, colored of brown with three septa, with poorly constriction, truncated base and mucronate apex, formed in black synnema, cylindrical, with apical dilatation, fasciculate, formed by anastomoses hyphae (Dubey and Rai 2020). The presence of these hiperparasitic fungi in plants from agroecological transition areas reinforces the importance of crop diversification, which, like native areas, occur ecological process of populational control of pathogens and plants disease (Meilhac *et al.* 2019). These fungi also were recorded as epi-parasites at native forests in different Brazilian biomes, such as Cerrado (Brazilian Savanna), Atlantic rainforest, and Amazon rainforest (GBIF), which have a dense canopy.

Prillieuxina winteriana is an important target for biological control, due the defoliation caused in soursop trees in monoculture in Bahia, Brazilian northeast (Caliman *et al.* 2021). However, in species of *Annona* recorded between August 2014 and October 2015, we did not observe this defoliation process. The crops under frequent prunes, with organic matter replacement on soil and the crop diversification could have inhibited the pathogenicity of *P. winteriana*. The plant diversification favors coexistence between epi-parasitic fungi and their hosts without decreasing both host populations.

We report the first occurrence of *Meliola* spp. and *Prillieuxina winteriana* in agroecological transition areas and we provided the first sequences in NCBI for *M. anacardii*, *M. mangiferae*, *M. trichostroma,* and *P. winteriana*, the first report of *M. rhoina* in Brazilian northeast and *M. annonacearum* in South America and the epiparasites *T. reptans* and *Spiropes* sp. on *M. annonacearum* and *M. mangifera*, respectivelly. This research also reinforces the importance of crop diversification like an ecosystem with population balance among hosts and parasites.

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