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NEW DATA OF *Meloidogyne arenaria* ON *Dioscorea cayenensis* IN BRAZIL

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ABSTRACT: Yam tubers with symptoms of root-knot disease were collected from a farm in the municipality of Goiana, State of Pernambuco, Brazil. *Meloidogyne* species were identified through morphological, biochemical, and molecular profiling based on the ITS and 28S ribosomal DNA (rDNA) regions. *Meloidogyne arenaria* was identified as the causal agent. This is the first report of *M. arenaria* in *D. cayenensis* in Pernambuco state, Brazil.

KEYWORDS: Molecular biology, Root-knot nematode, Yam, Identification

NOVOS DADOS DE *Meloidogyne arenaria* EM *Dioscorea cayenensis* NO BRASIL

RESUMO: Túberas de inhame com sintomas de meloidoginose foram coletadas em uma propriedade agrícola localizada no município de Goiana, PE, Brasil. Foi feito o processo de identificação de espécies de *Meloidogyne* por meio da caracterização morfológica, bioquímica e do perfil molecular com base nas regiões ITS e 28S do DNA ribossomal (rDNA). *Meloidogyne arenaria* foi identificado como agente causal. Este é o primeiro relato de *M. arenaria* em *D. cayenensis* no estado de Pernambuco, Brasil.

PALAVRAS-CHAVE: Biologia molecular, Nematoide das galhas, Inhame, Identificação

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The yam, *Dioscorea cayenensis* Lam., belongs to the Dioscoreaceae family and the genus *Dioscorea* L. It is grown mainly in tropical and subtropical regions worldwide, with the species *D. cayenensis* prevalent (Ngo-Ngwe et al., 2014). In Brazil, the Northeast region is the largest producer, consumer, and trader of yam, which plays an essential social, cultural, and economic role. The state of Pernambuco is among the highlights in production nationally (Brito et al., 2011).

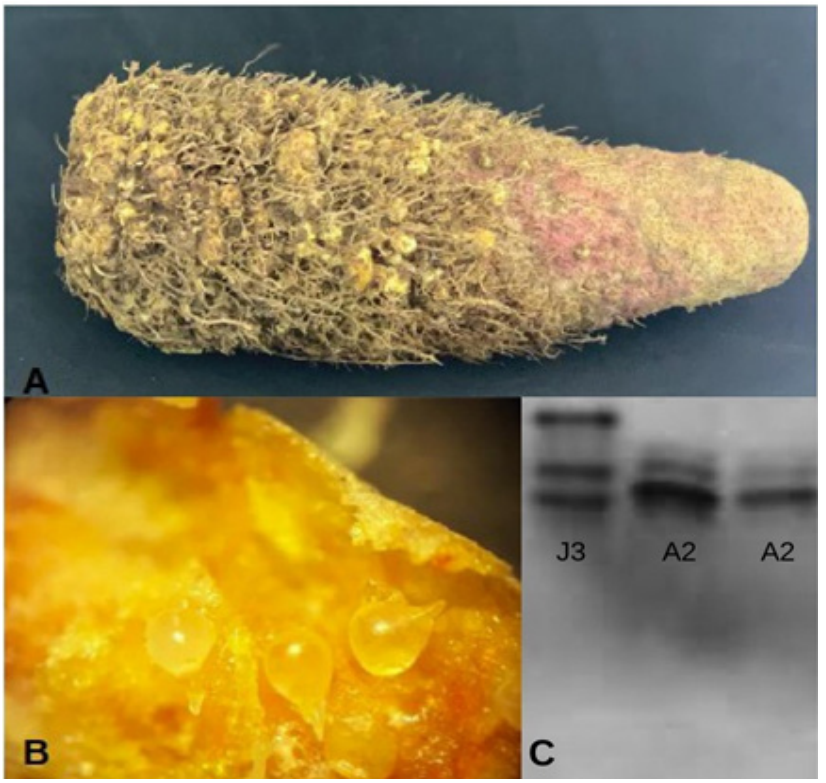
For yam culture, the occurrence of diseases becomes a limiting factor in production, primarily those caused by root-knot nematodes (*Meloidogyne* spp.) (Mudiope et al., 2012), root lesion nematodes (*Pratylenchus* spp.) (Assunção et al., 2023; Kolombia et al., 2021), and tuber dry rot nematode (*Scutellonema bradys* Steiner & LeHew) (Humphreys-Pereira et al., 2014). The genus *Meloidogyne* Goeldi occupies the first position in the world ranking of nematodes harmful to crops (Jones et al., 2013). Its significant importance in the production areas stems from its ability to cause substantial economic losses to producers, mainly because it parasitizes almost all agricultural plant species and has a wide geographical distribution (Moens et al., 2009).

