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SHORT COMMUNICATION

Presence and distribution of the African citrus psyllid in São Tomé island

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Abstract

The African citrus psyllid, *Trioza erytreae* (Hemiptera: Triozidae), is a natural vector of *Candidatus* Liberibacter spp., the causal agents of the citrus greening disease or Huanglongbing (HLB). Despite the global losses of the Citrus industry due to HLB, the vector is rapidly spread to new areas threatening citrus crops and raising concern for stakeholders. Until now, there was a single old register of *T. erytreae* from the São Tomé island (Bulletin of the British Museum of Natural History, Entomology, 49, 1–102). In this work, we provide up-to-date occurrence data on the presence of *T. erytreae* throughout the isle of São Tomé. The identity of the captured adults was assessed and confirmed through molecular identification by sequencing a portion of the mitochondrial COI. The psyllid was found in lemon, orange and tangerine orchards. Further monitoring is advisable to manage potential outbreaks.

KEYWORDS
Africa, distribution, HLB, occurrence, *Trioza erytreae*

1 | INTRODUCTION

The 'citrus greening disease' or *Huanglongbing* (HLB) is currently the most devastating citrus disease worldwide (Gottwald, 2010). HLB has so far caused significant losses or threatened the production in the USA (Hodges & Spreen, 2012), Brazil (da Costa et al., 2021; Oliveira et al., 2013), China (Gottwald, 2007), Indonesia (Bové, 2006), India (da Graça, 1991) and east Africa (Djeddour et al., 2021).

Trioza erytreae, the African citrus psyllid (AfCP), is one of the vectors of the putative causal agent of HLB, *Candidatus* Liberibacter spp. *Trioza erytreae* is considered native to south-eastern Africa and was initially described from individuals collected from *Citrus limon* (L.) (see Cocuzza et al., 2017). Until 2002, *T. erytreae* was reported from Sub-Saharan Africa, Saudi Arabi, Yemen, the islands of St. Helena, Mauritius, Reunion, Madagascar (EPPO, 2022) and Macaronesia (West Palaearctic) (Carvalho & Aguiar, 1997; González-Hernández, 2003). In 2014, *T. erytreae* reached mainland Europe through north-western Spain and spread throughout the Iberian Peninsula (mainland Spain and Portugal) (DGAV, 2021; Pérez-Otero et al., 2015). To the best of our knowledge, *T. erytreae* was reported a single time from the São Tomé island until now (see Hollis, 1984). Here we provide up-to-date occurrence data for AfCP throughout São Tomé.

2 | MATERIAL AND METHODS

A total of 46 localities distributed throughout the central and northern area of the São Tomé island were selected and a series of *C. limon* (lemon), *Citrus sinensis* (L.) (orange), *Citrus tangerina* Tanaka

(tangerine) and *C.×paradisi* Macfad (grapefruit) orchards were visually inspected from 14 to 30 June 2021 for the presence of *T. erytreae*, either eggs, nymphs or adults (only symptomatic leaves/shoots were not considered). An extra locality was inspected in Principe Island (Belo Monte; 326825, 186297; zone 32N; 140 m.a.s.l.). The occurrence sites of *T. erytreae* in the isle were projected on the land cover map GlobCover 2009 (ESA-UCLouvain, 2010). A pool of captured adults was preserved and prepared for morphological and molecular identification. Morphological identification followed OEPP/EPPO (2005) whereas molecular identification was conducted by sequencing a portion of the mitochondrial COI. The genomic DNA was extracted from the whole adult psyllid using the Speedtools tissue DNA extraction kit (Biotools) and followed the manufacturer's tissue DNA extraction protocol. All the extracted DNA was stored at −20°C. PCR amplification of the 710 bp mtDNA COI barcode sequences was performed using the universal primers LCO1490/HCO2198 (Folmer et al., 1994). PCR amplification in a total volume of 20 µl was performed using the following components: 14.2 µl of H2O, 2 µl of 1× buffer, 0.4 µl of dNTPs, 0.4 µl of forwarding primer (10 µM), 0.4 µl of reverse primer (10 µM), 0,1 µl of Taq DNA polymerase (BIORON, Gmbh) and 2 µl of template DNA. The PCR cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 45 s, extension at 72°C for 45 s and a final extension at 72°C for 10 min. Identification of PCR products was performed at 1% (v/v) agarose gel stained with 1X Gel Red™ nucleic acid gel dye (Biotium), and amplified products were then purified and sequenced at Macrogen Inc. The DNA sequences were analysed and

