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First report of *Robillarda sessilis* associated with *Anacardium occidentale* and *Beaucarnea recurvata* leaf spots in Brazil

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Cashew tree (*Anacardium occidentale* L.) is a plant native to the Brazilian northeastern region and is one of the most important cash crops of this semi-arid region. Approximately 700,000 hectares are planted with this crop, giving employment to more than 100,000 people and providing an annual turn-over of 200 million dollars. Processed kernels are the principal commodity exported to the USA, Europe and Japan (Freire, F.C.O.; Kozakiewick, Z.; Paterson, R.R.M. Mycoflora and mycotoxins of Brazilian cashew kernels. Mycopathologia, v.145, p.95–103, 1999). As part of an Embrapa project in course to study fungal bioactive secondary metabolites produced by endophytic fungi of plants typical of this region, an interesting fungus was consistently isolated as endophyte or from leaf spots of cashew plants collected in Maranhão State (Barra do Corda county). Simultaneously, a fungus isolated from leaf spots of the ornamental plant *Beaucarnea recurvata* Lem. was morphologically identical to *Robillarda sessilis* (Sacc.) Sacc. *B. recurvata*, known as elephant's foot tree and ponytail palm, is an ornamental plant species belonging to the family Asparagaceae. Despite its name of ponytail palm, it is not closely related to the true palms (family Arecaceae). This plant is native to the states of Tamaulipas, Veracruz and San Luis Potosí, in eastern Mexico. It is a curious evergreen perennial plant, growing to 6-10 m tall, with a noticeable heavily swollen expanded caudex, which has the purpose of storing water inside, and grass-like foliage giving it a unique appearance. According to the Institute of Ecology in Xalapa, state of Veracruz, which runs the Jardín Botánico Francisco Javier Clavijero, there are 10 different species of this plant. Since it was discovered in Mexico in 1870, it became a popular ornamental plant in Europe and in many other countries. There are 350-year-old Beaucarneas registered in Mexico (Irish, M.; Irish, G. Agaves, yuccas, and related plants: A Gardener's guide. Portland: Timber Press, 2000. 384p.). In Brazil, this species is very appreciated as a potted ornamental plant, mainly in its juvenile phase (Lorenzi, H.; Mello Filho, L.E. The tropical plants of R. Burle Max. São Paulo: Instituto Plantarum de Estudos da Flora, 2001. 488p.).

Leaf fragments of both plants were surface disinfected and planted in Potato-Carrot-Agar medium (PCA). Plates were kept at 25-28ºC (in an alternate regime of 12 hours light and darkness) and, 2 weeks later, the fungus formed 3cm round colonies, presenting a felt mat of hyphae, which was white at first and subsequently became grey (Figure 1C), producing scattered pycnidial conidiomata, unilocular, dark-brown to black, superficial to partly immersed, ostiolate, ovoid to globose, glabrous, with prismatic texture, 244.5-445.8 (X=353.1) µm (Figure 1D); conidiogenous cells were usually ampulliform, lining the cavity of the conidioma, invested in mucus. Conidium was 1-septate, mostly fusiform, straight, of smooth wall and often slightly constricted at the median septum, almost colorless, and presenting an apical cell with 2 to 4 appendages, measuring 7.4-13.5 X 2.3-3.1 (X=10.8 X 2.9) µm (Figure 1E and 1F). To confirm the morphological characterization, DNA of the fungus was extracted from the mycelium by using the UltraClean Microbial DNA Isolation Kit V (Mo-Bio Laboratories) at the Central Bureau of Schimelcultures (CBS), Utrecht, The Netherlands. Fragments containing the 26S ribosomal RNA gene, Large Subunit D1 and D2 region (LSU), were amplified by using the primers LR0R (ACCCGCTGAACTTAAGC) and LR5 (TCCTGAGGGAAACTTCG). Fragments containing the Internal Transcribed Spacer 1 and 2 and the 5.8S gene (ITS) were amplified by using the primers LS266 (GCATTCCCAAACAACTCGACTC) and V9G (TTACGTCCCTGCCCTTTGTA). These fragments were sequenced with the ABI Prism® Big DyeTM Terminator v.3.0 Ready Reaction Cycle sequencing Kit. Samples were analyzed on an ABI PRISM 3700 Genetic Analyzer and countings were performed by using the forward and reverse sequences with the program SeqMan from the LaserGene package. A multigene analysis was carried out by comparing part of the 26S ribosomal RNA gene, Large Subunit D1 and D2 region and Internal Transcribed Spacer 1 and 2 and the 5.8S gene. The sequencing result was subjected to BLAST analysis and, when aligned with the deposited sequences from GenBank, revealed 99 to 100% sequence identity with *R. sessilis* strains (*HQ608017, FJ825378, FJ825373 and FJ825368)*.

