

Pseudocercospora piperis associated with leaf spots on *Piper aduncum* in Brazil

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Received: 1 April 2013 / Accepted: 3 June 2013 / Published online: 4 July 2013
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Abstract *Piper aduncum* (Piperaceae) is a neotropical plant that has been widely exploited as a medicinal plant in its native range but has recently become an important invasive plant species in Papua New Guinea. A cercosporoid fungus was recently found associated with leaf spots on this plant in the Amazon. The morphology of the fungus is typical of *Pseudocercospora piperis*, a fungus that has never been recorded on this host species in Brazil.

Keywords Cercosporoid · Mycosphaerellaceae ·
Piperaceae · Taxonomy

Piper aduncum L. (aperta-ruão, pimento-de-macaco, amongst many other common names in Brazil) is widely used in folk medicine, and is known to have antimicrobial (Morandim-Giannetti et al. 2010) and insecticidal (Misni et al. 2011) properties. It is a perennial and erect shrub with articulate stalk originally occurring under the shade of tropical forests in Central and South America (Lorenzi 2000). In its native ranges it is never a dominant species, however *Piper aduncum* has been recently reported as an invasive species in Papua New Guinea (Hartemink 2010) probably owing to the absence of natural enemies in the exotic situation. Fungal pathogens associated with *Piper aduncum* may become of relevance for classical biological control. Nevertheless, little is known about the mycobiota of *Piper aduncum* in Brazil. Recently a fungus was found in association with severe spotting of leaves of *Piper aduncum* on the campus of the Instituto Nacional de Pesquisas da Amazônia (INPA),

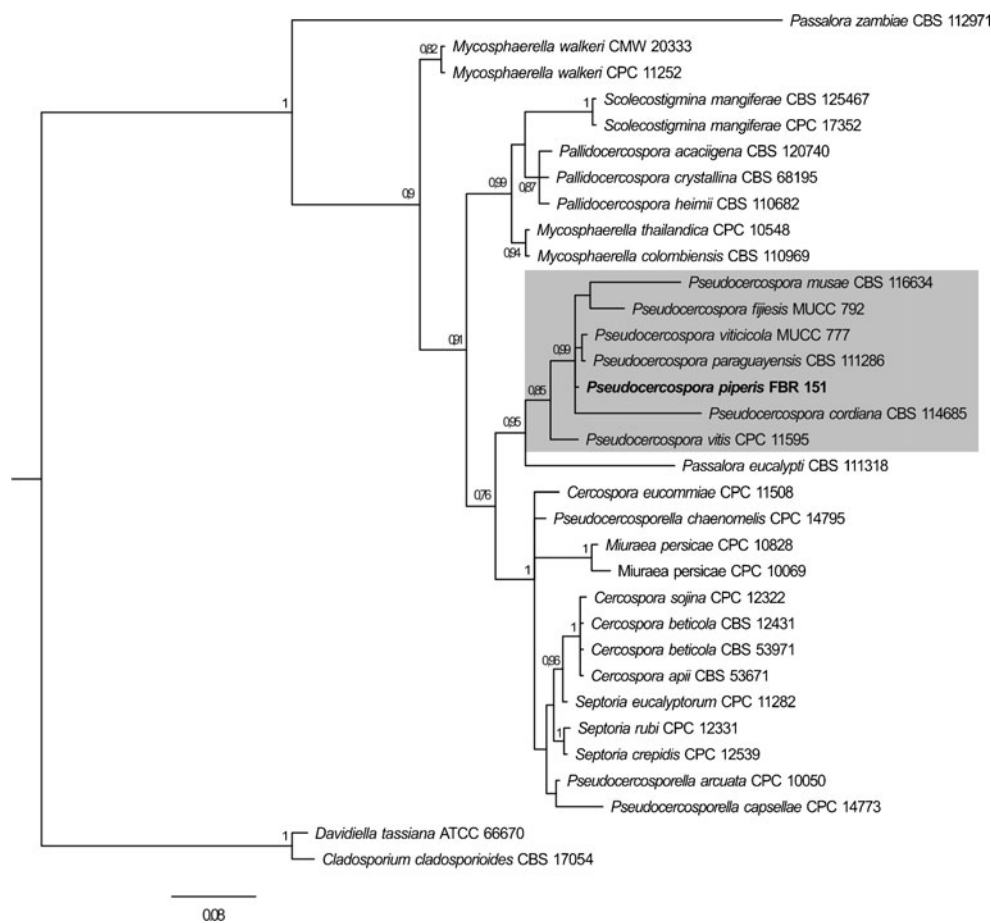
Manaus, state of Amazonas, Brazil. The fungus was readily recognized as a member of *Pseudocercospora* as delimited by Crous and Braun (2003). This publication provides an account of this occurrence and a molecular and morphological characterization of the specimen of the fungus found associated with *Piper aduncum* in Brazil.

