New menace to horticultural and staple food crops: Identification of an emerging thrips pest (Thysanoptera: Thripidae) infesting cassava

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Abstract

Cassava (*Manihot esculenta* Crantz) is a key staple crop in Central and Western Africa, yet its production in the Democratic Republic of Congo (DRC) is increasingly threatened by insect pests. Among these, an emerging “cassava thrips” has caused substantial yield losses, but its identity remained unresolved. This study aimed to determine the species identity of this pest and assess its host range using an integrative morphological and molecular approach. Thrips were collected from cassava fields in the DRC and from *Tithonia diversifolia* in Kenya. Morphological examination of slide-mounted specimens revealed diagnostic characters consistent with *Frankliniella borinquen* Hood, including antennal segment III morphology, body colouration, and metanotal and abdominal features. Molecular analyses targeting ITS2 and mitochondrial COI-5P markers were performed, with ITS2 providing limited resolution, whereas COI-5P sequencing unambiguously confirmed species identity. Phylogenetic reconstruction and genetic distance analyses demonstrated strong conspecificity among *F. borinquen* samples and clear divergence from other *Frankliniella* species. The presence of *F. borinquen* on both cassava and *T. diversifolia* suggests a broader host range and potential host-shift dynamics, possibly facilitated by agricultural intensification and proximity of host plants. The establishment of *F. borinquen* on cassava raises concerns for crop productivity and food security, particularly given the genus’s potential as vectors of tospoviruses. These findings provide a foundation for monitoring, risk assessment, and the development of targeted, sustainable management strategies for this emerging pest.

1. INTRODUCTION

Cassava, *Manihot esculenta* Crantz, is a major staple food crop in Central and Western Africa (Ndjouenkeu 2018; Mahungu et al. 2022). In the Democratic Republic of Congo (DRC), cassava is widely consumed in diverse forms, including chikwangue, fufu, and garri (Yamaguchi, 2015; Taelon et al. 2019; Mahungu et al. 2022), and contributes to the daily caloric intake of more than 70% of the population (Jansens 2001; Mahungu et al. 2015;2022; Moloba et al. 2019a). Beyond its role in food security, cassava is a key source of income for smallholder farmers and supplies raw materials for local industries producing starch, flour, ethanol, and provitamin A-enriched products (Moloba et al. 2019b; Lukombo et al. 2022; Borku et al. 2025). Its year-round availability, drought tolerance, and capacity to thrive on marginal soils make cassava one of the most climate-resilient crops in sub-Saharan Africa (Githunguri et al. 2014; Amelework et al. 2021; Adebayo 2023; Borku et al. 2025).

Despite these advantages, cassava production in the DRC has increasingly been constrained over the past three decades by a resurgence of disease and insect pests (Mfuti et al. 2011; Muengula et al. 2012; Tata Hangy et al. 2012; Bekelana et al. 2019; Tata Hangy et al. 2022; Casinga et al. 2025). Among these emerging pests, a thrips pest locally referred to as “cassava thrips” has become a major concern due to its widespread occurrence and severity of damage observed in farmers’ fields. Thrips damage on cassava was first reported in 1998 at the Mvuazi Research Centre (MRC) in western DRC (Mfuti 2005; Tata Hangy et al. 2012). However, the symptoms were initially misattributed to infestation by the cassava green mite (*Mononychellus tanajoa* Bondar) (Tata Hangy et al. 2012). Subsequent investigations demonstrated that the characteristic symptoms, including shortened internodes, proliferation of lateral shoots resulting in witches’ broom, and leaf scarring, were attributable to thrips feeding activity (Fig. 1).

Yield losses associated with this pest on cassava crops range from 10 to 64.8%, depending on cassava variety (Tata Hangy et al. 2012), highlighting its economic impact. Although morphologically similar thrips species have been reported on Mexican sunflower weed, *Tithonia diversifolia* (Hemsl.) in Kenya (Moritz et al. 2013), no confirmed records of thrips infesting cassava have been reported outside the DRC. This observation raised concerns that the cassava-associated thrips may represent either a host-shifted population or an emerging pest species of broader regional importance. Such concerns are further amplified by the fact that several species within the genus *Frankliniella* are known vectors of economically important plant viruses affecting horticultural and staple crops (Ullman et al. 2005; Scholthof et al. 2011; Wangai et al. 2012; Zhao and Rosa 2020).

Accurate species identification is therefore a critical prerequisite for understanding biology, epidemiological risks, and potential invasion pathways of this pest, as well as for developing effective and sustainable management strategies. However, prior to this study, the ‘cassava thrips’ in the DRC had never been conclusively identified to species level. This study, therefore, aimed to identify this emerging pest using an integrative taxonomic approach that combined detailed morphological examination with molecular characterisation based on nuclear (ITS2) and mitochondrial cytochrome oxidase subunit I (COI-5P) markers.

2. MATERIALS AND METHODS

2.1 Study site

Thrips samples were collected primarily from Mvuazi Research (MRC) located in Kongo Central province, southwestern DRC, approximately 207 km away from the capital city, Kinshasa (5°26’7.69" S, 14°53’18.85” E 492 m above sea level (asl)). Additional collections were conducted at two sites in the vicinity of MRC: the Kongo village site (05˚ 26' 28.44" S, 14˚ 54' 57.24" E 459 m asl) and Mvela site (05˚ 24'58.08" S, 14˚ 49' 10.92" E 412 m asl) (Fig. 2).

