

First report of *Dematophora bunodes* causing root rot of taro (*Colocasia esculenta*) and leatherleaf fern (*Rumohra adiantiformis*) in Brazil

Adans A. Colmán¹, Helcio Costa², Inorbert M. Lima² and Robert W. Barreto¹.

¹Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, 36570-000, Brazil

²Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, 29375-000, Venda Nova do Imigrante, ES, Brazil

Author for correspondence: Robert Barreto, e-mail: rbarreto@ufv.br

Colocasia esculenta, taro (T), is a major staple food crop in the tropics, including Brazil. *Rumohra adiantiformis*, leatherhead fern (LF), is broadly cultivated for its ornamental fronds that are used as a component of flower arrangements. Soft root rot of T and LF, and accompanying rapid plant wilt and death, was observed in plantations in Espírito Santo (Brazil), at Venda Nova do Imigrante, in April 2014 (LF) and July 2015 (T). Great losses were observed. Firstly, a few individual scattered plants showed symptoms of disease in the plantations, then aggregates of plants and, after a few seasons, the majority of the plants in the field died before harvest, leading to the abandonment of the activity by farmers. A white mycelial matt was observed on the crown and roots of dying T and LF plants. Infected corms become necrotic and dark brown mycelial strands were observed internally in tissues. Diseased organs were carefully washed and surface sterilized in 10% sodium hypochlorite. Samples of tissue were removed from the boundary of necrotic tissues and placed on potato dextrose-agar (PDA) plates and incubated at 23±2 C in the dark. Homogeneous mycelial colonies were isolated from both T and LF and, upon observation of microscope mounts under an Olympus BX 53 light microscope, pear-shaped hyphal swellings at the septae (Castro et al. 2013) were observed. A representative isolate from each host was deposited in the local culture collection as COAD 2911 (LF isolate) and COAD 2912 (T isolate). Additionally, DNA was extracted from each culture using the Wizard Genomic DNA Purification Kit (Promega) and the internal transcriptional spacer region was PCR amplified using the primers ITS5 and ITS1 (White et al. 1990). The amplicons were sequenced by MACROGEN (<http://www.macrogen.com>). Consensus sequences were deposited in GenBank: MW561595 (LF), MW561596 (T). Consensus regions were compared against other sequences available in Genbank. A BLASTn analysis resulted in LF and T sequences respectively 99% (526/531bp) and 98% (412/420 bp) identity with that of *Dematophora bunodes* (MN984619). Additionally, a phylogenetic analysis of a selected sequence alignment was performed on the CIPRES webportal (Miller et al., 2010) using MrBayes v.3.1.1 (Ronquist & Huelsenbeck, 2003). A phylogenetic tree was generated showing that the placement of LF and T isolates is in *D. bunodes* (Wittstein et al. 2020).

Pathogenicity tests were performed for LF and T isolates against their original hosts. For inoculum, bags of twice-autoclaved parboiled rice were seeded separately with each isolate, which were allowed to colonize the rice for two weeks. Four healthy young LF and T plants were utilized. Two extra healthy plants grown in the same conditions, but not inoculated, served as controls. Thirty g of *Dematophora*-colonized rice was placed in direct contact with stems or roots of each LF or T plant. Plants were maintained in a dew chamber for 48 h after inoculation and then transferred to a greenhouse bench. All inoculated plants developed wilt and root rot and died after 15-20 days. Controls remained healthy. White mycelial colonies were formed over tissues of diseased LF and T and upon observation under the microscope, typical pear-shaped swellings were observed in slides prepared from newly obtained pure cultures from LF and T. *Dematophora bunodes* (formerly *Rosellinia bunnodes*) has a worldwide distribution and is well known as a polyphagous plant pathogen (Farr and Rossman, 2020) but has never been reported as a pathogen either of LF or T before in Brazil and worldwide. Its report on LF and T further expands an already large host-range and resolves the etiology of the disease on LF and T.