Genomic DNA was extracted from pure cultures growth in liquid potato-dextrose (PD) for 1 week. Three genomic regions (Elongation Factor - EF-1 α , Internal Transcribed Spacer - ITS and 28S rDNA Large Subunit - LSU) were sequenced. The sequences were edited with DNA Dragon (<http://www.dna-dragon.com/>). A BLAST search (Zhang et al. 2000) was performed to check for similarity with other sequences. The Bayesian analysis (Fig. 1) were performed with ITS dataset (TreeBASE: S13481, <http://www.treebase.org/>) to confirm its placement in *Pseudocercospora* strictu sensu using *Cladosporium cladosporioides* (Fresen.) G.A. de Vries and *Davidiella tassiana* (De Not.) Crous & U. Braun (GenBank accessions: AY213640 and DQ780400, respectively) as outgroup. The MrModeltest 2.3 was used to select the best nucleotide substitution model (Nylander 2004). The Bayesian analysis was performed in CIPRES Science Gateway (Miller et al. 2010; <http://www.phylo.org/index.php/portal/>) by MrBayes on XSEDE. The sequences used in the study were selected from a phylogenetic tree provided in Crous et al. (2012). The sequence alignment was performed with MAFFT online version (<http://mafft.cbrc.jp/alignment/server/>). Samples of *Piper aduncum* bearing leaf spots symptoms were dried in a plant press and deposited in the herbarium at the Universidade Federal de Viçosa under the accession number VIC 31889, and a pure culture was deposited in the culture collection of the same university as COAD 1111. The colonies were described after 19 days incubated on PDA (potato dextrose-agar) and PCA (potato carrot-agar) at 25 °C under two different light regimes, 12 h of light and 24 h in the dark. The fungus structures were scraped with a scalpel from infected leaves

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Fig. 1 Phylogenetic tree based on the Bayesian analysis of ITS sequence. The highlighted clade represents *Pseudocercospora* sensu strictu



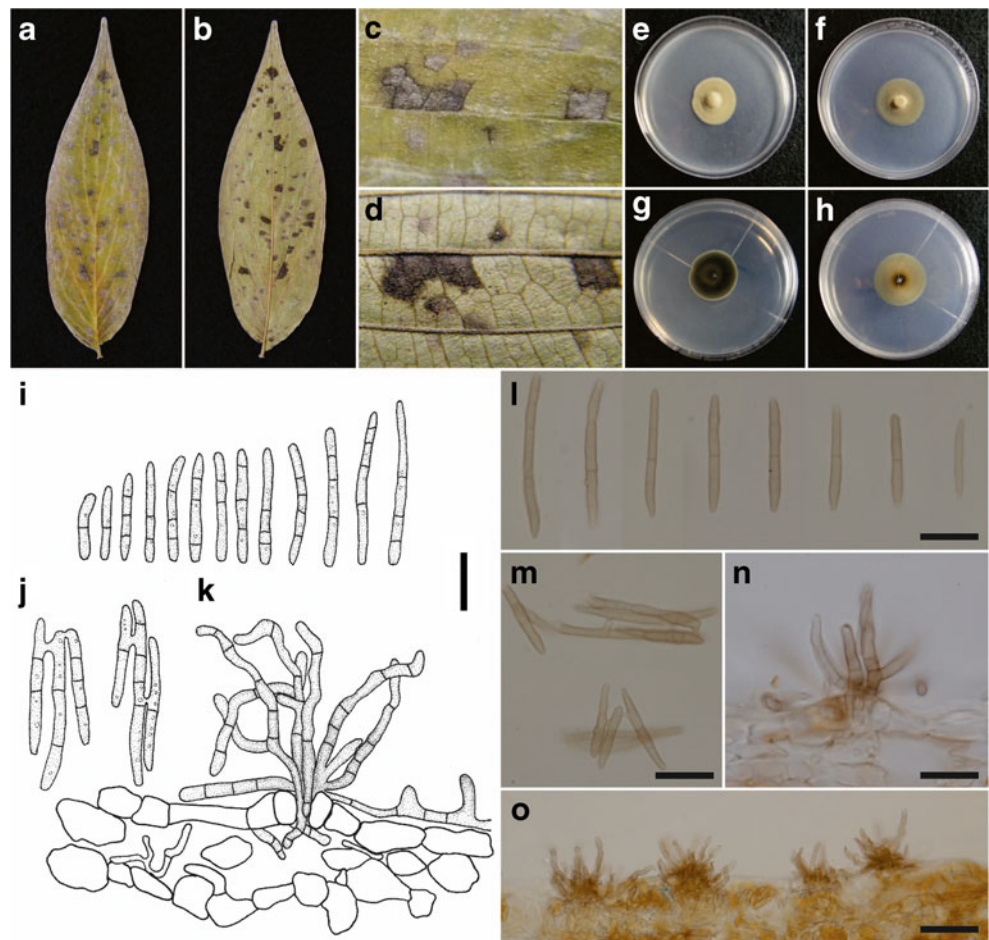
or sections were prepared and mounted in either lactophenol or lactofuchsin and examined with an Olympus BX 51 light microscope fitted with an Olympus E330 digital camera. A morphological description was elaborated and compared with descriptions of others *Pseudocercospora* species associated with Piperaceae found in literature.