The MRC landscape is predominantly characterized by savannah vegetation dominated by *Pennisetum purpureum* Schumach, *Panicum maximum* Jacquin, *Roettbolia exaltata* L.f., *Setaria megaphylla* (Steud.) T.Durand & Schinz, *Psophocarpus palustris* Desv., *Mucuna pruriens* var. *utilis* Baker, *Dioscorea bulbifera* L. and *Sorghum arundinaceum* (Desv.) Stapf. This savannah vegetation is mixed with some wood species such as *Strychnos lokua* Rich, *Entadopsis abyssinica* (Steud), *Erythrina tomatosa* Rich, and *Cussomia angolensis* Hiern (Denisoff and Devred 1954).

For comparative purposes, additional thrips samples were collected from *Tithonia diversifolia* in Kenya at three locations, namely: Nairobi (-1° 13' 18.39"S, 36° 53' 44.23"E; 1616 m asl), Muranga (0° 47' 24.3018" S, 37° 8' 13.1706" E; 1450 m asl) and Mwea (0° 36' 57.8124" S, 37° 21' 51.4902" E; 1401 m asl) (Fig. 2).

2.2 Sample collection and morphological identification

The first batch of thrips samples was collected between 1 and 30 June 2012 from ten cassava fields at two sites within the MRC: the Mvuazi plateau and valley. These sites were selected due to their intensity of cassava cultivation. A second batch of samples was collected during subsequent surveys conducted between July and August 2017, and again in September 2023, from experimental field trials of the National Cassava Program located at the MRC, Kongo village, and Mvela sites. All sample fields were separated by a minimum distance of 500 m to reduce spatial autocorrelation. Thrips sampling was done using the whole-plant or branch tapping method onto a white enamel tray, following Pearsall and Myers (2000). Approximately 20–25 thrips specimens were collected from five plants using a fine camel hair brush and immediately transferred into labelled vials containing 95% ethanol (150 mL). Each vial was labelled with geographic coordinates, date of collection, and name of the collector.

All thrips samples collected from the MRC were transported to the International Centre of Insect Physiology and Ecology (*icipe)*, Nairobi, Kenya, for identification and further processing. For morphological characterization, the specimen was macerated in 5% NaOH for 4 h and rinsed three times in distilled water. The rinsing solution was subsequently replaced with 60% ethanol, and specimens were stored for 24 h. The samples were then dehydrated through a graded ethanol series comprising of 70% (1 h), 80% (20 min), 95% (10 min), and absolute ethanol (5 min) and followed by clearing in clove oil for at least 30 min. Specimens were slide-mounted in Canada balsam following standard protocols (Muvea et al., 2014). Mounted specimens were identified using the *Lucid Key*: *Pest Thrips of the world* (Moritz et al., 2004) and *Pest thrips of East Africa* (Moritz et al., 2013). Digital imaging of diagnostic morphological characteristics was conducted following the procedures described by Gikonyo (2016).

Additional samples collected from Mwea, Muranga, and Nairobi in Kenya were confirmed morphologically following the same procedure as described above.

2.3 Molecular characterisation

2.3.1 DNA extraction and amplification

Collected samples of thrips were processed individually for molecular analyses. Genomic DNA was extracted using the Isolate II Genomic DNA Kit (Meridian Biosciences, London, United Kingdom), following the manufacturer's instructions. DNA was eluted in a final 50 µl, after which DNA quality and quantity were assessed using the Nanodrop 2000 Spectrophotometer (Thermo Fischer Scientific, Wilmington, USA). The intergenic transcribed spacer 2 (ITS2) and the mitochondrial cytochrome oxidase subunit I (COI-5P) gene regions were amplified by polymerase chain reaction (PCR). Each PCR was performed in a total reaction volume of 20 µL containing 5X MyTaq Reaction Buffer (providing 5 mM dNTPs, 15 mM $MgCl_2$, stabilisers, and enhancers), 0.5 pmol μl^{-1} of each primer (Table 1), 0.5 mM $MgCl_2$, 0.0625 U μl^{-1} MyTaq DNA polymerase (Meridian Biosciences) and 10 ng μl^{-1} of DNA template. These reactions were set up in the Nexus Mastercycler gradient PCR machine (Eppendorf, Hamburg, Germany). PCR amplification was conducted under the following thermal cycling conditions: an initial denaturation at 95°C for 2 min, 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C for ITS2 primers or 52°C for COI-5P primers for 40 s, and extension at 72°C for 1 min, followed by a final elongation step at 72°C for 10 min.

