Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 17 (5): 1243–1248
https://doi.org/10.15560/17.5.1243

PENSOFT

First occurrence of *Nigrospora lacticolonia* Mei Wang & L. Cai (Xylariales, Ascomycota) in the Neotropical Region

Deyse Viana dos Santos[1], Rejane Maria Ferreira da Silva[1], Paula Aguilera[2], Gladstone Alves da Silva[1], José Luiz Bezerra[3], Rafael José Vilela de Oliveira[1]

1 Federal University of Pernambuco, Graduate Program in Biology of Fungi (Department of Mycology), Av. Professor Nelson Chaves s/n, 50670-420, Recife, PE, Brazil • DVS: deysevianas25@gmail.com https://orcid.org/0000-0003-1303-5360 • RMFS: re.biologicas@gmail.com • GAS: gladstonesilva@yahoo.com • RJVO: rafaelvilela87@gmail.com https://orcid.org/0000-0002-8854-9449

2 Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Temuco, Chile • paulaaguilera08@gmail.com

3 Federal University of Recôncavo da Bahia, Rui Barbosa Street 710, 44.380-000, Cruz das Almas, BA, Brazil • jlulabezerra@hotmail.com

* Corresponding author Deyse Viana dos Santos,

Abstract

During a study on fungal endophyte diversity, *Nigrospora lacticolonia* Mei Wang & L. Cai was isolated from healthy leaves of *Guarea macrophylla*, a shade tree in the Cocoa agroecosystem (cabruca) in Brazil. We confirmed the identity of the specimens using morphological data and a phylogenetic reconstruction based on molecular markers (internal transcribed spacer region (ITS), β-tubulin (TUB2), and translation elongation factor 1-α (TEF) sequences). The specimen presented black globose or slightly ellipsoidal conidia, and the conidiophores were reduced to conidiogenous cells. This is the first report of *N. lacticolonia* in the Neotropical Region.

Keywords

Atlantic Forest, Cocoa crop, endophytic fungi, *Guarea macrophylla*, Sordariomycetes

Academic editor: Renan Barbosa | Received 4 May 2021 | Accepted 31 Jul 2021 | Published 9 September 2021

Citation: Santos DV, Silva RMF, Aguilera P, Silva GA, Bezerra JL, Oliveira RJV (2021) First occurrence of *Nigrospora lacticolonia* Mei Wang & L. Cai (Xylariales, Ascomycota) in the Neotropical Region. Check List 17 (4): 1243–1248. https://doi.org/10.15560/17.4.1243

Introduction

Nigrospora was described by Zimmermann (1902) to accommodate *N. panici* Zimm. The genus belongs to the family Apiosporaceae, and it comprises 35 species according MycoBank (data from 2021). *Nigrospora* is characterized as presenting micronematous or semi-macronematous conidiophores, which are branched, flexuous, hyaline to brown, smooth, and usually reduced to conidiogenous cells; conidiogenous cells are monoblastic, dolliform, ampulliform, subcylindrical to clavate, and hyaline. Conidia are solitary, spherical or broadly ellipsoidal or pyriform, compressed dorsiventrally, black, shiny, smooth, and aseptate (Rathod et al. 2014; Donayre and Dalisay 2016; Wang et al. 2017; Raza et al. 2019).

Nigrospora species have been found to be pathogenic (Dutta et al. 2014a; Kwon et al. 2016; Kee et al. 2019), saprobic (Brown et al. 1998), and endophytic (Pawle and Singh 2014; Thanabalasingam et al. 2015). Endophytic fungi live inside plant tissues and do not harm their host (Azevedo and Araújo 2007); they produce secondary metabolites that promote plant growth (Khan et al. 2015), protect against disease (Dutta et al. 2014b), and

provide tolerance to ambient stresses (Jia et al. 2016). Endophytic species of *Nigrospora* have been reported in several hosts, such as *Cocos nucifera* (Oliveira et al. 2021), *Emblica officinalis* (Rathod et al. 2014), and *Artemisia* spp. (Cosoveanu 2016).

