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Fungal diseases of non-conventional food plants: first report of *Stagonosporopsis caricae* causing leaf spots on *Vasconcellea monoica*

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Abstract
For the first time, *Stagonosporopsis caricae* is reported causing leaf spots on the non-conventional fruit crop *Vasconcellea monoica* (Caricaceae). The fungus was identified in Brazil based on morphological and molecular features and its pathogenicity was demonstrated. This is the first pathogen to be reported on *V. monoica*.

Keywords Ascomycota · Didymellaceae · Koch's postulates · Morphology · New record

Many edible plants are locally grown but poorly known, and, despite being undomesticated, have been attracting public attention lately in Brazil. These are referred to under the label PANC (plantas alimentícias não convencionais – non-conventional edible plants) (Kinupp and Lorenzi 2014). Such plants have received little attention from scientists in general and plant pathologists in particular. In order for these to become viable crops as well as sustainable sources of income for farmers it is necessary to build a body of scientific knowledge about them.

The family Caricaceae is mostly known because of papaya (*Carica papaya*), which is a highly important tropical fruit crop. However, it also includes other plants producing smaller but edible fruits with different tastes, textures and smells (Badillo 2000; Damasceno Junior et al. 2015). *Vasconcellea* is the largest genus in the Caricaceae and it includes several PANCs. *Vasconcellea monoica* is commonly known as babaco-mirim, or mamãozinho do cerrado in Brazil. Fruits of *V. monoica* as well as other members of *Vasconcelllea* have been used by traditional communities for the preparation of desserts (Kinupp and Lorenzi 2014).

A small representative stand of babaco-mirim was established in the Infectarium (plant disease garden) of the Universidade Federal de Viçosa (Viçosa, state of Minas Gerais, Brazil). Leaf spots leading to necrosis of foliage appeared on all plants of the stand. Lesions which developed on the margin and centre of leaves were subcircular (6,5–20 cm) with a brown necrotic centre and surrounded by a chlorotic halo. As the leaves matured, the central portion of the lesion fell out and severe defoliation followed, interrupting plant growth and fruit production. A fungus producing spherical bodies within the necrotic tissues was regularly found and a preliminary observation under the microscope showed that both conidia and ascospores were present inside those bodies. Here its identity and pathological status were investigated for the first time.

Diseased leaves were collected and subsamples were dried in a plant press and deposited in the herbarium of the Universidade Federal de Viçosa (Acc Ns VIC 47334 and VIC 47335). Part of the material was examined while still fresh. Fragments of lesions taken from VIC 47334 were attached with vaseline jelly to the inner side of plate lids and left over a layer of potato dextrose-agar (PDA) overnight, in order to induce ascospore ejection. VIC 47335 was treated as follows: 5 mm wide fragments were taken from the margins of lesions and left for 1-min in ethanol (70%), 6 min in sodium hypochlorite (1%) and rinsed three times in distilled water. The fragments were then placed on sterile blotter paper to dry and then transferred to plates containing PDA. The plates were left in an incubator adjusted to 25 °C under a 12 h light/12 h dark daily regime. Cultures of both samples were obtained by excising hyphal tips of colonies formed on the medium and transferring these to fresh plates. Two representative isolates were deposited in the local culture collection Octávio de Almeida Drummond of University Federal of Viçosa (COAD) – (Accession number COAD 2909 and COAD 2910).

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Colonies on PDA: fast-growing, (9 cm diam in 7 days), flat, aerial mycelium white, cottony, sporulation of asexual morph observed within three weeks, perithecia formed within 5 weeks.

Sections of the fungal structures formed in necrotic tissues were prepared with a razor blade and mounted in lactoglycerol on slides. The morphology of the fungus was examined under a light microscope (Olympus BX 51) equipped with differential interference contrast and to which a digital capture system (Olympus Q-Color 3 ™) was attached. The fungus produced both sexual and asexual morphs. The morphology of the fungus was as follows: Sexual morph - ascomata pseudothecioid, subglobose, 58 125 × 50 100 μm, ostiolate, walls of brown *textura angularis*, smooth, asci bitunicate, cylindrical to clavate, 17 31 × 4 10 μm, 8-spored, ascospores elliptical, straight to slightly curved, 9 16 × 3 6 μm, 1-septate, constricted at the septum, sub-hyaline, smooth; Asexual morph - conidiomata pycnidial, subglobose, 63 150 × 65 168 μm, ostiolate, wall of dark brown to black *textura angularis*, smooth, conidia short cylindrical or slightly reniform, 2 5 × 1 2 μm, 0 1 septate, hyaline, smooth (Fig. 1). This morphology was found to match that of *Mycosphaerella caricae* described in Sivanesan (1990) and *Phoma caricae*, described in Punithalingam (1979). More recently Aveskamp et al. (2010) placed these names as synonyms of *Stagonosporopsis caricae*.