Table 1
Primers used for molecular identification of thrips species collected in this study

Primer	Sequence 5'- 3'	Primer Cocktail	Gene region	Annealing Temp (°C)
MLepF1	GCTTTCCCACGAATAAATAATA	MLepF1/LepR1/HCO2198	COI-5P	52
LepR1	TAAACTTCTGGATGTCCAAAAAATCA			
HCO2198	TAAACTTCAGGGTGACCAAAAAATA			
28Z	AGACTCCTTGGTCCGTGTTTC	28Z/P1	ITS2	58.8
P1	ATCACTCGGCTCGTGGATCG			

The PCR products were resolved by electrophoresis on 1.2% agarose gels. DNA bands were visualised and documented using a KETA GL imaging system trans-illuminator (Wealtec Corp, Meadowvale Way, Sparks, Nevada, USA). Target amplicons were excised from the gel and purified using

Isolate II PCR and Gel Kit (Meridian Biosciences) following the manufacturer's instructions. Purified PCR products were subsequently shipped to Macrogen Europe BV (Meibergreef, Amsterdam, the Netherlands) for bi-directional Sanger sequencing.

2.3.2 Sequence analyses

Sequences obtained were assembled and edited using Geneious Version 8 (http://www.geneious.com) (Kearse et al. 2012). The primer sequences were identified and trimmed from the consensus sequences generated from both the forward and reverse reads. For species-level identification using both molecular markers, similarity searches were performed by querying the edited consensus sequences against BLAST (Basic Local Alignment Search Tool) at the GenBank database hosted by the National Centre for Biotechnology Information (NCBI). The BLAST analyses were used to identify regions of local sequence similarity by comparing the query sequences to reference sequences deposited in GenBank, thereby enabling assignment of the most closely related taxa.

2.3.3. Phylogenetic analysis

Evolutionary relationships were inferred using the Maximum Likelihood method under the Kimura 2-parameter (K2P) substitution model (Kimura 1980), in MEGA X (Kumar et al. 2018), utilising representative publicly available *Frankliniella* spp. sequences from GenBank while comparing to sequences generated from this study. The ITS2 and COI-5P phylogenetic analyses involved 245 and 331 nucleotide sequences, respectively, with the trees drawn to scale (highest log likelihood of − 56499.49 and − 6690.30, respectively), and the percentage of the associated taxa clustered together is shown next to the branches. Furthermore, pair-wise genetic distances among thrips species for both ITS2 and COI-5P gene sequences were estimated using the Maximum Composite Likelihood model (Tamura et al. 2004). Ambiguous positions were excluded on a pairwise basis using the pairwise deletion option, resulting in a final dataset of 2,239 and 799 positions for the ITS2 and COI-5P datasets, respectively. The evolutionary analyses were carried out in MEGA X (Kumar et al. 2018). In addition, Principal Coordinates Analysis was performed to visualise patterns of genetic differentiation among thrips taxa. The PCoA and associated visualisations were generated in R Studio (version 4.3.3; R Core Team 2023), using the '*ape* package'.

3. RESULTS

3.1 Morphological identification

Microscopic examination revealed that the majority of thrips specimens collected from the Democratic Republic of Congo (DRC) and Kenya, approximately 90.3% and 100%, respectively, conformed to the diagnostic morphological characters of *Frankliniella borinquen* Hood as described by Moritz et al. (2013). The remaining 9.7% of specimens from the DRC were identified as *Megalurothrips* spp. and other *Frankliniella* spp. The entire body colour of the insect was nearly uniformly pale yellow, with slightly deeper colour in the pterothorax (Fig. 3D). The legs were paler than the body, while the major setae were dark brown (Fig. 3D). The antennal segments I and II were whitish yellow, whereas segments III and IV were pale yellow basally and brown apically. Segment V was pale with faint shades at the base and the tip, while segment VI was yellowish-brown at the base, otherwise like VII and VIII brown (Fig. 3D). The forewings were uniformly pale (Fig. 3D & F).

The antenna had eight segments with segments III and IV bearing a forked sense cone (Fig. 3B), segment III had a pedicel distal to the basal ring with a convex swelling and an ill-defined collar (Fig. 3C), and segment VIII was equal to or slightly shorter than segment VII (Fig. 3B). The pedicel between antennal segments II and III was distinctly swollen, forming an edged ring surmounted by a distinctive swelling and a slightly flared collar giving a cup and saucer-shaped appearance (Fig. 3A &C). The head was wider than long (Fig. 3E); with three pairs of ocellar setae, pair III being longer than the distance between the midpoints of hind ocelli and arising on anterior margins of or just within anterior margins of the ocellar triangle (Fig. 3E). The postocular setae pair I was present, while pair IV was longer than the remaining postocular setae and approximately equal in length to the distance between hind ocelli (Fig. 3E). The Mesofurca had a well-developed spinula. The metanotal median area was transversely sculptured anteriorly and exhibited irregular reticulations on the posterior half. The median setae were longer than the lateral setae, and arose from the anterior margin; campaniform sensilla were present (Fig. 3G). Abdominal sternites III-VII possessed transverse glandular areas. Male and female specimens, with no pronounced sexual dimorphism, were observed.

3.2 Molecular characterisation

Amplification and sequencing of the ITS2 region successfully generated high-quality sequences from representative thrips samples. However, similarity searches against the NCBI GenBank database yielded matches only to closely related *Frankliniella* spp. (Table 2), with no conclusive species-level identification. This outcome reflects two inherent limitations of ITS2 for *Frankliniella* taxonomy. First, ITS2 exhibits limited interspecific divergence among closely related *Frankliniella* spp, reducing its discriminatory power (Moritz et al., 2002; Rugman-Jones et al., 2006). Second, at the time of analysis, validated ITS2 reference sequences for *F. borinquen* were absent from GenBank, precluding direct sequence matching and definitive species assignment.