Wang et al. (2017) described *Nigrospora lacticolonia* for the first time in leaves of *Camellia sinensis* and *Musa paradisiaca* in China. It has been reported as a pathogenic fungus causing reddish brown spots on the stem of dragon fruit in Malaysia (Kee et al. 2019), and it is associated with leaf spots of sugarcane in China (Raza et al. 2019). Recently, *N. lacticolonia* has been newly reported as a leaf pathogen in the date palm in Oman, causing dark brown to black spots (Al-Nadabi et al. 2020). Our study represents the first report of *N. lacticolonia* in the Neotropical Region, where it was found living as an endophyte in healthy leaves of *Guarea macrophylla*, which is planted as a shade tree in the agroecosystems of Cocoa (*Theobroma cacao* L.) cultivation in Brazil.

Methods

Isolation and purification. *Nigrospora lacticolonia* (Table 1) was obtained from healthy leaves of *G. macrophylla* in a Cocoa agroecosystem (14°47′42″S, 039°10′ 20″W), Bahia, Brazil (Fig. 1). Following the methodology proposed by Araújo et al. (2002), the leaves were washed in running water and neutral detergent and later fragmented into leaf discs (6 mm in diameter) and subjected to surface disinfection with 70% alcohol (1 min), 3% sodium hypochlorite (NaOCl) (2 min and 30 s), again with 70% alcohol (30 s), and then washed with sterile distilled water. The discs were transferred to Petri dishes containing Malto-Dextrose Agar (MEA) plus chloramphenicol (50 mg/L) and incubated at room temperature (28 ± 2 °C).

Morphological study. The endophytic fungus was cultivated on potato dextrose agar (PDA) (Gams et al. 1998) and synthetic nutrient-poor agar (SNA) (Nirenberg 1976) for six days (28 ± 2 °C), and microscopic characteristics

Table 1. Isolate numbers, host, locality, lifestyle, and GenBank accession numbers of *Nigrospora lacticolonia* samples analysed.

Isolate number	Host	Locality	Lifestyle	GenBank accession numbers		
				ITS	TUB2	TEF1-α
LC 3324 = CGMCC3.18123	*Camellia sinensis*	China	—	KX985978	KY019458	KY019291
URM 8360	*Guarea macrophylla*	Brazil	Endophytic	MW838186	MW901250	MW886097
PC KS6B1 A R2	*Hylocereus polyrhizus*	Malaysia	Pathogenic	MK408578	MK408562	MK408567
LC 7009	*Musa paradisiaca*	China	—	KX986087	KY019594	KY019454
SQUCC 2269	*Phoenix dactylifera*	Oman	Pathogenic	MN173587	MN205976	MN205981
LC 12061	*Saccharum officinarum*	China	Pathogenic	MN215785	MN329949	MN264024