Correctly diagnosing nematode species in production areas is an important tool for developing efficient management programs. Therefore, conventional methods based on morphometry and molecular analysis must be integrated.

In September 2019, samples of *D. cayenensis* tubers with symptoms of root-knot disease (Fig. 1A-B) were collected on a farm

located in the municipality of Goiana (7° 38' 28" S 34° 57' 16" W) in Pernambuco state, Brazil. The tubercules were processed for nematode extraction according to the method proposed by Coolen and D'Herde (1972). Perineal cuts were made using 20 females, according to Taylor and Netscher's methodology (1974). According to Alfenas et al. (1991), the esterase profile was created for biochemical characterization, using one female per sample with 20 repetitions (n = 20).

Figure 1. A. Yam tuber with symptoms of *Meloidogyne arenaria*; **B**. M. *arenaria* females viewed under a stereoscopic microscope; **C**. Esterase profiles of *M. arenaria* (A2) detected in *Dioscorea cayenensis* and reference isolate *M. javanica* (J3).



Molecular identification was performed through the amplification and sequencing of the D2-D3 regions of the 28S rDNA segment using the primers D2A (5´-ACAAGTACCGTGAGGGAAAGTTG-3´) and D3B (5´-TCGGAAGGAACCAGCTACTA-3´) (De Ley et al., 1999), and ITS using the primers VRAIN2F (5´-CTTTGTACACACCGCCCGTCGCT-3´) and VRAIN2R (5´-TTTCACTCGCCGTTACTAAGGGA ATC-3´) (Vrain et al., 1992). PCR amplifications were performed as described in Powers and Harris (1993). The PCR products were visualized on an agarose gel 2%, purified with the kit AxyPrep™ PCR Cleanup (Axygen®) and sequenced in both directions with the same primers used in the amplification. Sequences of ex-type isolates were used as a reference, obtained from GenBank (www.ncbi.nlm.nih.gov/), and multiple sequence alignments were performed with the MAFFT (Katoh and Toh, 2013) manually adjusted to allow maximum alignment and maximum similarity between sequences. The ambiguously aligned regions were excluded from the analysis. For each locus alignment, the Maximum Likelihood (ML) method was used to infer the phylogenetic relationships among species, using the RAxML-HCP2 v.8.2.8 (Stamatakis, 2014) implemented in CIPRES Portal v.2.0 (https://www.phylo.org/portal2/home.action) with 1,000 repetitions.

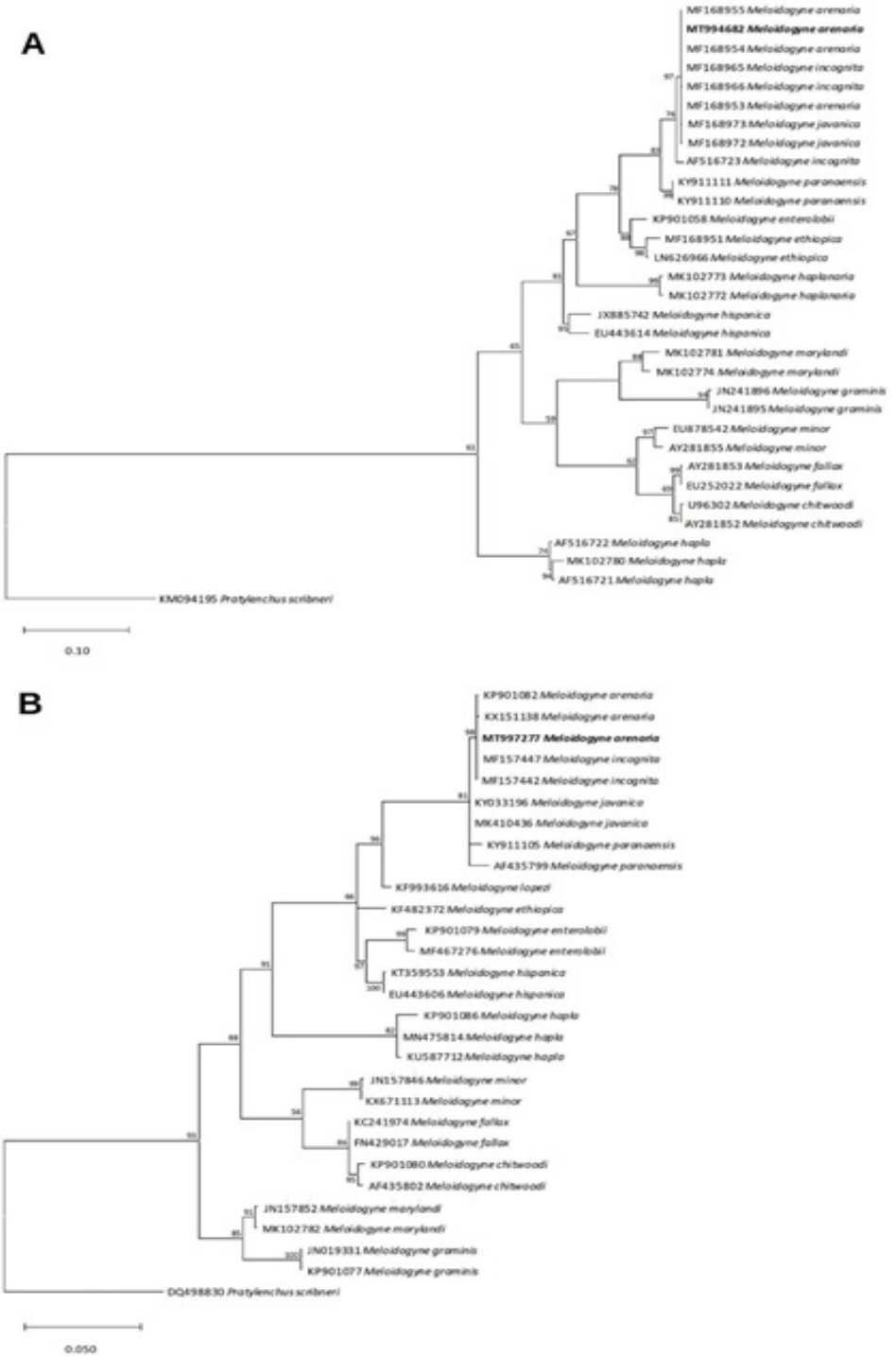
The perineal patterns of females from the *Meloidogyne* (PE07) population showed a low, flat dorsal arch with smooth and continuous streaks gently folded to the tip of the tail on the lateral line and a distinct, slightly irregular lateral field.