Table 2
Summary of characterised thrips samples using the ITS2 region

Sample name	Morphological ID using Lucid Key	ID from GenBank	ID %	Gene region	Country/Location
MKW 2-4b	Frankliniella borinquen	Frankliniella minuta (GU980276.1)	80.46	ITS2	DRC
MVL 4-6b	Frankliniella borinquen	Frankliniella tenuicornis (AJ308592.1)	91.91	ITS2	DRC
MVL 2-8a	Frankliniella borinquen	Frankliniella cephalica (AB775359.1)	85.14	ITS2	DRC
MVL 2-4b	Frankliniella borinquen	Frankliniella tenuicornis (AJ308592.1)	91.96	ITS2	DRC
MVL 4-7a	Megalurothrips spp.	Hercinothrips femoralis AJ308596.1	82.84	ITS2	DRC
F1	Frankliniella spp.	Frankliniella xanthaner GU980325.1	96.09	ITS2	Nairobi, Kenya - Females
F2	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	92.77	ITS2	Nairobi, Kenya - Females
F3	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	90.89	ITS2	Nairobi, Kenya - Females
F6	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	93.14	ITS2	Muranga, Kenya - Females
F7	Frankliniella borinquen	Frankliniella occidentalis GU980322.1	93.99	ITS2	Muranga, Kenya - Females
F8	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	92.48	ITS2	Muranga, Kenya - Females
F11	Frankliniella spp.	Frankliniella xanthaner GU980325.1	97.22	ITS2	Mwea, Kenya - Females
F12	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	82.63	ITS2	Mwea, Kenya - Females
F13	Frankliniella borinquen	Frankliniella tritici KC513061.1	92.32	ITS2	Mwea, Kenya - Females
MKW 4-4b	Frankliniella borinquen	Frankliniella occidentalis	93.61	ITS2	DRC
KG 2-5a	Frankliniella borinquen	Frankliniella occidentalis	92.75	ITS2	DRC
MKW 4-10a	Frankliniella spp.	Frankliniella brunnea KY605020.1	95.77	ITS2	DRC
M13	Frankliniella borinquen	Frankliniella tritici KC513061.1	96.14	ITS2	Mwea, Kenya - Males
M12	Frankliniella borinquen	Frankliniella tritici KC513061.1	95.22	ITS2	Mwea, Kenya - Males
M11	-	Frankliniella tenuicornis AJ308592.1	92.27	ITS2	Mwea, Kenya - Males
M8	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	96.63	ITS2	Muranga, Kenya - Males
M7	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	92.43	ITS2	Muranga, Kenya - Males
M6	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	92.34	ITS2	Muranga, Kenya - Males
M3	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	88.88	ITS2	Nairobi, Kenya - Males
M1	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	96.11	ITS2	Nairobi, Kenya - Males
Fbm5	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	92.34	ITS2	Mwea, Kenya - males
Fbm4	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	88.88	ITS2	Mwea, Kenya - males
Fbm3	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	92.34	ITS2	Mwea, Kenya - males

Sample name	Morphological ID using Lucid Key	ID from GenBank	ID %	Gene region	Country/Location
Fbm2	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	92.34	ITS2	Mwea, Kenya - males
Fbm1	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	92.34	ITS2	Mwea, Kenya - males
FbF1	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	82.63	ITS2	Mwea, Kenya - females
FbF2	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	82.63	ITS2	Mwea, Kenya - females
FbF3	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	82.63	ITS2	Mwea, Kenya - females
FbF2	Frankliniella borinquen	Frankliniella tenuicornis AJ308592.1	82.63	ITS2	Mwea, Kenya - females
FbF1	Frankliniella borinquen	Frankliniella tenuicornis	82.63	ITS2	Mwea, Kenya - females
Fbm5	Frankliniella borinquen	Frankliniella cephalica MN129042.1	80.6	ITS2	Mwea, Kenya - males
FbF1	Frankliniella borinquen	Frankliniella cephalica MN129042.1	82.81	ITS2	Mwea, Kenya - females
FbF2	Frankliniella borinquen	Frankliniella cephalica MN129042.1	82.81	ITS2	Mwea, Kenya - females
FbF3	Frankliniella borinquen	Frankliniella cephalica MN129042.1	82.81	ITS2	Mwea, Kenya - females
FbF2	Frankliniella borinquen	Frankliniella cephalica MN129042.1	82.81	ITS2	Mwea, Kenya - females
FbF1	Frankliniella borinquen	Frankliniella cephalica MN129042.1	82.81	ITS2	Mwea, Kenya - females

Consistent with the BLASTn query results, phylogenetic reconstruction based on ITS2 placed the sequences generated in this study within the *Frankliniella* clade but showed that they clustered separately from available reference *Frankliniella* spp (Fig. 4A), failing to resolve them with confidence at the species level. Pair-wise genetic distance analysis further highlighted both closely related and divergent thrips taxa (Table 4). The smallest intraspecific genetic distance of 0.0805 was observed between the representative *F. borinquen* samples from this study, indicating strong genetic similarity. Similarly, a low intraspecific genetic distance (0.0572) was observed between *F. tritici* (AB973013.1) and *F. xanthaner* (AB775363.1). In contrast, interspecific genetic distances were markedly higher, with the greatest divergence (0.8007) observed between FbF3 and *T. tabaci* (AB775449.1), followed by 0.1570 between Fbm3 and *F. williamsi* (LC416215.1) and 0.1315 between *F. tritici* (AB973013.1) and *F. williamsi* (LC416215.1) (Table 4).

