## plant disease

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## First Report of 'Candidatus Liberibacter asiaticus' Associated with Huanglongbing on Citrus latifolia in Martinique and Guadeloupe, French West Indies

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## Open Access.

Huanglongbing is an unculturable vascular citrus pathogen transmitted from infected to healthy plants through grafting or by citrus psyllids, *Diaphorina citri* mainly in Asia and America and *Trioza erytreae* in Africa. This phloem limited gram-negative bacterium causes dramatic yield losses and is classified into three species based on 16S rDNA sequence analysis (2): (i) 'Candidatus Liberibacter asiaticus' (Las), the most epidemiologically active, widespread and heat tolerant species; (ii) 'Ca. L. africanus' (Laf), only found in Africa; and (iii) the newly described 'Ca. L. americanus' (Lam), which appeared in 2005 in Brazil (5). Considered as a quarantine organism in America and Europe, Las is actively affecting North America and Asia, and research is leading toward psyllid management and resistance breeding. Despite the fact that Reunion Island has successfully controlled Las by introducing a psyllid parasitoid, *Tamarixia radiata* (1), this strategy was less effective or reproducible

within other territories. D. citri was first detected in Guadeloupe in 1998, where the control of the the psyllid population has been effective with T. radiata (3); and was first detected in Martinique in 2012. Following the outbreak in the United States and the Caribbean, and also supported by reports of symptoms in citrus orchards, local National Plant Protection Organizations (NPPO) organized a detection survey across both islands to verify the occurrence of Huanglongbing. Since 2012, 450 sites were prospected each year in Martinique and Guadeloupe, where 20 leaves from 10 to 30 trees were analyzed. DNA extraction was performed (DNeasy Plant Mini Kit, Qiagen) on fresh or dried leaf midribs, along with negative control midribs (Citrus paradisi 'Star Rubis') and PCR amplification was done with the species-specific primers A2/J5 (4) and GB1/GB3 (5). Only Las-specific 703-bp amplicons were obtained (n = 43) and 20 were sequenced (Beckman Coulter Genomics, United Kingdom; sequences available through GenBank Accession Nos. KF699074 to KF699093) and blasted against the National Center for Biotechnology Information non-redondant database (NCBI-nr). BLAST analysis revealed 100% identity with the 50S ribosomal protein subunit L1 (rplA) and L10 (rplJ) of 'Ca. L. asiaticus' (all strains), and no significant homology to other organisms. Additionally, sequence assembly on a reference genome (NC 012985) showed 100% homology. Huanglongbing was detected in Guadeloupe on March 2012 at Le Moule (East coast) in a Tahiti lime orchard (C. latifolia) and crossed the island in 6 months. Las was detected in Martinique on May 2013 on Tahiti lime (C. latifolia) at Bellefontaine (Northwest) in a private garden and at Le Lorrain (Northeast) in an orchard. Other species from the Rutaceae family were affected by HLB (C. reticulat and C. sinensis) on both islands; however, few of the positive samples showed HLB symptoms (blotchy mottle patterns and green islands on leaves), but presented symptoms similar to nutrient deficiencies. Despite the former presence of T. radiata in Guadeloupe and its detection in Martinique a few weeks after the detection of D. citri, where it had a mean parasitism rate of 70%, an outbreak of HLB spread across both islands. These analyses confirm the presence of HLB in Martinique and Guadeloupe and to our knowledge represent the first report of Las in the French West Indies. Introduction events remain unclear, but this report raises the importance of plant certification, psyllid population control, and surveillance of territories close to the French West Indies, with regards to the risk that HLB presents to citrus production worldwide.

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