References:

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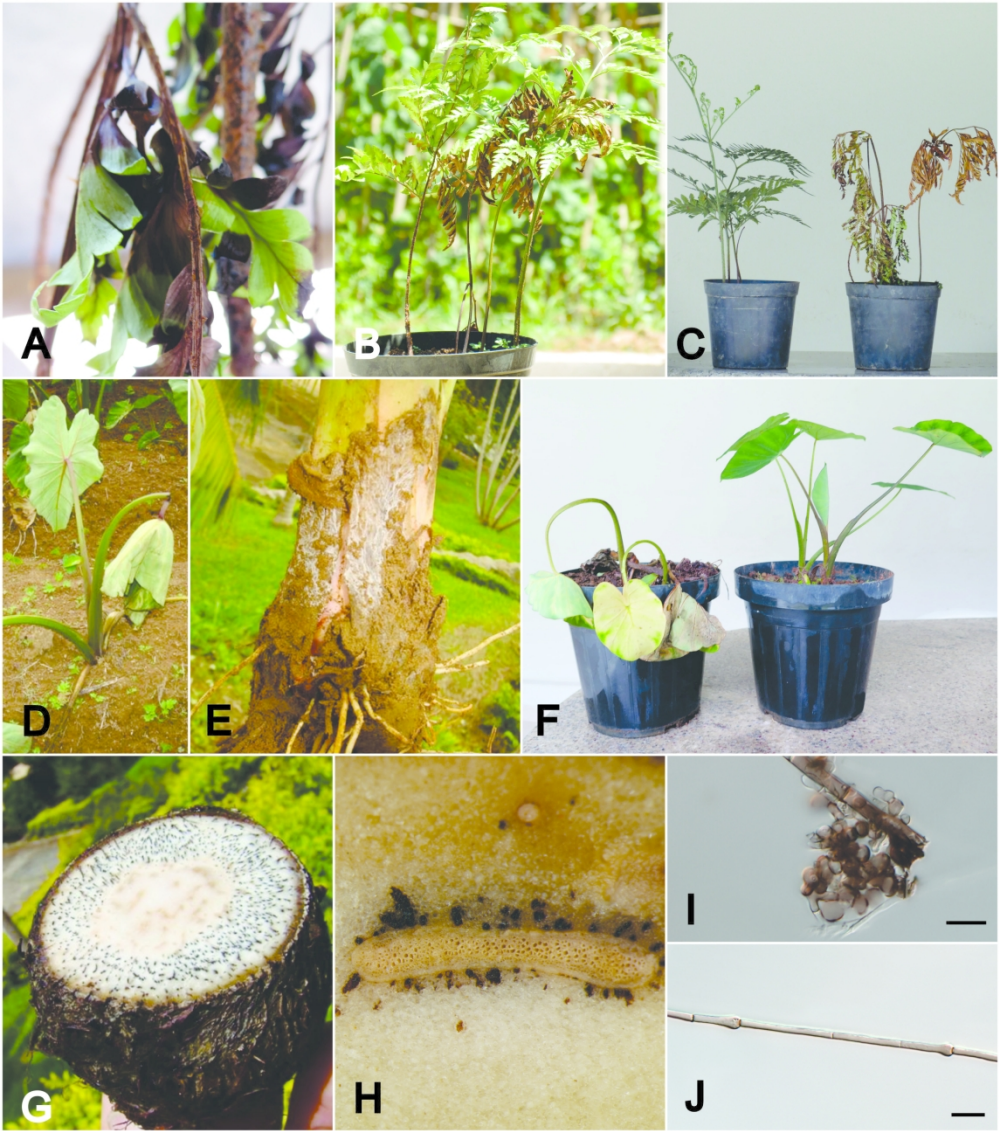


Figure 1. *Dematophora bunodes* on *Rumohra adiantiformis* (leatherhead fern – LF) and on *Colocasia esculenta* (taro – T). A-B. Foliage blight resulting from stem rot on LF. C. Potted LF showing results of Koch’s postulates after fifteen days: left - healthy control vs. right - wilted inoculated plant. D. Wilted dying T plant in plantation. E. Collar rot on T uprooted individual. F. Potted T showing results of Koch’s postulates after fifteen days: right - healthy control vs. left - wilted inoculated plant. G and H. Transversal section of diseased taro corm showing discoloration and soft root rot of central part and dark internal mycelial strands. I. Apresorial-pad of COAD 2911 (LF isolate) on PDA J. Hypha bearing typical *Dematophora* pyriform-swellings next to septae on COAD 2912 (T isolate).