Table 4
Pair-wise genetic distances of the ITS2 gene region sequences of different thrips species

		1	2	3	4	5	6	7	8	9	10
		FbF3	**Fbm3**	***F. tritici***	***F. cephalica***	***F. williamsi***	***F. occidentalis***	***F. xanthaner***	***F. tenuicornis***	***F. schultzei***	***T. tabaci***
1	FbF3										
2	Fbm3	0.0805									
3	AB973013.1_*F. tritici*	0.1115	0.0739								
4	AB775363.1_*F. cephalica*	0.1003	0.0633	0.0572							
5	LC416215.1_*F. williamsi*	0.1966	0.1570	0.1315	0.1565						
6	AB973007.1_*F. occidentalis*	0.1957	0.1568	0.1437	0.1320	0.1324					
7	GU980435.1_*F. xanthaner*	0.3069	0.2880	0.2532	0.2367	0.1879	0.1974				
8	KM886245.1_*F. tenuicornis*	0.2767	0.2464	0.1971	0.1980	0.1640	0.1814	0.1837			
9	LC416211.1_*F. schultzei*	0.5624	0.5338	0.4796	0.4374	0.4950	0.4340	0.7211	0.4922		
10	AB775449.1_*T. tabaci*	0.8007	0.7859	0.6747	0.6156	0.6609	0.6083	0.8816	0.7021	0.6561	

The intergenic transcribed spacer 2 (ITS2) and mitochondrial COI-5P gene sequencing provided conclusive species-level identification. BLAST analysis of the COI-5P sequences revealed high similarity to *Frankliniella borinquen*, with query coverage ranging from 93–100% and sequence identity values between 97.7% and 99.4%, accompanied by extremely low E-values (≤ 1.0×10^{-144}; Table 3). The majority of the samples matched the *F. borinquen* reference sequence ON970306.1, recently deposited in GenBank, thereby providing an authoritative molecular anchor for species confirmation. Moreover, the thrips samples in this study clustered tightly within the *F. borinquen* clade, supported by high bootstrap values (Fig. 4B). The other *Frankliniella* species formed distinct, well-resolved clades separate from *F. borinquen*, demonstrating clear phylogenetic distinction from the *F. borinquen* clade (Fig. 4B).

Table 3
Summary of characterised thrips samples using the COI-5P gene region

Sample name	Morphological ID using Lucid Key	ID from GenBank	ID %	Gene region	Country/Location
F14	Frankliniella borinquen	Frankliniella borinquen ON970306.1	99.41	COI-5P	Nairobi, Kenya - Females
F8	Frankliniella borinquen	Frankliniella borinquen ON970306.1	99.4	COI-5P	Nairobi, Kenya - Females
MVL 2–46 22	Frankliniella borinquen	Frankliniella borinquen ON970306.1	99.41	COI-5P	DRC
F6	Frankliniella borinquen	Frankliniella borinquen ON970306.1	99.4	COI-5P	Nairobi, Kenya - Females
KG 2-5a 23	Frankliniella borinquen	Frankliniella borinquen ON970306.1	99.12	COI-5P	DRC
F12	Frankliniella borinquen	Frankliniella borinquen ON970306.1	98.37	COI-5P	Nairobi, Kenya - Females
M13	Frankliniella borinquen	Frankliniella borinquen ON970306.1	99.1	COI-5P	Mwea, Kenya - Males
F15	Frankliniella borinquen	Frankliniella borinquen ON970306.1	98.98	COI-5P	Nairobi, Kenya - Females
M11		Retithrips syriacus PV029932.1	94.67	COI-5P	Mwea, Kenya - Males
F2	Frankliniella borinquen	Frankliniella borinquen ON970306.1	98.5	COI-5P	Nairobi, Kenya - Females
F9	Frankliniella borinquen	Frankliniella borinquen ON970306.1	99.4	COI-5P	Nairobi, Kenya - Females
F5	Frankliniella borinquen	Frankliniella borinquen ON970306.1	98.09	COI-5P	Nairobi, Kenya - Females
MVL 2-4a 21	Frankliniella borinquen	Frankliniella borinquen ON970306.1	99.41	COI-5P	DRC
F4	Frankliniella borinquen	Frankliniella borinquen ON970306.1	97.72	COI-5P	Nairobi, Kenya - Females
F13	Frankliniella borinquen	Frankliniella borinquen ON970306.1	97.75	COI-5P	Nairobi, Kenya - Females
MVL 3-8a 27	Frankliniella borinquen	Frankliniella borinquen ON970306.1	99.41	COI-5P	DRC
F10	Frankliniella borinquen	Frankliniella borinquen ON970306.1	97.84	COI-5P	Nairobi, Kenya - Females
M3	Frankliniella borinquen	Frankliniella borinquen ON970306.1	97.79	COI-5P	Mwea, Kenya - Males
MVL 2-8b 20	Frankliniella borinquen	Frankliniella borinquen ON970306.1	99.38	COI-5P	DRC

