Agrotrópica 33(3): 173 - 180. 2021.
Centro de Pesquisas do Cacau, Ilhéus, Bahia, Brasil

NEW SPECIES OF *Meliola* AND *Irenopsis* (ASCOMYCOTA: SORDARIOMYCETES) FOUND IN BAHIA, BRAZIL

Jérsica dos Santos Silva, Leonardo de Oliveira Barbosa, Ana Cristina Fermino Soares, José Luiz Bezerra

Center of Agricultural, Environmental and Biological Sciences, Federal University of Reconcavo of Bahia, 44380-000 Cruz das Almas, Bahia, Brazil. jersicaagro@live.com; barbosleonard@gmail.com; ferminosoares@gmail.com; zelulabezerra@yahoo.com.br

Meliola Fr. and *Irenopsis* F. Stevens are fungal genera classified as Fungi, Dikarya, Ascomycota, Pezizomycotina, Sordariomycetes, Meliomycetideae, Meliolales and they are agents of black mildews on plants. *Meliola* is the largest genus within the group and *Irenopsis* is the fourth largest. Representatives of these fungi were collected on leaves of *Croton urucurana* Baill., *Clitoria fairchildiana* R.A. Howard and *Myrsine* sp. in the following Bahian municipalities: Santa Teresinha, Cruz das Almas and Barra da Estiva, respectively. Permanent and semi- permanent microscopic preparations were used for the morphological characterization of the three fungi. As their characteristics did not fit any described species, the following new species: *Meliola crotonifolia*, *M. myrsines* and *Irenopsis cruzalmensis* are proposed to designate them.

Key words: Ascomycetes, Meliolales, black mildews, tropical plant parasitic fungi

Novas espécies de *Meliola* e *Irenopsis* (Ascomycota: Sordariomycetes) encontradas na Bahia, Brasil. *Meliola* Fr. e *Irenopsis* F. Stevens são gêneros de fungos classificados como Fungi, Dikarya, Ascomycota, Pezizomycotina, Sordariomycetes, Meliomycetideae, Meliolales e são agentes do míldio preto nas plantas. *Meliola* é o maior gênero dentro do grupo e *Irenopsis* é o quarto maior. Representantes desses fungos foram coletados em folhas de *Croton urucurana* Baill., *Clitoria fairchildiana* R.A. Howard e *Myrsine* sp. nos seguintes municípios baianos: Santa Teresinha, Cruz das Almas e Barra da Estiva, respectivamente. Preparações microscópicas permanentes e semipermanentes foram utilizadas na caracterização morfológica dos três fungos, que não se enquadraram em nenhuma das espécies descritas. Como suas características não correspondem a nenhuma espécie descrita, as seguintes novas espécies: *Meliola crotonifolia*, *M. myrsines* e *Irenopsis cruzalmensis* são propostas para designá-las.

Palavras-chave: Ascomicetes, Meliolales, míldios pretos, fungos parasitas de plantas tropicais

Received for publication on November 05, 2021. Accepted on December 03, 2021.
DOI: 10.21757/0103-3816.2021v33n3p173-180

Introduction

The order Meliolales (Ascomycota: Sordariomycetes) comprises biotrophic foliicolous fungi, common in tropical and subtropical regions. Because of their dark colonies they have the common name of black mildews. Although they occur mainly on leaves, they may be found occasionally on petioles, green twigs and fruits (Assis et al., 2010; Carvalho et al., 2015). Leaves and branches infected by Meliola species rarely dry out and become completely dead, with brownish spots usually being observed. Hansford, (1961) reports that perhaps the most parasitic member of the entire group is *Meliola plumbaginis* in East Africa, which can cause the death of whole leaves and branches of heavily attacked individual host plants. The Meliolales are characterized by superficial colonies formed by hyphae with hyphopodia from which haustoria are sent into the host cells. The term appressorium has been used for capitate hyphopodia but it is not adequate (Hongsanan et al., 2015). The term appressorium should be used when dealing with fixation structures formed in germ tubes of fungal spores, as occurs in *Colletotrichum*. Spermogonia (phialides) flask shaped are present in the same mycelium and they were known in the past as 'mucronate hyphopodia' (Saenz and Taylor, 1999; Thomas, Alex & Thomas, 2013; Justavino, Kirshner & Piepenbring, 2015). These structures form "conidia-like" endogenous cells incapable to germinate or infect the host (Hongsanan et al., 2015) and propably act as spermatia during spermatization.