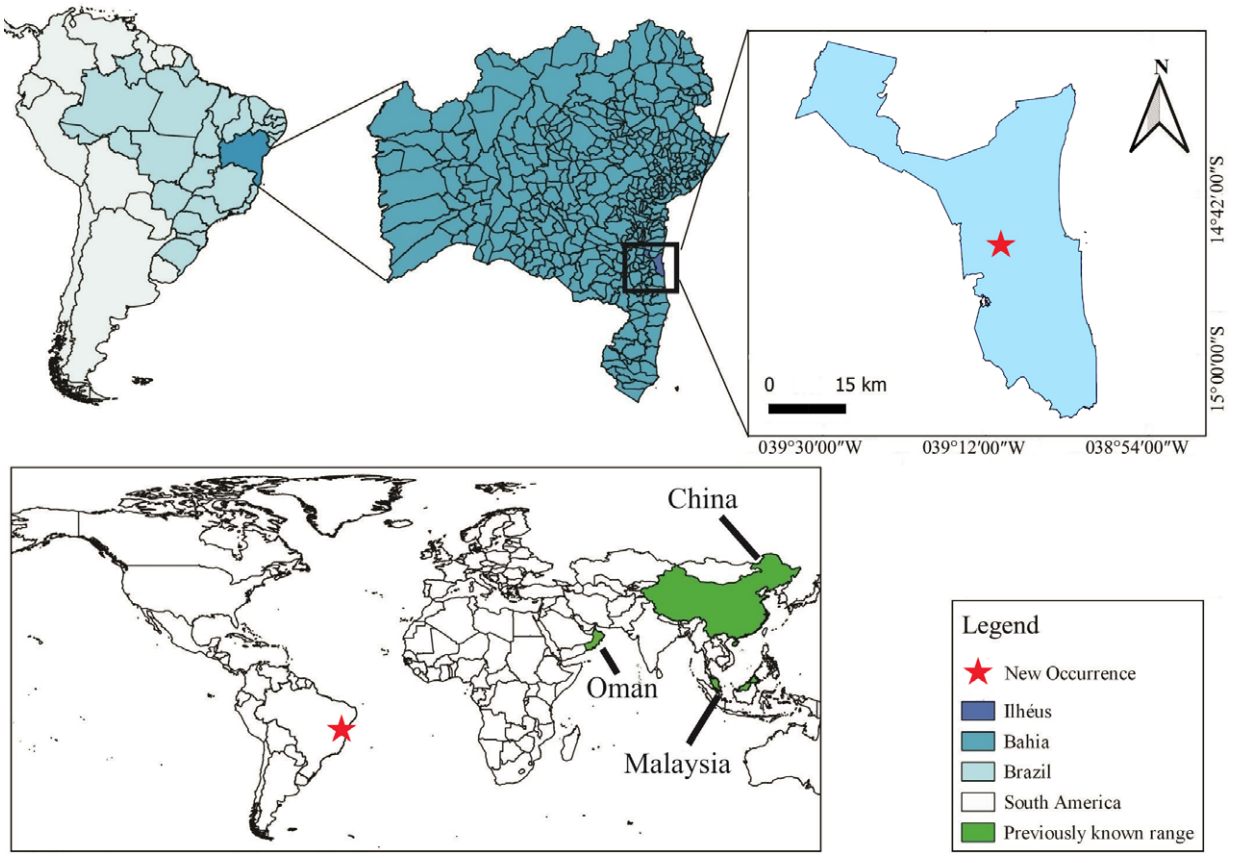

Figure 1. New occurrence of *Nigrospora lacticolonia* URM 8360 and previous distribution.

analysis was performed in SNA, to check the size of at least 20 conidia, 10 conidiogenous cells, and 10 hyphae. The isolate used in this study was maintained in the URM culture collection (Micoteca URM Profa. Maria Auxiliadora de Queiroz Cavalcanti) at the Universidade Federal de Pernambuco, Recife, Brazil.

Molecular analysis. For DNA extraction, fungal biomass was cultivated in a Petri dish containing PDA for seven days at 28 °C. The protocol used followed the methodology described by Oliveira et al. (2016). The primers ITS1/ITS4 (White et al. 1990) were used to amplify the rDNA ITS region using the parameters described by Oliveira et al. (2014). The primers Bt2a/Bt2b (Glass and Donaldson 1995), EF1-728F (Carbone and Kohn 1999) and EF-2 (Odonnell et al. 1998) were also used to amplify the TUB2 and TEF1-α genes, respectively. The sample was purified using NucleoSAP® (Cellco Biotec, São Carlos, SP, Brazil), according to the manufacturers' recommendations using the GeneAmp® PCR System 9700. Subsequently, the sample were sequenced using the same PCR primers sets by Multi-user DNA Sequencing Platform at the Biosciences Center, Universidade Federal de Pernambuco, Recife, Brazil. The consensus sequences were computed and visually inspected using the Staden software package (Staden et al. 1998).

