

Title Using molecular analysis to investigate phylogenetic relationships in two tropical pathosystems: witches' broom of cacao, caused by *Moniliophthora perniciosa*, and mango anthracnose, caused by *Colletotrichum* spp.

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Abstract

The increasing availability of DNA sequence data has enabled rapid advances in molecular systematics. This is especially true for the Fungi, where systematics and taxonomy relied previously on largely artificial, morphologically based systems. Many of the advances in fungal systematics have been made with phytopathogens. Characterizing the relationships of lineages among phytopathogenic genera provides insight into not only basic systematic information, but can also address applied questions on host specificity, life strategy and pathogenicity. With DNA sequence-based phylogenetic analyses, two broad topics were investigated: (1) relationships among biotypes of *Moniliophthora perniciosa*, causal agent of witches' broom of cacao, and (2) host and tissue specificity of *Colletotrichum gloeosporioides sensu lato* on mango. Sequences from the ITS1-5.8S-ITS2 ribosomal DNA region (ITS), intergenic spacer (IGS) region, and RNA polymerase large subunit (RPB1) regions were analyzed for 36 accessions of *M. perniciosa* from all reported biotypes of the pathogen. Maximum parsimony and maximum likelihood analyses resolved three major clades within the species: a clade that contained all isolates from *Theobroma* spp. and most isolates from *Solanum* spp.; a clade that contained most isolates from malpighiaceae hosts; and a clade that contained three isolates from *S. cernuum*, a bignoniaceous liana, and an unknown host. Analysis of morphological characters did not reveal striking differences among the clades. The molecular findings indicate that *M. perniciosa* likely evolved from a saprophytic ancestor and that pathogenicity may have evolved with a switch from a heterothallic to homothallic lifestyle. Host jumps have resulted in distinct lineages within the pathogen. Sequences from the ITS, mating type (MAT) 2 gene, and a cloned region from a randomly amplified DNA fragment were analyzed to examine 58 accessions of *C. gloeosporioides sensu lato* that represented all anthracnose-affected organs of mango, as well as avocado, banana, carambola and guava. Phylogenies from maximum parsimony and maximum likelihood analyses revealed a mango-specific clade that comprised all blossom blight and leaf anthracnose agents, and some fruit anthracnose agents. Other mango fruit anthracnose and peduncle isolates resolved in two general clades that also contained isolates from other fruit hosts (avocado, guava,

carambola, banana). The pathogenicity of representative isolates supported the phylogenetic findings, in that the mango-specific clade isolates cause blossom blight, leaf anthracnose, and fruit anthracnose, and the general clade isolates caused only fruit anthracnose. There were no differences among clades with respect to conidium size and shape, and hyphopodium size. Hyphopodia produced by isolates from the mango-specific clade were clavate and smooth, while isolates from other clades produced irregular, lobed hyphopodia.