The genus *Meliola* Fr. is the most important of the order Meliolales and has more than 3000 described species, while the genus *Irenopsis* F. Stevens is the fourth largest with 204 species (Kirk et al., 2008; INDEX FUNGORUM, 2021). These genera are morphologically similar, however *Meliola* has sterile setae in the mycelium and in *Irenopsis* the setae are in the perithecium (Hansford, 1961; Hosagoudar and Archana, 2009).

The taxonomy of Meliolales is based mainly on morphology because they are biotrophic fungi and cannot be cultured (Soares and Dianese, 2013; Maharachchikumbura, Hyde & Jones, 2015; Hongsanan et al., 2016). DNA can be extracted from fruiting bodies scraped directly from leaves, but this method requires clean, fresh material not always available (Zeng et al., 2018). For this reason, there are few sequences for the species of Meliolaceae (Maharachchikumbura, Hyde & Jones, 2015). Currently at the National Center for Biotechnology Information (NCBI) there are only 35 sequences *Meliola* and 7 of *Irenopsis*. It is widely accepted that Meliolales spp. are host specific, but Hongsanan et al. (2015) reported *Meliola thailandicum* occurring on host plants of two different families. Whether all species of Meliolales are host specific needs more verification using molecular techniques (Maharachchikumbura, Hyde & Jones, 2015). Presently, new species of Meliolales are being described based on the combination of morphology, host association and where possible phylogeny (Soares and Dianese, 2013; Zeng et al., 2018; Zeng et al., 2020; Barbosa et al., 2021). In this paper two new species of *Meliola* and one of *Irenopsis* are described parasitizing leaves of *Croton urucurana* Baill. (Euphorbiaceae), *Clitoria fairchildiana* R.A.Howard (Fabaceae) e *Myrsine* sp. (Myrsinaceae).

Materials and Methods

Leaves of *Croton urucurana, Clitoria fairchildiana* R.A. and *Myrsine* sp. with signs of black mildews were collected in the municipalities of Santa Teresinha, Cruz das Almas e Barra da Estiva, state of Bahia, Brazil. The materials were sent to the Microbiology Laboratory of the Federal University of Recôncavo da Bahia (UFRB) for microscopic analysis and drying at 45 °C for 72 hours. Exsicates were deposited at UFRB Herbarium (HURB).

Microscope slides were prepared direct from the fungal colonies using dissecting needles to remove ascomata from the colonies. Intact mycelium was examined by dropping transparent nail polish on colony edges and drying for 24 h to form a pelicle. Ascomata and mycelium pellicles were mounted in polyvinyl-alcohol lactoglycerol (PVLG) (Mueller, Bils & Foster, 2004). Morphological analysis and measurements were done in a LEICA ICC50 HD microscope with the LAS Version 4.5.0 program. For each species, the Beeli formula was calculated

acording to Hansford (1961). This formula represents the morphometric characteristics of each species. Identifications were performed based on morphology and host association according to specific literature.

Results and Discussion

Three new species were identified, two of them belonging to the genus *Meliola* and one to *Irenopsis*. The results are discussed after each description.

Species descriptions

***Irenopsis cruzalmensis* J. S. Silva & J. L. Bezerra sp. nov. (Figure 1).**

Etymology: Referring the collection site (Cruz das Almas - Bahia-Brazil; HURB 14769)