DNA was extracted with the Wizard Genomic DNA Purification Kit, according to the instructions of the manufacturer. The actin (ACT), the β-tubulin (BTUB) and the internal transcribed spacer (ITS) regions were amplified using the primer pairs ACT-512F/ACT-783R, Bt2Fd/Bt4R, ITS4/ITS5, respectively for both isolates (White et al. 1990; Carbone and Kohn 1999; Woudenberg et al. 2009). The resulting sequences (COAD2909 - ACT MT084555, TUB MT084557, ITS MN947335; COAD2910 - ACT MT084556, TUB MT084558, ITS MN947336) were compared with additional sequences from GenBank in a BLASTn search and similar sequences to those obtained for the isolates from *V. monoica* were chosen for a phylogenetic study. A direct comparison with the reference isolate CBS 248.90 (Aveskamp et al. 2010), resulted in 100% identity for all three regions.

The alignments were checked, and manual adjustments were made when necessary. The tree was constructed using two different algorithms: the Bayesian Inference (BI) and Neighbor Joining (NJ) (Fig. 2). BI analysis was conducted in the MrBayes v. 3.2.6 on XSEDE platform available on

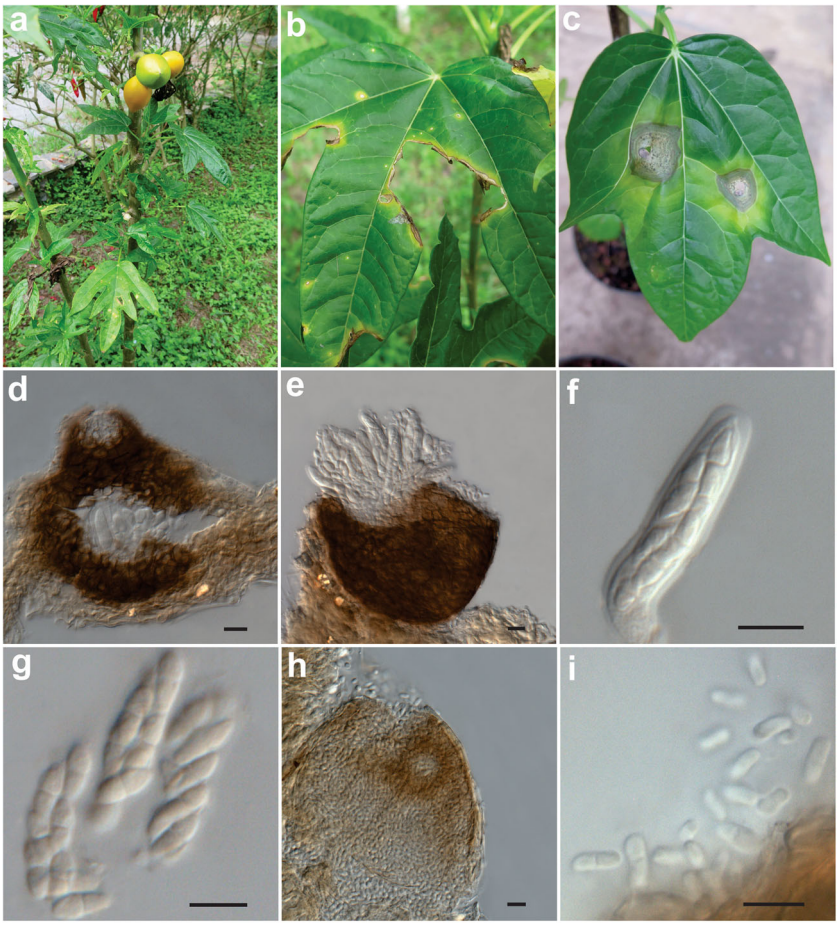

Fig. 1 *Stagonosporopsis caricae* on *Vasconcellea monoica* **a** *V. monoica* individual in the field. **b** Close up of a young lesion on a naturally infected leaf. **c** Leaf spots resulting from inoculation of plants under controlled conditions. **d** Perithecium. **e** Perithecium containing asci. **f** Bitunicate ascus. **g** Asci containing eight ascospores. **h** Pycnidium. **i** Conidia. Scale bar = 10 μm