The fungus had the following features (Fig. 2): *Lesions* on leaves, initially pale grey reduced to small spots less than 2.5 mm, becoming vein-delimited and angular or occasionally circular, up to 7 mm diam, dark brown to black abaxially and dark grey with black edge adaxially. *Internal mycelium* intra and intercellular, 2–3 μm diam, branched, septate, pale olivaceous to hyaline. *External mycelium* arising from stomata, hypophyllous, 2.0–3.0 μm , sparingly branched, septate, pale brown, smooth. *Stromata* absent. *Conidiophore* hypophyllous, fasciculate, subcylindrical, 20–80 \times 4.5–5 μm , 0–4 septate, rarely branched, erect or decumbent, pale brown, paler towards the apex, smooth, occasionally reduced to conidiogenous cell when arising from stomata, and solitary, 525 \times 36 μm , reduced to the conidiogenous cell when arising from external mycelium. *Conidiogenous cell* terminal, integrated, holoblastic, sympodial sometimes percurrent, subcylindrical, 4–29 \times 4–6 μm , pale brown sometimes olivaceous, smooth. *Conidiogenous loci* indistinct. *Conidia* isolate, subcylindrical, straight or slightly

curved, 17–65 \times 3–5 μm , base obtuse, 1.5–3.5 μm , 1–7 septate, hilum unthickened, guttulate, pale olivaceous to pale brown, sometimes yellowish brown, occasionally anastomosing to neighboring conidia. *Colonies* very slow growing (2.53 mm diam. after 20 days), circular, flat to slightly convex, velvety, smoke grey to grey olivaceous, reverse smoke grey to olivaceous black, with diurnal zonation (visible only on PCA under 12 h light regime), not sporulating.

Among the species of fungi that have been reported in association with *Piper aduncum* there is one cercosporoid species, namely *Pseudocercospora piperis* (Patouillard) Deighton (Farr and Rossman 2013). Here we provided the first sequences deposited in GenBank for a species of *Pseudocercospora* associated with Piperaceae (GenBank: EF-1 α -JX896123; ITS-JX875062; LSU-JX875063). The Bayesian analysis presented here (Fig. 1) showed that *P. piperis* found on *Piper aduncum* belongs to the genus *Pseudocercospora* sensu strictu. Crous et al. (2012) recommended that a search for novel loci is still necessary for more robust phylogeny analysis to be produced for *Pseudocercospora* at the species level. There are seven species of *Pseudocercospora* associated with Piperaceae (Rocha et al. 2008). The morphology of the fungus on *Piper aduncum* matched well with *P. piperis* (Chupp 1954). It is possible that phylogenetic analyses will reveal cryptic speciation amongst the

Fig. 2 *Pseudocercospora piperis* associated with *Piper aduncum*. **a, b.** Leaf spots on adaxial and abaxial leaf side, respectively, with which *P. piperis* is associated. **c, d.** Close up of the leaf spots located on adaxial and abaxial side, respectively. **e, f.** Upper view of colonies culture on PDA and PCA, respectively. **g, h.** Bottom view of colonies culture on PDA and PCA, respectively. **i, l.** Conidia. **j, m.** Anastomosing conidia. **k, n, o.** Conidiophores arising from stomata. Bars: i-n=20 μ m, o=50 μ m



species of *Pseudocercospora* on Piperaceae, as several have very close morphology. As *P. piperis* was found in association with damaging spots on *Piper aduncum* leaves, it is possible that this fungus warrants investigation as a classical biocontrol agent against this plant in weedy situations in Papua New Guinea and elsewhere it has become invasive.

Acknowledgments The present work was supported by CAPES, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil, and CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico – Brazil

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