Beeli formula: 3301- 3220

MycoBank: MB842015

Colonies superficial, epiphyllous, 3-4 mm diam., dark brown to black, round, subdense, velvety, scattered. External mycelium dark brown. Hypha substraight to flexuous, septate, brown, oppositely branched in acute angles, cells 22.5–37.5 × 6.25–7.5 µm. Appressoria capitate, brown, alternate or unilateral, straight to subantrose, 13.75–20 × 10–12.5 µm, abundant; stalk cell cylindrical to cuneate 3.75–7.5 × 6.25–7.5 µm; head cell globose, entire, 12.5–17.5 × 10–12.5 µm. Spermogonia (phialides) opposite or unilateral, mixed with appressoria, ampuliform 15–20 × 7.5–10 µm. Perithecia scattered, slightly verrucose, setose, brown, 70–135 µm diam.; walls of angular texture, formed by irregular cells, 5–17.5 µm diam.; perithecial setae, 3–9 per perithecium, thick walled, slighty curved, non-septate, 50–102 µm long, 7.5–10 µm diam. in the median part. Asci evanescent, bisporic. Ascospores oblong, 4-septate, brown, constricted at the septa, 37.5–40 × 15–20 µm.

Specimens examined - Brazil, Bahia: Cruz das Almas, on leaves of *Clitoria fairchildiana* (Fabaceae), 24. VIII. 2015, J.S. Silva & J.L. Bezerra (HURB 14769)

Comments: *Irenopsis cruzalmensis* differs from *Irenopsis chamaecristicola* by its larger perithecia (up to 170 µm diam.), straight, septate and smaller perithecial setae (up to 80 µm long); and from *Irenopsis toruloidea* by its larger perithecia (up to 170 µm), septate, smaller perithecial setae (up to 80 µm) and smaller ascospores (29–35 × 14–16 µm) (Hansford, 1961; Stevens, 1927). This new taxon is supported by morphometric differences from *Irenopsis* species recorded on Fabaceae. *Clitoria fairchildiana* is also a new host for *Irenopsis*. No black mildew was reported on this plant so far.

***Meliola crotonifolia* J.S. Silva & J.L. Bezerra, sp. nov. (Figure 2).**

Etymology: referring to the host plant (*Croton*)

Beeli formula: 3121- 5233

MycoBank: MB842018

Colonies superficial, hypophyllous, 4–15 mm in diam, dark brown to black, opaque, effuse, isolated, confluent, sparse, velvety. External mycelium dark brown, consisting of hyphae flexuous, septate, dark brown, with opposite or irregular branching at acute angles, cells 22.5–32.5 × 5–7.5 µm. Appressoria capitate, plentiful, dark brown, alternate or unilateral, rarely opposite, straight to antrorse, 11.25–15 × 7.5–10 µm; stak cell 2.5–5 × 5–7.5; head cell globose, entire, 7.5–10µm in diam. Spermogonia (phialides) opposite, escarce, mixed with appressoria, ampuliform, 15–20 × 5–12.5 µm. Setae simple mycelium, brown, septate, straight to curved, scattered or clustered around the perithecium, 350–740 µm in length and 7.5–10 µm in width in the median part. Perithecia, globose, glabrous, dispersed, dark brown, 150–250 µm diam.; walls of angular texture, formed by irregular cells, 7.5–25 µm diam. Ascospores oblong, 4-septate, brown, constricted at the septa, 50–57.5 × 15–22.5 µm.

Specimens examined - Brazil, Bahia: Santa Teresinha, on leaves of *Croton urucurana* (Euphorbiaceae), 04. I. 2011, J. T. Souza (HURB 14771).

Comments: Many *Meliola* spp. are reported on *Croton* spp., such as: *M. anfracta* on *Croton* sp., *C. angustatus,* and *C. linearis ; M. brevidentata* on *C. curranii*; *M. crotonis-macrostachydi* on *C. macrostachyus*; *M. jamaicensis* on *Croton* sp.; *M. janeirensis* on *Croton.* sp.*;* *M. longispora* on *Croton* sp..*;* and *M. micropoda* on *C. sylvaticus.* However,