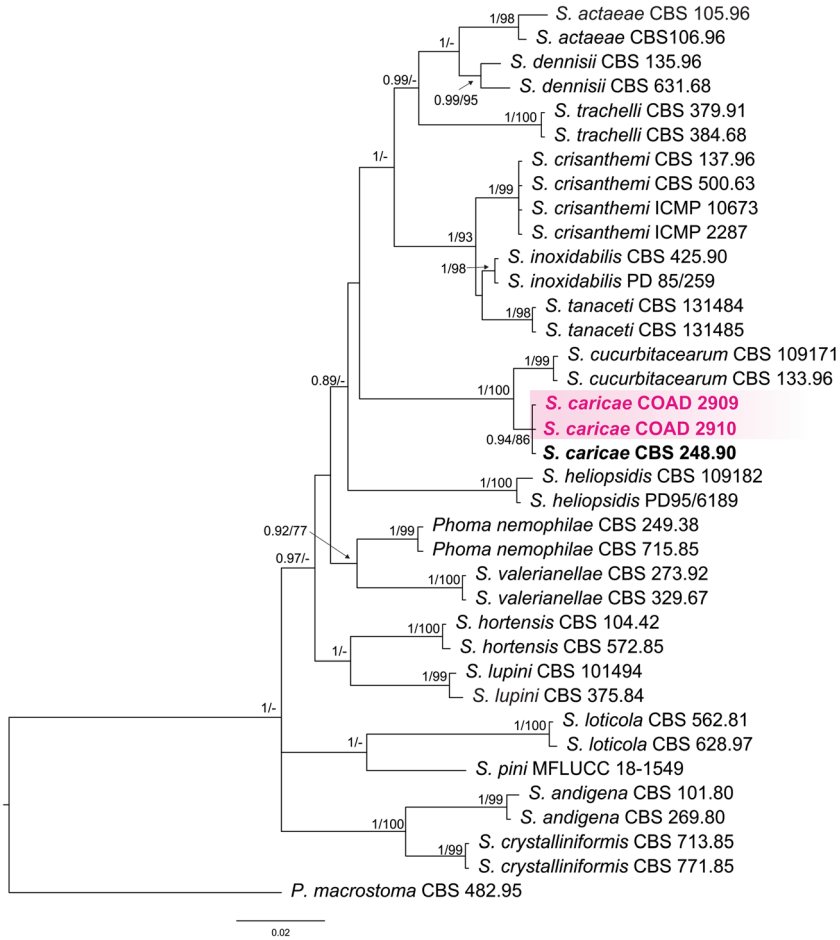

Fig. 2 Phylogenetic tree constructed from Bayesian Inference analysis using combined data sets of sequences from the ACT, β-tubulin and ITS gene regions of isolates belonging to *Stagonosporopsis*, including COAD 2909 and COAD 2910 obtained from *Vasconcellea monoica* (indicated in pink). Bootstrap support values (≥ 70%) and later probabilities (≥ 0.90)

CIPRES Science Gateway Portal (Miller et al. 2012). NJ analysis was conducted in the MEGA X 10.1 software (Kumar et al. 2018).

The three-locus analysis included 1135 bp (ACT 302 pb, BTUB 340 pb and ITS 493 bp) with 35 representative taxa sequences obtained from GenBank. Each gene was analyzed separately prior to multilocus analysis. The two isolates obtained from this study were grouped in a monophyletic clade along with the material CBS 248.90 of *S. caricae* described by Aveskamp et al. (2010) with high support of posterior probability and bootstrap (0.94/86, respectively). The phylogenetic study further confirmed the morphology-based identification of the fungus on *V. monoica* as *S. caricae* (Fig. 2).

Pathogenicity was tested on healthy 40-day-old babacomirim plants. Four plants were inoculated by depositing culture disks obtained from the margin of actively growing COAD 2910 on PDA (5-days-old) on the adaxial side of young leaves. Two plants were used as controls and received PDA disks with no fungus. After inoculation, each plant was covered with a plastic bag wetted inside and a pad of water-soaked cotton wool was placed at the base of each plant to keep humidity high for 24 h. After that period the plants were incubated on a greenhouse bench at 25 ± 2 °C and irrigated regularly. Plants were observed daily for the emergence of disease symptoms. After 10 days, a necrotic area was observed surrounding the sites of inoculation, but not around PDA-only disks on controls. *Stagonosporopsis caricae* was reisolated from newly formed lesions and morphology of colony and fungal structures confirmed the identity of such isolates as *S. caricae*, hence fulfilling Koch's postulates.

Stagonosporopsis caricae has been reported many times in association with papaya (Aveskamp et al. 2010; Chen et al. 2015, 2017; Li et al. 2017; Babaahmadi et al. 2018) and is one of the most important fungal pathogens of this crop, causing leaf spots and significant post-harvest losses (black rot). It is difficult to control with fungicide sprays and there is ongoing research aimed at developing papaya varieties resistant to this disease (Poltronieri et al. 2019). Nevertheless, *S. caricae* also attacks plants belonging to other families: *Brassica* sp. (Brassicaceae) (Aveskamp et al. 2010; Chen et al. 2015, 2017; Babaahmadi et al. 2018), *Citrullus lanatus* (Cucurbitaceae) (Li et al. 2017; Rennberger et al. 2018) and *Cucumis sativus* (Cucurbitaceae) (Garampalli et al. 2016; Li et al. 2017). This is the first report of *S. caricae* on *V. monoica* or any other member of Caricaceae besides *C. papaya* anywhere in the world.

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