**Legend: MKW: Mankewa location in Mvuazi (DR Congo); MVL: Mvela location (DR. Congo); KG: Kongo location (DR. Congo); M: Males; F: Females; FbF: *Frankliniella borinquen* females; Fbm: *Frankliniella borinquen* males

Furthermore, the COI-5P pairwise genetic distance analysis revealed a clear separation between intraspecific and interspecific variation among the analysed thrips taxa (Table 5). The two *F. borinquen* representative samples from this study, FbF3 and Fbm3, showed near-zero genetic divergence (0.003) and clustered closely with the reference *F. borinquen* sequence (ON970305.1), exhibiting very low genetic distances (0.003–0.006). In contrast, genetic distances between *F. borinquen* and other *Frankliniella* species were substantially higher, ranging from 0.174 with *F. cephalica* to 0.292 with *F. schultzei*. The greatest divergence was observed between *F. borinquen* and *T tabaci* (0.289–0.301). These results demonstrate a distinct genetic gap between *F. borinquen* and other thrips species, while confirming the genetic consistency of the study samples with the reference *F. borinquen* sequence.

Table 5
Pair-wise genetic distances of COI-5P gene region sequences of different thrips species

		1	2	3	4	5	6	7	8
		T_tabaci	***F_schultzei***	***F_tenuicornis***	***F_occidentalis***	***F_cephalica***	***F_borinquen***	**FbF3**	**Fbm3**
1	PP069763.1_*T_tabaci*								
2	MF075860.1_*F_schultzei*	0.264							
3	EF213761.1_*F_tenuicornis*	0.292	0.291						
4	KY369940.1_*F_occidentalis*	0.306	0.265	0.222					
5	AB277217.1_*F_cephalica*	0.287	0.238	0.240	0.202				
6	ON970305.1_*F_borinquen*	0.289	0.292	0.229	0.231	0.174			
7	FbF3	0.296	0.305	0.253	0.241	0.191	0.006		
8	Fbm3	0.301	0.312	0.260	0.244	0.195	0.003	0	

***Fbm3** – *Frankliniella borinquen*, male (this study). ****FbF3** - *Frankliniella borinquen*, female (this study).

These COI results unequivocally resolved the cassava-associated thrips as *Frankliniella borinquen* and validated the morphological identifications. One sample was identified as *Retithrips syriacus*, indicating the presence of minor non-target thrips species within the sampled populations. The robustness of the COI-5P data reflects the higher evolutionary rate and greater interspecific divergence of mitochondrial genes, which make COI the standard barcode marker for insect species identification (Hebert et al. 2003).

Visualisation of genetic relationships using Principal Coordinates Analysis (PCA; Fig. 5A) corroborated these findings. The first principal coordinate (PC1) accounted for 71.42% of the total genetic variance, while the second principal coordinate (PC2) explained 24.44%. The representative *F. borinquen* samples (FbF3 and Fbm3) clustered closest together and showed intermediate genetic distances relative to *F. tritici*, *F. cephalica*, *F. occidentalis*, *F. tenuicornis* and *F. williamsi*. In contrast, *F. schultzei* and *F. xanthaner* appeared as outliers, while *Thrips tabaci* was the most genetically divergent taxon (Fig. 5A).

Additionally, the PCA based on the COI-5P genetic distance data confirmed the clear structuring among the analysed thrips taxa (Fig. 5B). Principal Coordinate 1 (PC1) explained 41.5% of the total genetic variation, while PC2 accounted for 23.2%, together capturing 64.7% of the overall variance. The representative *F. borinquen* samples from this study clustered tightly together and in close proximity to the *F. borinquen* reference sequence.

Sequences of the samples collected from Mvuazi, Mankewa, and Kongo sites (DR. Congo) and Mwea, Muranga, and Nairobi (Kenya) have been deposited in GenBank, NCBI, to serve as a reference for this pest.

4. DISCUSSION

This study provides the first definitive species-level identification of the ‘cassava thrips’ in the DRC as *Frankliniella borinquen* Hood, using an integrative taxonomic framework that combined detailed morphological diagnostics with molecular assays. Accurate species identification is a prerequisite for understanding pest biology and developing effective management strategies, particularly for emerging pests of economic importance. Our findings resolve a long-standing uncertainty surrounding the identity of this pest and provide a robust taxonomic foundation for future ecological and management studies.