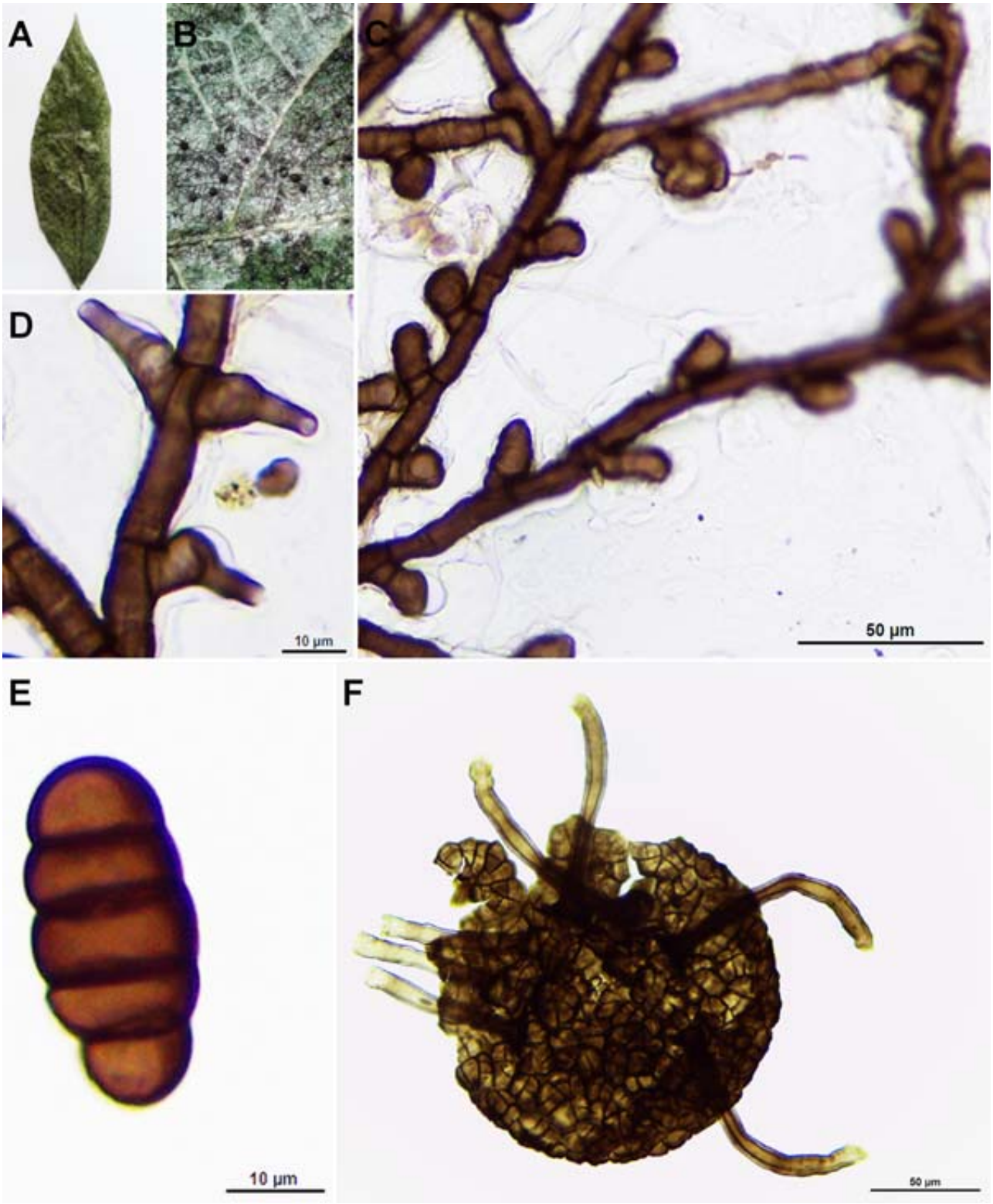

Figure 1. **A-F:** *Irenopsis cruzalmensis* on leaves *Clitoria fairchildiana.* **A.** Epiphyllous Colonies. **B.** Colony detail. **C.** Capitate hyphopodia. **D.** Spermogonia (phialides) **E.** Ascospores. **F.** Setose perithecium.

