First Report of a Huanglongbing-Like Disease of Citrus in Sao Paulo State, Brazil and Association of a New Liberibacter Species, "*Candidatus* Liberibacter americanus", with the Disease. D. C. Texeira and J. Ayres, Fundecitrus, Av. Dr. Adhemar Pereira de Barros, 201, CEP 14807-040, Araraquara, SP, Brazil; E. W. Kitajima, F.A.O. Tanaka, CEP 13418-900, Piracicaba, SP, Brazil; and L. Danet, S. Jagoueix-Eveillard, C. Saillard, and J. M. Bové, Institut National de la Recherche Agronomique and Université de Bordeaux 2, Laboratoire de Biologie cellulaire et moléculaire, BP 81, 33883-Villenave d'Ornon cedex, France. Plant Dis. 89:107, 2005; published on-line as DOI: 10.1094/PD-89-0107A, 2005. Accepted for publication 7 October 2004.

Huanglongbing (HLB) (ex-greening) is one of the most serious diseases of citrus. The causal agent is a noncultured, sieve tube-restricted a-proteobacterium, "*Candidatus* Liberibacter africanus" in Africa and"*Candidatus* Liberibacter asiaticus" in Asia (2). The disease has never been reported from the American continent. However, *Diaphorina citri*, the Asian psyllid vector of HLB, is found in South, Central, and North America (Florida and Texas). Early in 2004, leaf and fruit symptoms resembling those of HLB were observed in several sweet orange orchards near the city of Araraquara, Sao Paulo State. Leaf mottling on small and large leaves was the major symptom. Shoots with affected leaves were yellowish. Fruits were small and lopsided, contained many aborted seeds, and appeared more severely affected than were plants infected with classic HLB. Forty-three symptomatic samples and twenty-five samples of symptomless sweet orange leaves from five farms were analyzed for the presence of the HLB-liberibacters using polymerase chain reaction (PCR) with two sets of HLB-specific primers for amplification of 16S rDNA (2,3) and ribosomal protein genes (1). None of the 43 symptomatic leaf samples gave a positive PCR amplification, while HLB-affected leaves from the Bordeaux HLB collection produced the characteristic amplicons with both sets of primers. The 43 symptomatic and the 25 symptomless leaf samples were then analyzed using PCR with universal primers for amplification of bacterial 16S rDNA (4). All symptomatic leaf samples, but none of the symptomless leaf samples, yielded the same 16S rDNA amplification product, indicating the presence of a bacterium in the symptomatic leaves. This was confirmed using the observation of a sieve tube restricted bacterium by electron microscopy. The 16S rDNA product was cloned, sequenced, and compared with those of "*Ca.* L. africanus" and "*Ca.* L. asiaticus". While the 16S rDNAs of these two liberibacter species have 97.5% sequence identity, the 16S rDNA sequence of the new bacterium shared only 93.7% identity with that of "*Ca.* L. asiaticus" and 93.9% with that of "*Ca.* L. africanus". The 16S rDNA sequence of the new bacterium had a secondary loop structure characteristic of the a subdivision of the proteobacteria and possessed all the oligonucleotide signatures characteristic of the liberibacters. For these reasons, the new bacterium is a liberibacter and is sufficiently different phylogenetically from known liberibacters to warrant a new

species, “*Candidatus* Liberibacter americanus”. Specific PCR primers for amplification of the 16S rDNA of the new species have been developed. They were able to detect “*Ca.* L. americanus” in 214 symptomatic leaf samples from 47 citrus farms in 35 municipalities, while the “old” species, “*Ca.* L. asiaticus”, has been found only four times within the 47 farms.

References: (1) A. Hocquellet et al. Mol. Cell. Probes, 13:373, 1999. (2) S. Jagoueix et al. Int. J. Syst. Bacteriol. 44:379, 1994. (3) S. Jagoueix et al. Mol. Cell. Probes 10:43, 1996. (4) W. G. Weisburg et al. J. Bacteriol. 173:697, 1991.