Phylogenetic analysis. Phylogeny was reconstructed based on the combination of the rDNA ITS region, TUB2, and TEF1-α genes. The sequence of *N. lacticolonia* was aligned with others retrieved from GenBank using MEGA v. 5.05 (Tamura et al. 2007). Prior to phylogenetic analyses, the optimal model of nucleotide substitution (TRN + G) was estimated using Topali v. 2.5 (Milne et al. 2004). A maximum likelihood (1.000 bootstraps) analysis was performed using the PhyMl (Guindon and Gascuel 2003), launched from Topali v. 2.5 (Milne et al. 2004). *Arthrinium malaysianum* CBS 102053 was including as the out-group.

Results

Nigrospora lacticolonia **Mei Wang & L. Cai, 2017**; Persoonia 39: 131.
Figure 2A–E

Material examined. BRAZIL – Bahia • Ilhéus, Universidade Estadual de Santa Cruz; 14°47′42″S, 039°10′20″W; 05.VII.2019; Deyse Viana dos Santos leg.; as

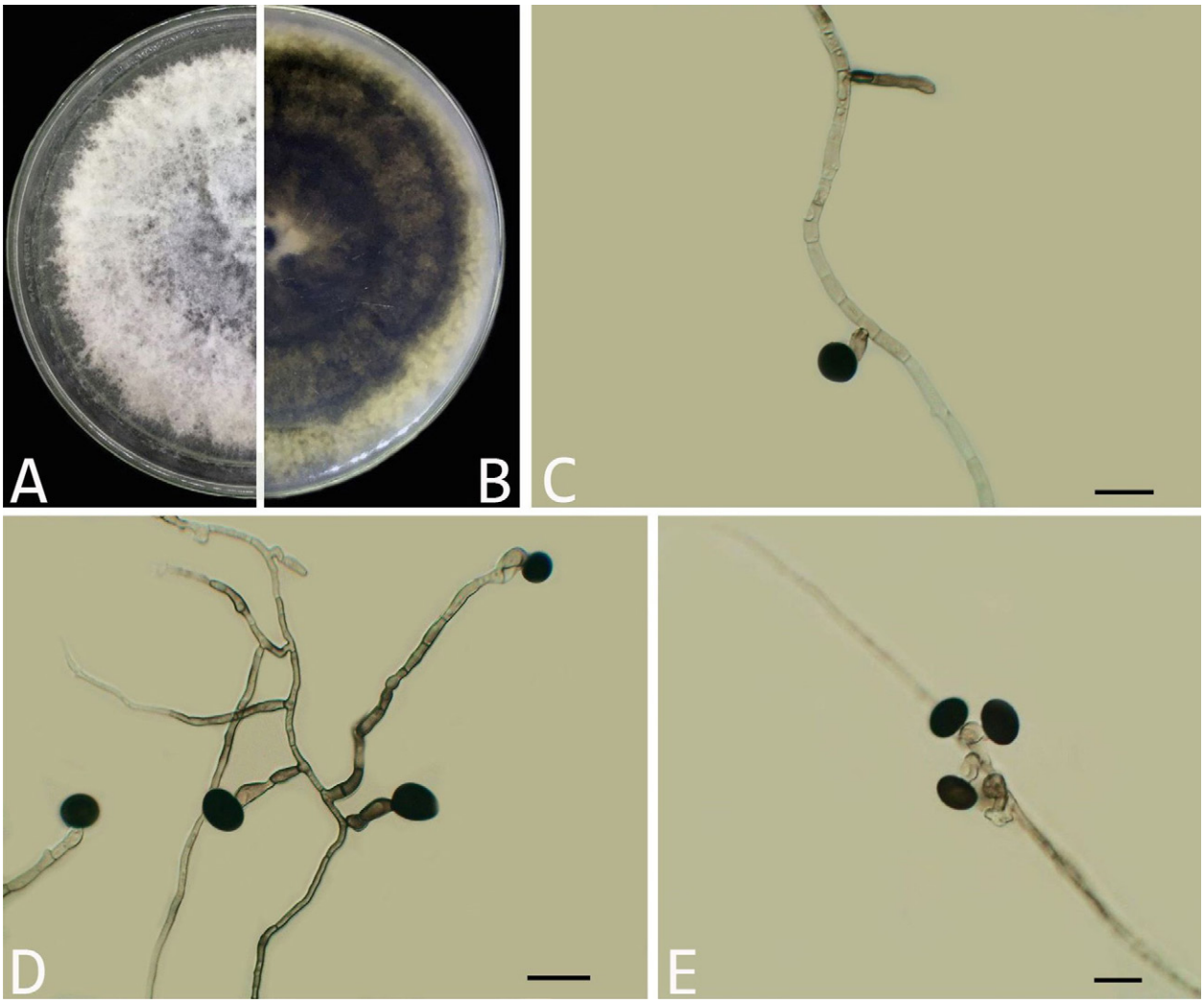

Figure 2. *Nigrospora lacticolonia*. **A, B.** Top and bottom view of the colony, six days after inoculation on PDA. **C, D, E.** Conidiogenous cells and conidia. Scale bars = 10 µm.

endophytic fungi in healthy leaves of *Guarea macrophylla*; URM 8360.

Identification. After 6 days at 28 ± 2 °C on SNA, hyphae smooth, hyaline to light brown, branched, septate, 2–4 µm in diameter. Conidiophores reduced to conidiogenous cells. Conidiogenous cells aggregated in clusters on hyphae, pale brown, globose to clavate to dolliform, 7.5–12.5 × 5–10 µm. Conidia were black, shiny, smooth, aseptate, globose, or slightly ellipsoidal 12.5–15.0 × 10–15 µm.