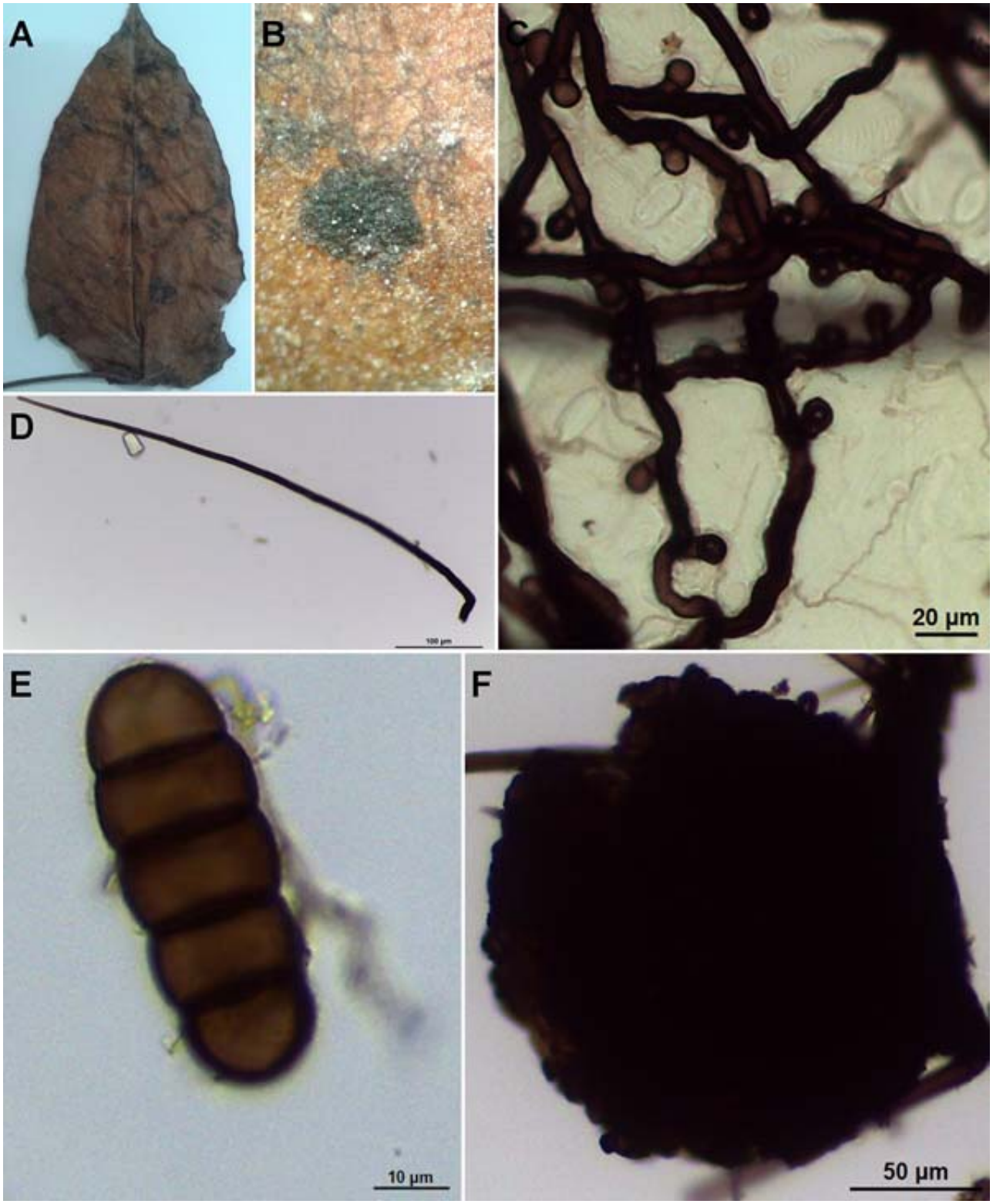

Figure 2. **A-F:** *Meliola crotonifolia* in leaves *Croton urucurana*. **A.** Epiphyllous **c**olonies. **B.** Colony detail. **C.** Capitate hyphopodia. **D.** Mycelial setae. **E.** Ascospore. **F.** Perithecia.

