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'*Candidatus* Liberibacter' in four indigenous Rutaceous species from South Africa

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'*Candidatus* Liberibacter africanus' (Laf), a phloem restricted, gram negative bacteria of the α-proteobacteria is the agent associated with Citrus greening disease in South Africa. A related bacterium '*Candidatus* Liberibacter africanus spp. capensis' (LafC) was previously described from an indigenous Rutaceae tree, *Calodendrum capense.* This led to the hypothesis that other indigenous Rutaceous trees may also be infected with Liberibacters related to either Laf or LafC. Samples from 289 *Vepris lanceolata,* 231 *Zanthoxylum capense,* and 234 *Clausena anisata* were collected from within the natural distribution of these trees in South Africa. Total DNA was extracted and tested for the presence of a Liberibacter using a generic Liberibacter real-time PCR. '*Candidatus* Liberibacters' present in positive samples were characterised by amplifying and sequencing the β-operon, 16S and *omp* gene regions. The percentage of Liberibacter positive samples differed per tree species with 6% *V. lanceolata*, 4% *Z. capense* and 11% *C. anisata* respectively, being infected. Phylogenetic analysis of the β-operon and *omp* gene regions, revealed unique phylogenetic clusters for Liberibacters associated with each tree species. Phylogenetic analysis from the 16S gene region however indicated that sequences obtained from *V. lanceolata* and *C. anisata* were similar to 16S sequences for LafC, whereas that obtained from *Z. capense* grouped on its own. Laf has not been identified from HLB-infected orchards from other Citrus producing countries other than Africa and the Mascarene Islands. The presence of related Liberibacters from indigenous Rutaceae species in South Africa may therefore suggest that Laf originated on the African continent.