TABLE 1 Results of the survey of *Trioza erytreae* conducted in São Tomé Island

Site	Coordinates				Altitude (m.a.s.l.)	Orchard			Life stage		
	X	Y	Zone	Hemisphere		Lemon	Orange	Tangerine	Eggs	Nymphs	Adults
Água Casada	236220	42940	32	N	100	×	×				
Água Casada	240772	41022	32	N	91	×	×				
Água Creola	242870	34282	32	N	206	×	×			×	
Belém	239976	32678	32	N	433	×	×			×	×
Belém	240766	33962	32	N	274	×	×			×	
Bernado Faro	240483	25620	32	N	227	×	×			×	
Boa Entrada	238909	37469	32	N	276	×			×	×	×
Boa Entrada	238854	37801	32	N	296	×	×	×		×	×
Capela	241924	34526	32	N	233	×	×	×		×	
Conde	241248	40917	32	N	287	×				×	×
Diogo Vaz	221282	35226	32	N	56	×	×				
Dona Agusta	235582	11613	32	N	46	×	×				
Enrique Santo	242618	22007	32	N	71	×	×				
Espainha Praia	223825	26690	32	N	14	×	×				
Fratenidade	237213	14179	32	N	43	×	×				
Guadalupe	240528	40815	32	N	107	×				×	
Lemba	246692	26667	32	N	38	×	×				
Lemba	217916	27868	32	N	26	×	×				
Madalena	238452	37243	32	N	327	×				×	
Madalena	238458	37229	32	N	119	×		×		×	×
Manuel Caroça	236181	13085	32	N	58	×	×				
Manuel Caroça	236006	13166	32	N	82	×	×				
Mende Silva	243725	24493	32	N	206	×	×				
Mesquita	243721	37878	32	N	100	×	×		×	×	
Micondo	241519	18863	32	N	51	×	×				
Monte Belo	245891	23657	32	N	27	×	×				
Monte Café	238656	33425	32	N	484	×				×	

(Continues)

TABLE 1 (Continued)

Site	Coordinates				Altitude (m.a.s.l.)	Orchard			Life stage		
	X	Y	Zone	Hemisphere		Lemon	Orange	Tangerine	Eggs	Nymphs	Adults
Monte Forte	225274	37548	32	N	94	×	×				
Novo Destino	238749	33361	32	N	516	×	×			×	×
Pontas das Palmeiras	241473	27006	32	N	169	×	×			×	×
Pontas das Palmeiras	241473	26557	32	N	142	×	×				
Ponte Samú	220524	32909	32	N	24	×	×				
Quimpo II	245853	26234	32	N	83	×				×	×
Quimpo II	246676	26669	32	N	62	×				×	
Quintal Castelo	245576	23238	32	N	65	×	×				
Ribeira Peixe	231043	10840	32	N	98	×	×				
Ribeira Peixe	244550	10935	32	N	42	×	×				
Ribeira Peixe	234304	09930	32	N	26	×	×				
Rio Less	227359	38510	32	N	89	×	×				
Roça Santa Clara	240436	31056	32	N	415	×	×	×	×	×	×
Roça Santa Lusia	238787	30401	32	N	451	×	×	×		×	
Santa Margarida	239417	35889	32	N	356	×	×	×	×	×	×
Santo Amaro	242425	40649	32	N	51	×	×				
Santo Amaro	242023	40702	32	N	59	×	×				
Solidade	236797	14448	32	N	172	×	×				
Vila Irene	247281	37607	32	N	40	×	×				

edited with MEGA v10.1.8 (Kumar et al., 2016) and the identification of each specimen was confirmed by querying the GenBank database using the Nucleotide Basic Local Alignment Search Tool (BLASTn) in NCBI's website (www.ncbi.nlm.nih.gov).