M. anfracta presents shorter setae (up to 280 µm), smaller perithecia (up to 150 µm), and shorter ascospores (33–39 µm long). *Meliola brevidentata,* differs by its smaller, alternate or opposite apressoria, shorter dentate setae (up to 300 µm), smaller perithecia (up to 190 µm), and smaller ascospores (30–38 × 12–15 µm). *Meliola crotonis-macrostachydi* presents shorter setae (up to 400 µm) subacute or dentate, perithecia smaller (up to 160 µm), and smaller ascospores (34–39 × 13–15 µm). *Meliola jamaicensis* has longer acute or dentate setae (up to 900 µm) and broader ascospores (23–27 µm). *Meliola janeirensis* has shorter dentate setae (up to 300 µm), smaller perithecia (up to 170 µm), and smaller ascospores (39–46 × 14–17 µm). *M. longispora* possesses short setae (up to 320 µm), small perithecia (up to 150 µm), and smaller ascospores (37–43 × 15–17 µm). *Meliola micropoda,* differs by its opposite and alternate apressoria, larger dentate setae (up to 900 µm), and smaller ascospores (34–38 × 11–15 µm). *Meliola glochidiicola* is the closest species of all, but presents shorter setae (up to 435 µm), and ascospores slighty smaller (46–52 × 14–19 µm) (Ciferri, 1938; Hansford, 1961; Stevens, 1928). The remaining species of *Meliola* recorded on Euphorbiaceae show morpho-dimensional features quite different from *M. crotonifolia.* All mentioned species possess Beeli formula discrepant in one or more numbers.

***Meliola myrsines* J. S. Silva & J. L. Bezerra, sp. nov. Figure 3.**

Etymology: referring to the host (*Myrsine*)

Beeli formula: 3131- 5221

MycoBank: MB842017

Colonies superficial, epiphyllous occasionally hypophyllous, 1.5–10 mm in diam, dark brown to black, opaque, circular for effuse, isolated or confluent, velvety. External mycelium dark brown, consisting of hyphae straight to flexuous, septate, brown, opposite lybranched in acute angles cells, 17.5–32.5 × 5–7.5 µm. Appressoria capitate, brown, alternate or unilateral, straight to subantrose, 22.5–27,5 × 10–15 µm; stak cell cylindric, 6.25–10 × 7.5–10 µm; head cell globose to obovoid, entire, 12.5–17,5 × 10–15 µm. Spermogonia (phialides) opposite or unilateral, mixed with appressoria, ampuliform, 15–25 × 7.5–10 µm. Setae mycelium dark brown, septate, straight to curved, scattered or clustered around the perithecium, 210–300 µm in length and 10–12.5 µm in width in the median part, apex simple or shortly branched, 2-7 denticulate, branches 11.25–17.5 × 5–10 µm, denticles 2.5–15 µm of lenght. Perithecia globose, glabrous, dispersed, brown for black, 100–230 µm diam.; walls of angular texture, formed by irregular cells, 5–20 µm diam. Ascospores oblong, 4-septate, brown, smooth, constricted at the septa, 50–60 × 17.5–22.5 µm.

Specimens examined - Brazil, Bahia: Barra da Estiva, on leaves of *Myrsine* sp. (Myrsinaceae), 11. I. 2011, J. T. Souza (HURB 14768)

Comments: Some species of *Meliola* on Myrsinaceae show similar morphology such as: *Meliola delicatula, M. armata, M. myrsinacearum,* and *M. transvaalensis*. *Meliola delicatula* was described on *Myrsine* sp., but it possesses smaller setae (up to 220 µm) not denticulate and smaller ascospores (45–50 × 11–12 µm). *M. armata* has mycelial setae shorter (up to 220 µm), acute, smaller perithecia (up to 180 µm), and shorter ascospores (45–53 × 20–22 µm). *M. myrsinacearum* occurs on *Ardisia guadelupensis* e *Myrsine africana*, but its setae are longer (up to 700 µm) not denticulate and the ascospores are slightly smaller (46–52 × 17–19 µm). Finaly, *M. transvaalensis* displays mycelial setae longer (up to 650 µm) not denticulate, larger perithecia (up to 290 µm) and smaller ascospores (44–50 × 16–19 µm) (Hansford, 1961; Stevens, 1916). Based on these differences and by adopting present criteria to identify *Meliola* we are dealing with a new species of this genus.

Conclusion

Beeli formula calculated from morphometric chacteristics of *Meliola* species combined with host association is adequate to identify taxa of this genus.

Acknowledgements

This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - (CAPES).

Figure 3. **A-G:** *Meliola myrsines* in leaves *Myrsine* sp. **A.** Epiphyllous colonies. **B.** Capitate hyphopodia. **C.** Colony detail. **D.** Spermogonia (phialides). **E.** Mycelial **s**eta. **F.** Ascospore. **G.** Perithecium.