Measurements and ratios of females were as follows, expressed in microns as mean ± standard deviation, with the range in parentheses. The body length was 1305.0±401.5 (1000.3-1750.5) µm; the stylet measured 23.5±1.6 (22.0-24.1) µm in length; the dorsal esophageal gland orifice (DEGO) was 4.8±0.7 (3.7-5.1) µm. For

second-stage juveniles (J2), the body length was 452.3±32.2 (410.2-489.6) µm; stylet length, 10.2±0.5 (9.6–11.0) µm; DEGO equal to 4.7±0.7 (3.2-6.2) µm; tail length was 45.8±0.5 (44.9-46.1) µm; hyaline tail terminus measuring 10.1±0.3 (9.9-10.5) µm.

Electrophoresis of esterase bands revealed the A2 phenotype (Rm 1.20, 1.30) typical of *M. arenaria* (Fig. 1C) (Carneiro et al., 2008). The sequences of the studied rDNA regions were submitted to GenBank (ITS: MT994682 and D2-D3 28S: MT997277). Research at BLAST showed 98% (ITS) to 97% (D2-D3) identity with sequences of *M. arenaria* isolates from Brazil. Phylogenetic analysis using ML placed the *Meloidogyne* (PE07) population isolated from *D. cayenensis* in a clade with the *M. arenaria* sequences from GenBank (Fig. 2).

Figure 2. Phylogenetic relationship of *Meloidogyne* species based on sequence alignment of the (**A**) ITS rDNA region and (**B**) 28S rDNA D2-D3 expansion fragments. Maximum likelihood estimated in the phylogenetic tree. The scale bar indicates the expected number of substitutions per site.



To test Koch's postulates, suspensions of eggs and juveniles of *M. arenaria* (PE07) obtained from the yam production area (Goiana-PE) evaluated in this research were added to 8 L pots with *D. cayenensis* plants grown in pasteurized soil in a greenhouse at an average temperature of 25.5±1°C. Approximately 10,000 eggs and juveniles of *M. arenaria* were added into holes approximately 4 cm deep in the rhizosphere of each plant. Inoculated plants developed gall symptoms, but non-inoculated plants did not. The pathogen was subsequently identified.

This is the first record of *M. arenaria* in *D. cayenensis* in Pernambuco state, Brazil. Integrative taxonomy based on morphological, biochemical, and molecular methods provides the correct identification of the species, which is paramount. These nematodes cause significant losses in the productivity of several crops (Ferraz and Brown, 2016). Thus, with precise characterization, the recommended management measures are more effective.

The occurrence of *M. arenaria* in the state of Pernambuco was previously described in the municipality of Aliança by Moura and Freitas (1983), parasitizing *D. cayenensis* tubers in infested production areas; however, identification was performed only based on the basic morphology of the perineal pattern. Furthermore, *Meloidogyne* species were also reported by Leal and Ponte (1980) in yam tubers in the state of Ceará.

The presence of *M. arenaria* in *D. cayenensis* updates information about this nematode, which damages this crop. It indicates a new paradigm about which species are associated with root-knot disease in agricultural areas of Pernambuco.