3 | RESULTS

Trioza erytreae was found in 19 out of the 46 inspected localities and in three out of the four types of citrus orchards inspected (lemon, orange and tangerine) (Table 1). All the life stages (eggs, nymphs and adults) were observed during the sampling. No presence of *T. erytreae* was detected in the locality of Principe Island. The central country zone encompassing the arable land was found to be colonized by the psyllid in areas mainly encompassed by mosaic vegetation (grassland, shrubland and forest), croplands and broadleaved evergreen and/or semi-deciduous forest (Figure 1).

Both the morphological and molecular analyses confirmed the species' identity. The two DNA sequences were submitted to NCBI under the following accession numbers: SUB11096367 ST1 OM746764 and SUB11096367 ST2 OM746765. Comparison of consensus sequences of COI gene fragments from the studied specimens with the ones publicly available in NCBI database showed regions of complete similarity with homologous *T. erytreae* sequences according to BLAST (Percentage of Identity = 100%).

4 | DISCUSSION

Trioza erytreae is an important citrus pest and vector of the most dangerous citrus disease (HLB) worldwide. We found *T. erytreae* throughout the central and north part of the island and in the three crop types inspected, that is lemon, orange and tangerine. Adults, eggs and nymphs were found suggesting that *T. erytreae* is yet established and can complete the lifecycle in the study area. The obtained information is of utmost importance to control the spread of both the disease and the vector. As far as we know, the disease is not yet present, although it cannot be discarded. Since São Tomé is an island with natural physical barriers, the occurrence data provided in this work should encourage government authorities to support measures aiming at vector eradication. On the other hand, the data provided also highlights the need to develop and implement

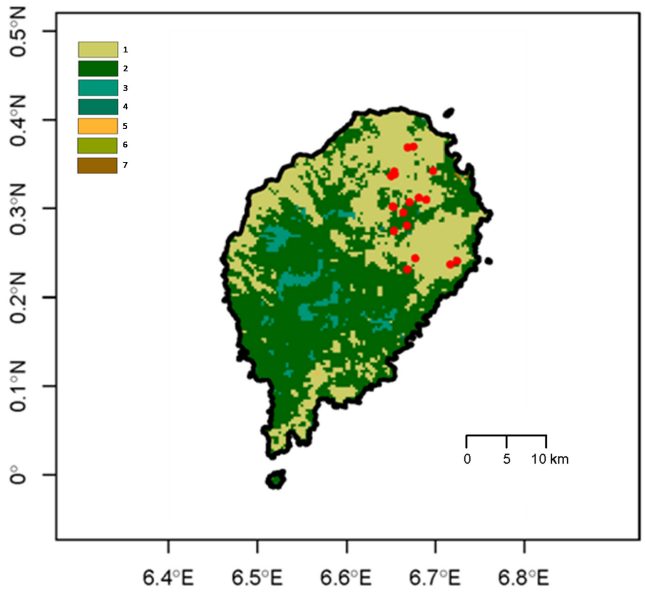

FIGURE 1 Geographical distribution of *Trioza erytreae* throughout São Tomé. Red dots represent occurrence points. Land use colours follow GlobCover 2009 (global land cover map). Legend: 1. Mosaic vegetation (grassland, shrubland, forest) (50%–70%)/cropland (20%–50%). 2. Closed to open (>15%) broadleaved evergreen and/or semi-deciduous forest (>5 m). 3. Closed (>40%) broadleaved semi-deciduous and/or evergreen forest regularly flooded - saline water. 4. Closed (>40%) broadleaved forest regularly flooded – fresh water. 5. Closed to open (>15%) grassland. 6. Mosaic forest/Shrubland (50%–70%)/grassland (20%–50%). 7. Closed to open (>15%) shrubland [Colour figure can be viewed at wileyonlinelibrary.com]

legislative measures toward the limitation of the uncontrolled circulation of foreign plant materials. This will allow avoiding reintroductions and future dispersions of *T. erytreae* and strengthen official controls to prevent the entry of other pests and diseases.

AUTHOR CONTRIBUTIONS

J.A.P., M.C. and J.B.-M. conceived the idea. D.F.-O. and P.B. conducted the genetic analyses. M.J.L.X and M.C. developed the fieldwork. All authors contributed to writing and reviewing the paper. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The coordinates for the occurrence dataset are provided in Table 1.

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