Culture characteristics. On PDA, colonies floccose, surface white, reverse light brown, reaching 9 cm in diameter after 6 days at 28 ± 2 °C. On SNA, surface and reverse mycelium black, colonies flat. The phylogenetic tree (Fig. 3) shows that the URM 8360 sequence formed a clade together with *N. lacticolonia* sequences, including the holotype (CGMCC3.18123).

Discussion

Based on morphological characteristics and phylogenetic analysis, *N. lacticolonia* has been identified and is reported for the first time in the Neotropical Region from a Cocoa agroecosystem in the Atlantic Forest domain. Wang et al. (2017) confirmed *Nigrospora* as a monophyletic genus and described *N. lacticolonia* as present on the leaves of *Camellia sinensis* and *Musa paradisiaca* in China. The morphological characteristics of the specimen *N. lacticolonia* URM 8360 were similar to those described by Wang et al. (2017): hypha size (1.5–4.0 µm in diameter (LC 3324) vs. 2.0–4.0 µm in diameter), conidia size (13.5–17.5 × 10.5–13.5 µm (LC 3324) vs. 12.5–15.0 × 10.0–15.0 µm), and conidiogenous cell size (6.5–11.5 × 5.5–9.0 µm (LC 3324) vs. 7.5–12.5 × 5.0–10.0 µm). According to our BLASTn analyses, the ITS rDNA, TUB2, and TEF1-α sequences obtained from our