The morphological characterisation of the thrips specimens collected from cassava in the DRC and from *Tithonia diversifolia* in Kenya revealed consistent diagnostic features of *F. borinquen*. Within the genus *Frankliniella*, *F. borinquen* is distinguished by the unique morphology of the pedicel on antennal segment III, which is similar to that of *F. invasor* but occurs in conjunction with a bicoloured body pattern (Sakimura 1972; Moritz et al. 2013; Wang et al. 2019). In addition, antennal segment III is distinctly swollen and bears an edged ring surmounted by a distinctive swelling and a slightly flared collar, a diagnostic feature that clearly differentiates *F. borinquen* from closely related species such as *F. occidentalis* (Moritz et al. 2013; Wang et al. 2019). Additional distinguishing characteristics include the antennal segment VIII being equal to or shorter than segment VII, the uniformly pale colouration of the antennal segment V relative to the darker segments IV and VI, and characteristic patterns of postocular setae, metanotal sculpture, and abdominal sternite II (Moritz et al. 2013). Although *F. borinquen* possesses similar morphological features to other members of the genus, particularly *F. cephalica*, the unique combination of these characters enabled reliable differentiation from closely related species.

Although morphological diagnostics strongly indicated *F. borinquen*, molecular confirmation based on the ITS2 marker alone proved insufficient due to limited sequence variability and paucity of reference sequences in public databases. The inability of ITS2 to reliably resolve *F. borinquen* underscores a broader limitation in thrips systematics, where nuclear ribosomal markers frequently lack the resolution necessary to discriminate among closely related species (Brunner et al. 2004; Mound and Morris 2007). In contrast, the incorporation of COI-5P sequencing substantially strengthened molecular identification. The high identity sequence scores and consistent matches across multiple independent samples and geographically distinct populations provided unambiguous confirmation of species identity. This highlights the critical importance of marker choice in molecular diagnostics and demonstrates that reliance on ITS2 alone would have resulted in under-identification of a pest with significant agricultural implications.

Furthermore, the detection of *F. borinquen* on cassava in the DRC and on *Tithonia diversifolia* in both Kenya and the DRC indicates a broader host range than previously documented and suggests possible host shifting. *Tithonia diversifolia*, widely used as a green manure and pesticidal field-margin plant, may function as a primary host, with cassava representing a secondary host following host expansion or shift. Such dynamics are likely facilitated by agricultural intensification, landscape modification, and the widespread cultivation of cassava in the DRC (Kandungu et al. 2013; Godifrey et al. 2018). In addition, human-mediated movement of planting material and the close spatial proximity of cassava fields to *T. diversifolia* stands may enhance pest spillover between hosts.

Given that species of *Frankliniella* are well-known vectors of tospoviruses, the establishment of *F. borinquen* on cassava raises serious concerns for plant health and food security. Although vector competence has not yet been demonstrated for *F. borinquen*, its presence on a major staple crop warrants urgent investigation into its potential epidemiological role (Ullman et al. 2005; Zhao and Rosa 2020). If confirmed as a virus vector, *F. borinquen* could affect cassava productivity not only through direct feeding damage but also through virus-mediated yield losses, substantially increasing its economic impact

Genetic distance analyses further reinforced species-level delimitation. Near-zero intraspecific genetic distances were observed among *F. borinquen* samples generated in this study, indicating strong genetic cohesion and confirming conspecificity. Similarly, a relatively low genetic distance between *F. tritici* and *F. cephalica* suggests a close evolutionary relationship and a likely recent common ancestor, consistent with previous findings that closely related species may exhibit limited molecular divergence due to insufficient evolutionary time (Hebert et al. 2003; Nei and Kumar 2000). Conversely, substantially higher interspecific genetic distances were observed between *F. borinquen* and other *Franklinell*a spp, as well as between *F. borinquen* and *T. tabaci*, indicating more pronounced evolutionary divergence and clearly defined species boundaries (Mound and Kibby 1998; Brunner et al. 2004; Crespi et al.1997; Mound and Morris 2007). These patterns are concordant with known morphological and ecological differentiation within the group.

Findings from both the phylogenetic analyses and the pair-wise sequence comparisons indicate that the Lucid key was instrumental in resolving the characterisation of *F. borinquen*. The sequences generated in this study will be deposited in GenBank, addressing a critical gap that previously hindered molecular identification of this species. Nevertheless, further research is warranted, particularly focusing on its genomics. For instance, Pakrashi et al. (2022) analysed the mitochondrial genomes (mitogenomes) of various thrips species, examining gene arrangement, phylogenetic relationships, and divergence time estimates, providing a framework that could be applied to *F. borinquen*. The study revealed unique gene orders within the thrips mitogenomes, which were conserved across different species but showed significant variations when compared to other insect orders. These findings highlighted the evolutionary significance of mitochondrial genome structure in thrips and reinforced the utility of mitogenomic data in resolving complex phylogenetic relationships. Moreover, the comparative analysis underscores the genetic diversity within thrips and supports the use of mitogenomes as a robust tool for species identification and phylogenetic inference (Chakraborty et al. 2018; Kumar et al. 2019).

5. CONCLUSION

This study conclusively identifies the cassava thrips in the DRC as *Frankliniella borinquen* Hood using integrative morphological and molecular characterisation approaches. Diagnostic morphological characters were consistent across specimens collected from cassava and *Tithonia diversifolia* in the DRC and Kenya. Although the nuclear ITS2 markers lacked sufficient resolution for species-level discrimination, mitochondrial COI-5P sequencing provided unambiguous identification, supported by phylogenetic and genetic distance analyses, confirming the robustness of mitochondrial barcoding for *Frankliniella* diagnostics. The confirmed occurrence of *F. borinquen* on cassava represents a previously undocumented host association in DRC and suggests ecological flexibility and potential host-shift dynamics, possibly facilitated by agricultural intensification and landscape modification. The establishment of this species on a major staple crop raises concerns about cassava productivity and food security. While vector competence has not yet been demonstrated, the known role of *Frankliniella* species as virus vectors highlights the need for further investigation. These findings provide a foundation for targeted surveillance, risk assessment, and the development of sustainable management strategies for this emerging cassava pest.