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REFERENCES

Alfenas, A.C.; Peters, I.; Brune, W.; Passador, G.C. Eletroforese de proteínas e isoenzimas de fungos e essências florestais. Viçosa, MG, UFV, SIF, **1991**, 242p.

Assunção, M.C.; Souza Junior, F.J.C.; Santos Neto, J.C.; Mello, A.A.R., Silva, L.R.B.O.; Blawid, R.; Pedrosa, E.M.R.; Guimarães, L.M.P. *Pratylenchus zeae* in yam, *Dioscorea cayenensis* and *D. alata*, in Brazil. *Australasian Plant Disease Notes*, **2023**, 18, 1, 26.

Brito, T.T; Soares, L.S.; Furtado, M.C.; Castro, A.A.; Carnelossi, M.A.G. Composição centesimal de inhame (*Dioscorea* sp.) in natura e minimamente processado. *Scientia Plena*, **2011**, 7, 1-7.

Carneiro, R.M.D.G.; Almeida, M.R.; Mota, F.C.; Gomes, A.C.; Tigano, M. Diversity of *Meloidogyne arenaria* using morphological, cytological and moleculares approaches. *Nematology*, **2008**, 10, 6, 819-834.

Coolen, W.A.; D´Herde, C.J. A method for the quantitative extraction of nematodes from plant tissue. State Agricultural Research Center, Ghent, **1972**; 77p.

De Ley, P.; Felix, M.A.; Frisse, L.M.; Nadler, S.A.; Sternberg, P.W.; Thomas, W.K. Molecular and morphological characterization of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology,* **1999**, 1, 591-612.

Ferraz, L.C.C.B.; Brown, D.J.F. Nematologia de plantas: fundamentos e importância. Norma Editora, Manaus, **2016**, 251p.

Humphreys-Pereira, D.A.; Williamson, V.M.; Lee, S.; Coyne, D.L.; Salazar, L.; Gomez-Alpizar, L. Molecular and morphological characterisation of *Scutellonema bradys* from yam in Costa Rica and development of specific primers for its detection. *Nematology*, **2014**, 16, 2, 137-147.

Jones, J.T.; Haegeman, A.; Danchin, E.G.J.; Gaur, H.S.; Helder, J.; Jones, M.G.K.; Kikuchi, T.; Manzanilla-López, R.; Palomares-Rius, J.E.; Wesemael, W.M.L.; Perry, R.N. Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, **2013**, 14, 946-961.

Katoh, K.; Toh, H. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, **2013**, 30, 772-780.

Kolombia, Y.A.; Ogundero, O.; Olajide, E.; Viaene, N.; Kumar, P. L.; Coyne, D. L.; Bert, W. Morphological and molecular characterization of *Pratylenchus* species from Yam (*Dioscorea* spp.) in West Africa. *Journal of Nematology*, **2021**, 52, 1, 1-25.

Leal, O.B.; Ponte, J.J. Meloidoginose em túberas de inhame *Dioscorea cayenensis* Lam., destinadas à exportação. *Sociedade Brasileira de Nematologia,* **1980**, 4, 115-118.

Moens, M.; Perry, R.N.; Starr, J.L. *Meloidogyne* species – a diverse group of novel and important plant parasites. In: Perry, R.N.; Moens, M.; Starr, J.L. (eds) Root-knot nematodes. CABI, Wallingford, **2009**, pp 1-17.

Moura, R.M.; Freitas, O.M.L. Observações sintomatológicas sobre a meloidoginose do inhame (*Dioscorea cayenensis*). *Fitopatologia Brasileira*, **1983**, 8, 243-249.

Mudiope, J.; Coyne, D.L.; Adipala, E.; Talwana, H.A.L. Damage to yam (*Dioscorea* spp.) by root-knot nematode (*Meloidogyne* spp.) under field and storage conditions in Uganda. *Nematropica*, **2012**, 42, 137-145.

Ngo-Ngwe, M.F.S.; Joly, S.; Bourge, M.; Brown, S.; Omokolo, D.N. Nuclear DNA content analysis of four cultivated species of yams (*Dioscorea* spp.) from Cameroon. *Journal Plant Breed Genet*, **2014**, 2, 87-95.

Powers, T.O.; Harris, T.S. A polymerase chain reaction method for identification of five major *Meloidogyne* species. *Journal of Nematology*, **1993**, 25, 1-6.

Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **2014**, 30, 1312–1313.

Taylor, A.L.; Netscher, C. An improved technique for preparing perineal patterns of *Meloidogyne* spp. *Nematologica*, **1974**, 20, 268-269.

Vrain, T.C.; Wakarchuk, D.A.; Levesque, A.C.; Hamilton, R.I. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental & Applied Nematology*, **1992**, 15, 563-573.