Pathogenicity tests were performed by placing plugs (0.5 cm^2) of a 10-day-old PCA culture of *R. sessilis* on leaves of cashew seedlings (Dwarf Clone CCP 76). Twenty leaves were wounded with a sterilized needle and 20 leaves remained unwounded. Twenty control leaves were inoculated with plugs of sterilized PCA medium. The same procedure was conducted for seedlings of *B. recurvata*. All seedlings were covered with plastic bags and incubated in a growth chamber at 26 ºC with 12 h of light for 3 days and then kept in the greenhouse. Fifteen days after inoculation, dark angular to oval spots were observed in 40% of inoculated leaves, but no symptoms were seen on unwounded leaves or on leaves which received only PCA plugs (Figure 1B). On wounded leaves of *B. recurvata*, spots were cream color with a dark brown halo (Figure 1A). Koch's postulates were fulfilled by re-isolation of *R. sessilis* from diseased leaves. To our knowledge, this is the first report of *R. sessilis* in Brazil. This is also the first global report of this fungus as an endophyte in cashew nut plant, as well as a pathogen in leaves of the ornamental plant *B. recurvata*.

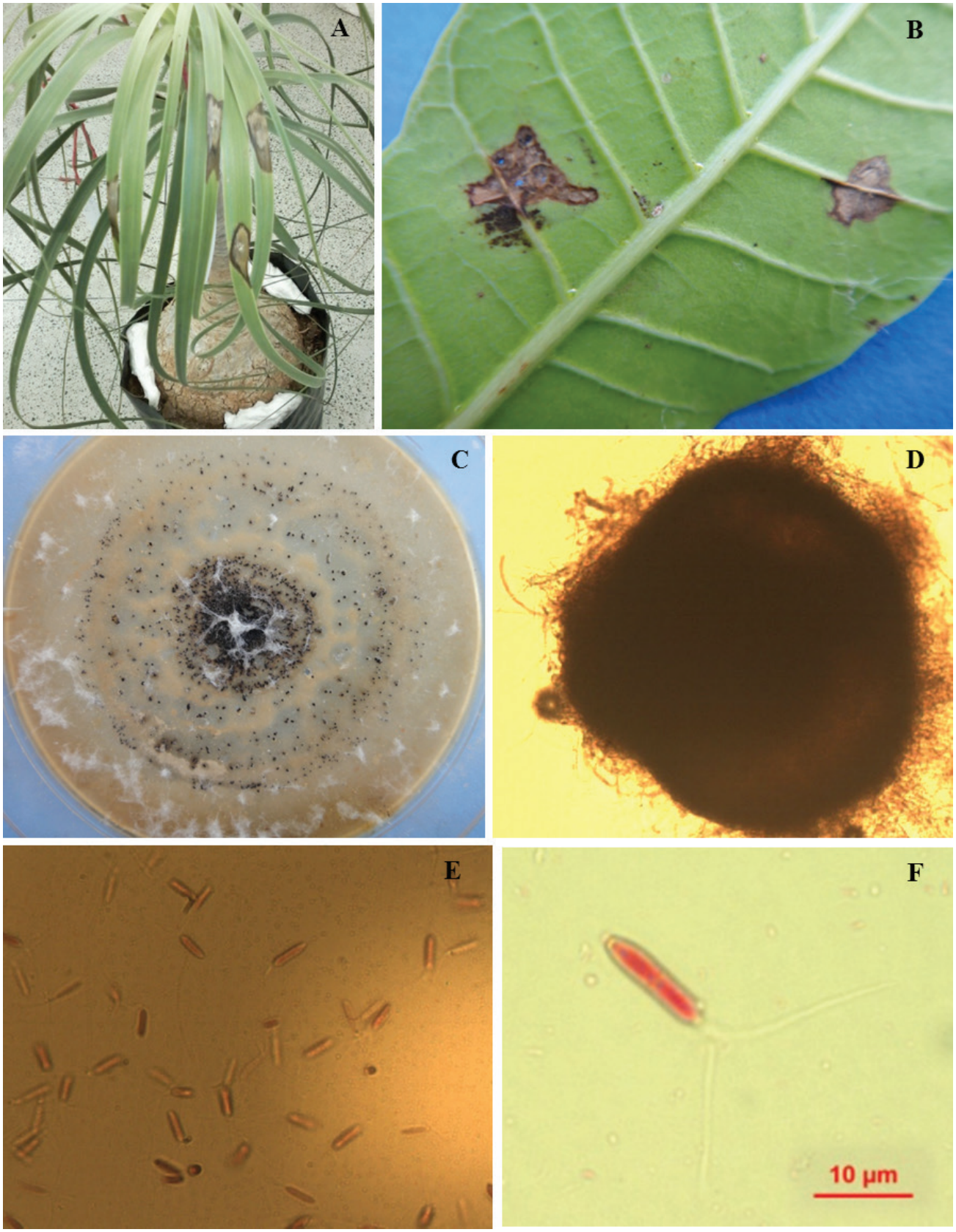

Figure 1. Symptoms of seedlings inoculated with *Robillarda sessilis* (*Beaucarnea recurvata* - A, and *Anacardium occidentale* - B); Culture, pycnidium and conidia of *R. sessilis* (C, D, E and F).