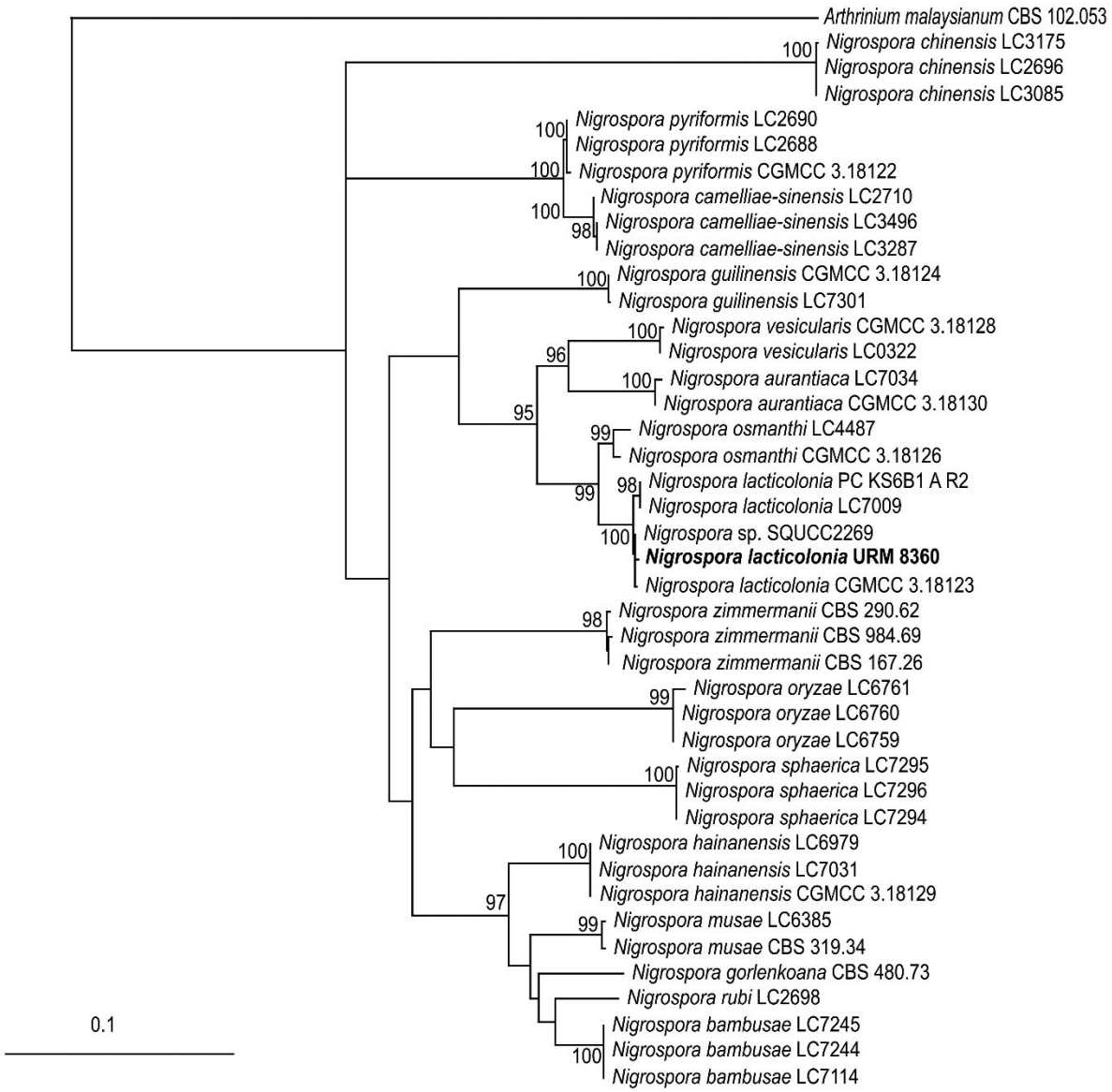

Figure 3. Phylogenetic tree of *Nigrospora lacticolonia* and related species, constructed using combined rDNA ITS region, TUB2, and TEF1-α genes. *Arthrinium malaysianum* CBS 102.053 was used as outgroup. Support values were obtained from maximum likelihood analysis. The sequence obtained in this study is annotated in bold.

specimens were 99.31%, 99.67%, and 100% identical to the KX986087 (LC 7009), K408564 (PC KS6B1 B R2), and KY019291 (CGMCC3.18123) sequences, respectively, of *N. lacticolonia* from GenBank.

There are reports of *N. lacticolonia* as a pathogenic fungus on leaves of Date Palm (*Phoenix dactylifera* L.) in Oman (Al-Nadabi et al. 2020), causing reddish brown spots on the stems of *Hylocereus polyrhizus* (Weber) Britton & Rose in Malaysia (Kee et al. 2019), and disease symptoms in leaves of sugarcane (*Saccharum officinarum* L.) in China (Raza et al. 2019). However, in this study, *N. lacticolonia* is reported as endophyte on *G. macrophylla* (family Meliaceae), a native tree of the Atlantic Forest (Flores 2020). It is used as a shade tree in the Cocoa crop system (Sambuichi 2003).