209x237mm (300 x 300 DPI)

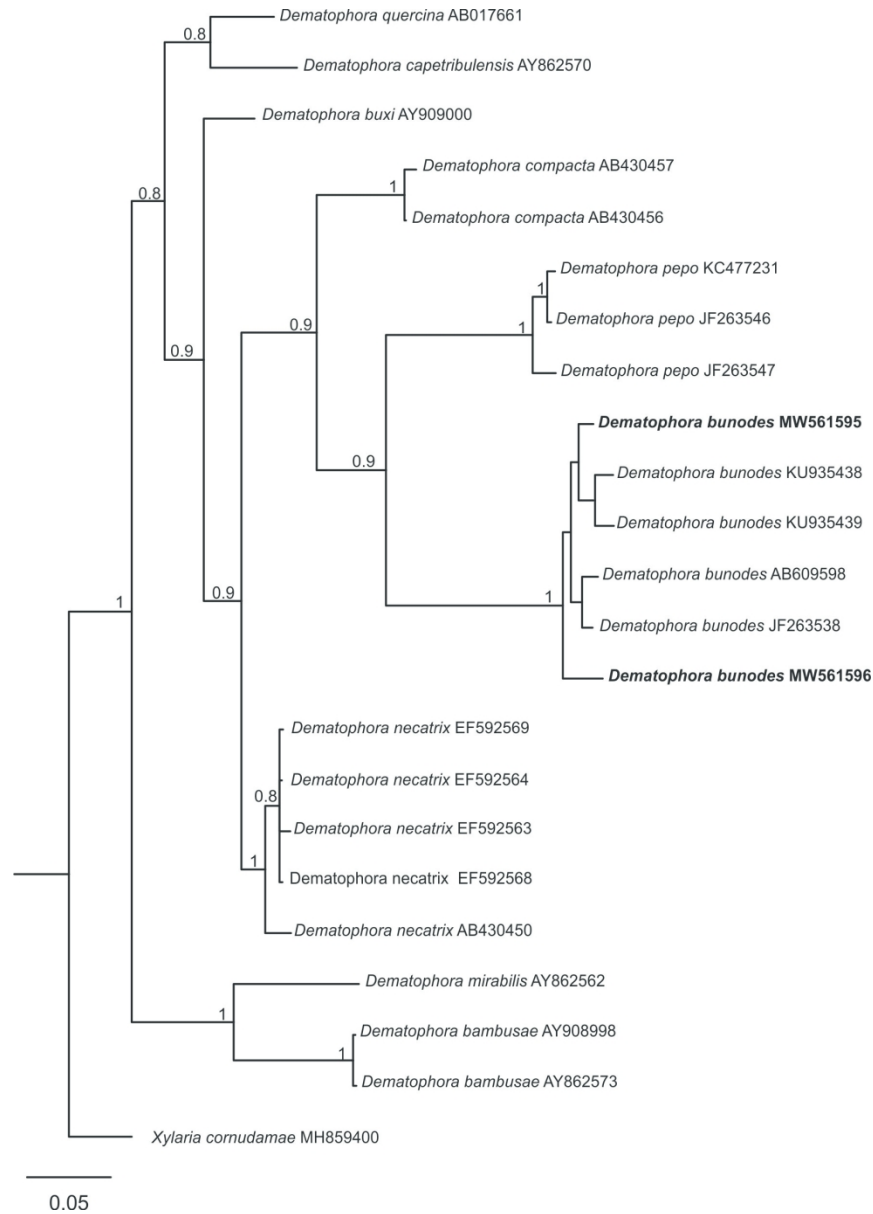


Figure 2. Phylogenetic tree generated by Bayesian analysis of ITS sequences showing the placement of COAD 2911 (leatherhead fern *Dematophora bunodes* isolate) and COAD 2912 (taro *D. bunodes* isolate) among species of *Dematophora*. Posterior probability values are indicated above the nodes. The isolates used in this study are highlighted in bold. The tree is rooted with *Xylaria cornudamae*.

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