Disease Note

First report of field population of *Trioza erytreae* carrying the Huanglongbing associated pathogen, *Candidatus* Liberibacter *asiaticus*, in Ethiopia

I. J. Ajene, † International Center of Insect Physiology and Ecology/ Department of Genetics, Stellenbosch University/Department of Crop Protection, Ahmadu Bello University Zaria, Nigeria; **F. Khamis, S. Mohammed, B. Rasowo and F. L. Ombura,** International Center of Insect Physiology and Ecology; **G. Pietersen and B. van Asch,** Department of Genetics, Stellenbosch University, Stellenbosch; **S. Ekesi,** International Center of Insect Physiology and Ecology.

African Citrus greening is a destructive disease of citrus that has been reported in South Africa since the 1920s. The disease is associated with "*Candidatus* Liberibacter africanus" (Laf) and is transmitted by *Trioza erytreae* (McClean 1974, Cook et al. 2014). The related bacteria *"Candidatus* Liberibacter asiaticus" (Las), which is associated with the much more severe Huanglongbing disease and is transmitted by *Diaphorina citri,* was recently reported in Ethiopia (Saponari et al. 2010). Experimentally, *T. erytreae* has been proven to transmit Las (Massonié et al., 1976), but natural occurrence of Las in field populations of this psyllid has not been reported. A survey was conducted for the citrus greening vector *T. erytreae* in the Amhara region of Ethiopia in November 2017. *Trioza erytreae* adults were identified as per the descriptions of OEPP/EPPO (2005). Sampling sites included large and small-scale citrus orchards (in both the highland and lowland areas), and citrus trees grown in backyard gardens. *T. erytreae* were found and collected from sweet orange, lemon and tangerine trees in backyard gardens and a small-scale commercial orchard (Supplementary Table S1). Voucher specimens were deposited at the International Centre of Insect Physiology and Ecology repository. Adult *T. erytreae* collected were screened for the presence of various strains of Liberibacter bacteria. The samples were surface-sterilised using 3% sodium hypochlorite and rinsed with distilled water. Genomic DNA was extracted from individual insects using the Isolate II Genomic DNA Kit (Bioline, United Kingdom), following the manufacturer's instructions. Leaf samples from citrus trees on which the psyllids were collected were also tested and plant total DNA was extracted from individual petioles. DNA quality and quantity checks were performed using a Nanodrop 2000/2000c Spectrophotometer (Thermo Fischer Scientific, USA). Conventional PCR assays were done to amplify the 50s ribosomal protein L10 gene region (*rplA-rplJ*) of Las and Laf using primers A2 and J5 (Hocquellet et al. 1999), generating the expected 650-bp product from 70 insect samples and the plant samples from each site. Amplicons were purified and bi-directionally sequenced. The *rplA-rplJ* sequences were aligned with reference Las sequences retrieved from GenBank (www.ncbi.nlm.nih.gov/genbank/) using the MUSCLE tool in MEGA X (Kumar et al., 2018). Sequences from this study (GenBank Accession No. MH809485) had a 100% base-pair match with Las (GenBank Accession No. MG418842.1). A maximum likelihood phylogenetic tree constructed for the ribosomal protein gene sequences

revealed that the Liberibacter obtained from *T. erytreae* clustered with Las and clustered separately from Laf and *Candidatus* Liberibacter solanacearum species (Supplementary Figure S1). To the best of our knowledge, this is the first report of field populations of *T. erytreae* carrying Las in Ethiopia. Furthermore, the detection of Las (initially reported to be solely transmitted by *D. citri*) in sweet orange, lemon and tangerine trees in an area with the presence of *T. erytreae* highlights the potential of this psyllid to transmit Las. Therefore, this study provides new insight into a possible alternate route of proliferation of Las in the absence of *D. citri* and it raises the need to determine the transmission efficiency, vector competency and the vector-pathogen relationships in field populations of the psyllid (*T. erytreae*).

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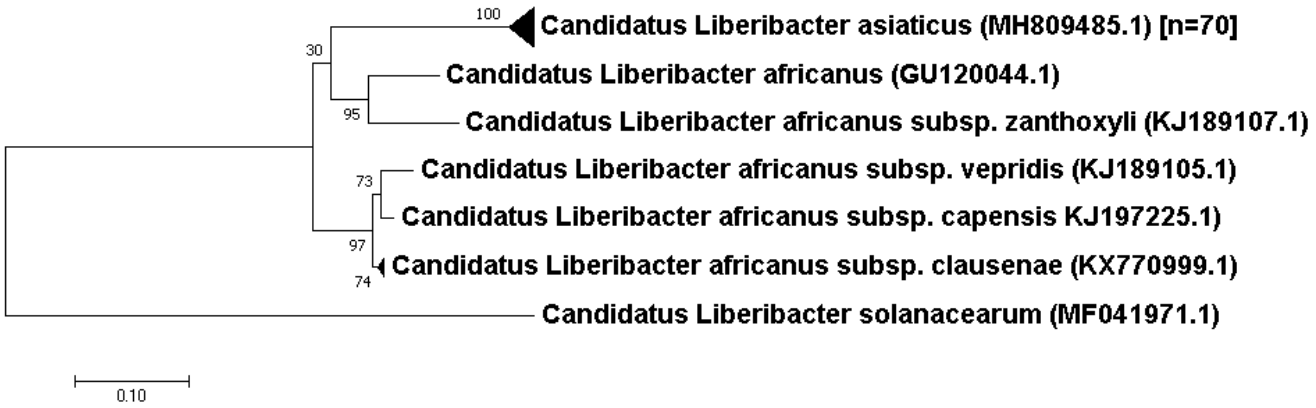
Supplementary Table S1: Detection of *Candidatus* Liberibacter *asiaticus* by conventional PCR in specimens of *Trioza* collected in Ethiopia

Region	Location	Latitude, Longitude	Altitude (m.a.s.l)	PCR +/Total psyllids tested	Plants Tested (PCR)	Host plant species	Symptoms
Amhara	Dangila	11.26105, 36.84836	2116	12/15	Positive	Sweet orange	Leaf galls
Amhara	Shuwabere	11.35555, 36.95422	1979	10/15	Positive	Lemon	Leaf galls
Amhara	Insude	11.41575, 37.16275	1959	10/15	Positive	Tangerine	Leaf galls, d
Amhara	Achabere	11.48809, 37.29512	1983	12/15	Positive	Sweet orange	Leaf galls, d
Amhara	Zenzelima	11.598, 37.44383	1868	11/15	Positive	Sweet orange	Leaf galls, y
Amhara	Sasaberete	11.60664, 37.45234	1892	15/15	Positive	Sweet orange	Leaf galls, d



Supplementary figure S1: Maximum-Likelihood tree based on a 650 bp alignment of 70 sequences of the 50S ribosomal protein L10 (rplA-rplJ) gene from Trioza erytreae samples collected from Ethiopia compared to species of the genus Liberibacter. Bootstrap values based on 1000 replicates are indicated at branches, GenBank accession numbers are shown on the tree for sequences included in analyses. The number of sequenced Liberibacter positive samples is indicated in brackets. Candidatus Liberibacter solanacearum was used as the outgroup.