We report here the first record of *N. lacticolonia* in the Neotropics and as endophyte in a new host plant species. Our increases the knowledge of the geographic distribution and lifestyle of this species and emphasizes the importance of further studies on fungal endophytes in agroecosystems worldwide.

Acknowledgements

We thank the Universidade Federal de Pernambuco for using their facility, which was financed by the "Foliar endophytic mycobiota in shade trees of a Cocoa plantation (cabruca) in south Bahia, Brazil" project, CNPq process no. 435273/2018-3 (MCTIC/CNPq - Universal).

References

Azevedo JL, Araújo WL (2007) Diversity and applications of endophytic fungi isolated from tropical plants. In: Ganguli BN, Deshmukh SK (Eds.) Fungi: multifaceted microbes. Anamaya Publishers, Nova Delhi, India, 189–207.

Araújo WL, Lima AOS, Azevedo JL, Marcon J, Sobral JK, Lacava PT (2002) Manual: isolamento de microrganismos endofíticos. Centro Acadêmico Luiz de Queiroz, Piracicaba, Brasil, 86 pp.

Brown KB, Hyde D, Guest DJ (1998) Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. Fungal Diversity 1: 27–51.

Cosoveanu A (2016) Fungi as endophytes in Chinese *Artemisia* spp.: juxtaposed elements of phylogeny, diversity and bioactivity. Mycosphere 7 (2): 102–117.

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91 (3): 553–556. https://doi.org/10.2307/3761358

Donayre DK, Dalisay TU (2016) Identities, characteristics, and assemblages of dematiaceous-endophytic fungi isolated from tissues of barnyard grass weed. Philippine Journal of Science 145 (2): 37–42.

Dutta J, Gupta S, Thakur D, Handique PJ (2014a) First report of *Nigrospora* leaf blight on tea caused by *Nigrospora sphaerica* in India. Plant Disease 99 (3): 417. https://doi.org/10.1094/PDIS-05-14-0545-PDN

Dutta D, Puzari KC, Gogoi R, Dutta P (2014b) Endophytes: exploitation as a tool in plant protection. Brazilian Archives of Biology and Technology 57 (5): 621–629.

Flores TB (2020) Meliaceae in Flora do Brasil 2020. Jardim Botânico do Rio de Janeiro, Brasil. https://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB9997. Accessed on: 2021- 3-9.

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52 (5): 696–704. https://doi.org/10.1080/10635150390235520

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied Environmental Microbiology 61 (4): 1323–1330.

Gams W, Hoekstra ES, Aptroot A (1998) CBS course of mycology. Centraalbureau voor Schimmelcultures, Delft, the Netherlands, 165 pp.

Al-Nadabi H, Maharachchikumbura SSN, Al-Gahaffi ZS, Al-Hasani AS, Velazhahan R, Al-sadi AM (2020) Molecular identification of fungal pathogens associated with leaf spot disease of date palms (*Phoenix dactylifera*). All Life 13 (1): 587–597. https://doi.org/10.1080/26895293.2020.1835740

Jia M, Chen L, Xin H, Zheng C, Rahman K, Han T, Qin L (2016) A friendly relationship between endophytic fungi and medicinal plants: a systematic review. Frontiers in Microbiology 7: 906. https://doi.org/10.3389/fmicb.2016.00906

Kee YJ, Hafifi ABM, Huda-Shakirah AR, Wong KL, Jin XL, Nordahliawate MSS, Zakaria L, Mohd MH (2019) First report of reddish brown spot disease of red-fleshed dragon fruit (*Hylocereus polyrhizus*) caused by *Nigrospora lacticolonia* and *Nigrospora sphaerica* in Malaysia. Crop Protection 122: 165–170.

Kwak YB, Kwak YS (2016) First report of *Nigrospora* sp. causing kiwifruit postharvest black rot. New Zealand Journal of Crop and Horticultural Science 45 (1): 1–5.