Literature Cited

ASSIS, L. A. G. de. et al. 2010. Parasitas fúngicos em espécies florestais nativas da Amazônia Central. Agrotrópica 22(3):137-144.

BARBOSA, L. de O. et al. 2021. A new species of X*ylaria* on *Salzmannia nitida* in Bahia, Brazil. Agrotrópica (Brasil) 33(2):127-130.

CARVALHO, N. B. de. et al. 2015. Ascomicetos foliícolar coletados aleatoriamente no dossel de árvores da mata atlântica no sul da Bahia. Agrotrópica (Brasil) 27(3):239-248.

CIFERRI, R. 1938. Mycoflora domingensis exciccata. Annales Mycologici 36(2-3):1938.

HANSFORD, C. G. 1961. The Meliolineae. A monograph. Beihefte zur Sydowia 2.806p.

HONGSANAN, S. et al. 2016. The evolution of fungal epiphytes. Mycosphere 7 (11):1690-1712, DOI: 10.5943/mycosphere/7/11/6.

HONGSANAN, S. et al. 2015. Meliolales. Fungal Diversity 74(1):91–141. DOI: https://doi.org/10.1007/s13225-015-0344-7.

HOSAGOUDAR, V. B.; ARCHANA, G. R. 2009. Host range of Meliolaceous fungi in India. Journal of Threatened Taxa 1(5):269-282. Doi: https://doi.org/10.11609/JoTT.o2061.269-82.

INDEX FUNGORUM, 2021. Available: http://www.indexfungorum.org/Names/Names.asp. Accessed: Out 13, 2021.

KIRK, P. M.; CANNON, P. F.; DAVID, J. C.; STALPERS, J. A. 2008. (eds.) Dictionary of the Fungi.10th edition. Wallingford: CABI Publishing. 784p.

JUSTAVINO, D. R.; KIRSHNER, R.; PIEPENBRING, M. 2015. New species and new records of Meliolaceae from Panama. Fungal Diversity 70(1):73-84. DOI: https://doi.org/10.1007/s13225-014-0292-7.

MAHARACHCHIKUMBURA, S. S. N.; HYDE, K. D.; JONES, E. B. G. 2015. Towards a natural classification and backbone tree for *Sordariomycetes*. Fungal Diversity 72:199–301. DOI: https://doi.org/10.1007/s13225-015-0331-z.

MUELLER, G. M.; BILS, G. F.; FOSTER, M. S. 2004. Biodiversity of Fungi – Inventory and monitoring methods. Burlington, San Diego and London: Elsevier Academic Press. 777p.

SAENZ, G. S.; TAYLOR, J. W. 1999. Phylogenetic relationships of Meliola and Meliolina inferred from nuclear small subunit rRNA sequences. Mycological Research 103(8):1049-1056. DOI: 10.1017/S0953756298008211.

SOARES, W. R. O.; DIANESE, J. C. 2013. New Meliola species on fabaceous hosts from the Brazilian Cerrado. Mycological Progress 13(2):321-331.

STEVENS, F. L. 1916. The Genus Meliola in Porto Rico. Illinois Biological Monographs 2(4):1-104.

STEVENS, F. L. 1927. The Meliolineae I. Annales Mycologici 25(5-6):405-469.

STEVENS, F. L. 1928. The Meliolineae II. Annales Mycologici 26(3-4):165-383.

THOMAS. J.; ALEX, T. E.; THOMAS, R. J. 2013. *Meliola marthomaensis* sp. nov., an addition to Meliolaceae from Western Ghat Region in Kerala State, India. Universal Journal of Plant Science 1(3):100-103. DOI: 10.13189/ujps.2013.010307.

ZENG, X. Y. et al. 2018. Simplified and efficient DNA extraction protocol for Meliolaceae specimens. Mycological Progress 17(3):403–415. DOI: https://doi.org/10.1007/s11557-017-1361-6.

ZENG, X. et al. 2020. Unravelling evolutionary relationships between epifoliar Meliolaceae and angiosperms. Journal of Systematics and Evolution. Doi:10.1111/jse.12643.

●