Khan AR, Ullah I, Waqas M, Shahzad R, Hong SJ, Park GS, Jung BK, Lee IJ, Shin JH (2015) Plant growth-promoting potential of endophytic fungi isolated from *Solanum nigrum* leaves. World Journal Microbiology Biotechnology 31 (9): 1461–1466.

MycoBank Database (2021) https://www.mycobank.org/. Accessed on: 2020-4-8.

Milne I, Wright F, Rowe G, Marshall DF, Husmeier D, McGuire G (2004) TOPALi: software for automatic identification of recombinant sequences within DNA multiple alignments. Bioinformatics 20 (11): 1806–1807. https://doi.org/10.1093/bioinformatics/bth155

Nirenberg HI (1976) Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion Liseola. Mitteilungen Biologische Bundesanstalt fur Land-Forstw 169: 1–117.

Oliveira RJV, Sousa NMF, Neto WPP, Bezerra JL, Silva GA, Cavalcanti MAQ (2021) Seasonality affects the community of endophytic fungi in coconut (*Cocos nucifera*) crop leaves. Acta Botanica Brasilica 34 (4): 704–711. https://doi.org/10.1590/0102-33062020abb0106

Oliveira RJV, Lima TEF, Cunha IB, Coimbra VRM, Silva GA, Bezerra JL, Cavalcanti MAQ (2014) *Cornicullariella brasiliensis*, a new species of coelomycetes in the rhizosphere of *Caesalpinia echinata* (Fabaceae, Caesalpinioideae) in Brazil. Phytotaxa 178 (3): 197–204. https://doi.org/10.11646/phytotaxa.178.3

Oliveira RJV, Bezerra JL, Lima TEF, Silva GA, Cavalcanti MAQ (2016) *Phaeosphaeria nodulispora*, a new endophytic coelomycete isolated from tropical palm (*Cocos nucifera*) in Brazil. Nova Hedwigia 103 (12): 185–192. https://doi.org/10.1127/nova_hedwigia/2016/0343

Odonnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences 95 (5): 2044–2049. https://doi.org/10.1073/pnas.95.5.2044

Pawle G, Singh SK (2014) Antimicrobial, antioxidant activity and phytochemical analysis of an endophytic species of *Nigrospora* isolated from living fossil *Ginkgo biloba*. Current Research in Environmental & Applied Mycology 4 (1): 1–9.

Raza M, Zhang Z, Hyde KD, Diao Y, Cai L (2019) Culturable plant pathogenic fungi associated with sugarcane in southern China. Fungal Diversity 99 (1): 1–104.

Rathod S, Dar MA, Gade AK, Rai MK (2014) Griseofulvin produc-

ing endophytic *Nigrospora oryzae* from Indian *Emblica officinalis* Gaertn: a new report. Austin Journal Biotechnology Bioengineering 1 (3): 5.

Sambuichi RHR (2003) Ecologia da vegetação arbórea de cabruca, Mata Atlântica raleada utilizada para cultivo de cacau na região sul da Bahia. Tese de Doutorado, Universidade de Brasília, Brasília, Brasil, 108 pp.

Staden R, Beal KF, Bonfield JK (1998) The staden package. In: Misener S, Krawetz SA (Eds.) Bioinformatics methods and protocols. Humana Press, Totowa, EUA, 115–130.

Thanabalasingam D, Kumar NS, Jayasinghe L, Fujimoto Y (2015) Endophytic fungus *Nigrospora oryzae* from a medicinal plant *Coccinia grandis*, a high yielding new source of phenazine-1-carboxamide. Natural Product Communications 10: 1659–1660.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24 (8): 1596–1599. https://doi.org/10.1093/molbev/msm092

Wang M, Liu F, Crous PW, Cai L (2017) Phylogenetic reassessment of *Nigrospora*: ubiquitous endophytes, plant and human pathogens. Persoonia 39: 118–142.

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds.) PCR protocols: a guide to methods and applications. Academic Press, San Diego, USA, 315–322.