Declarations

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Clinical trial number: not applicable.

Author Contribution

Conceptualization, Writing–original draft, Reviewing, Investigation, Data curation, Formal analysis, Methodology, D.K.M.; Writing–review & editing, Methodology, Conceptualization, F.K.; Writing–review & editing, Methodology, Data curation, F.L.O.O.; Writing– review & editing, Methodology, Data curation, N.M.; Methodology, Review & editing, A.O.K.; Review & editing, T.B.; Review & editing, M.Z.M., Review & editing, K.S.A.; Conceptualization, Methodology, Review & editing, S.N.; Conceptualization, Review & editing, Funding acquisition, SS.

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Data Availability

All data supporting the findings of this study are available within the paper.

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Figures

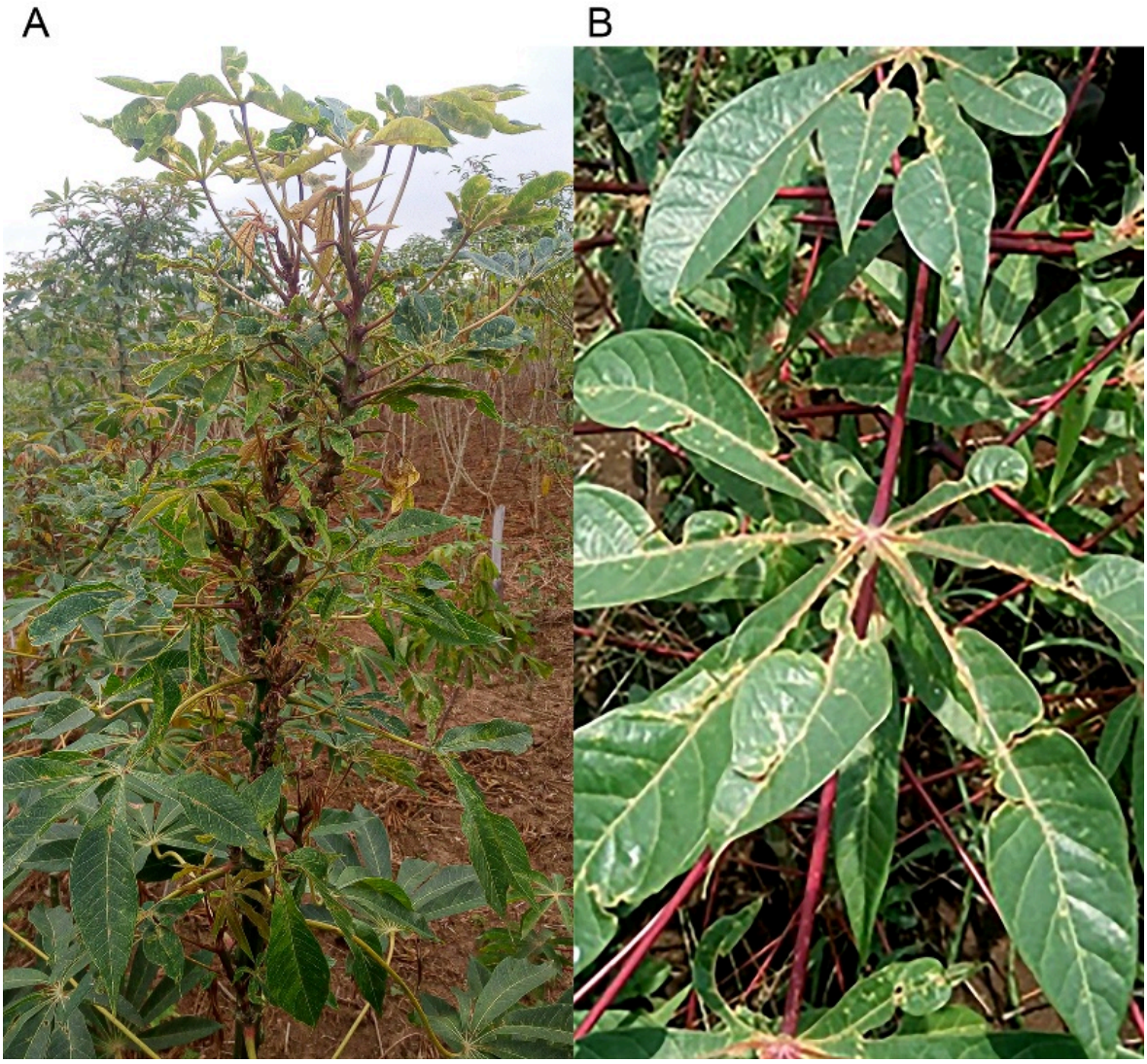

Figure 1

Symptoms caused by thrips on cassava: shortening of the internodes, lateral shoots emergence (A); holes, tears, and scars on cassava leaves (B).

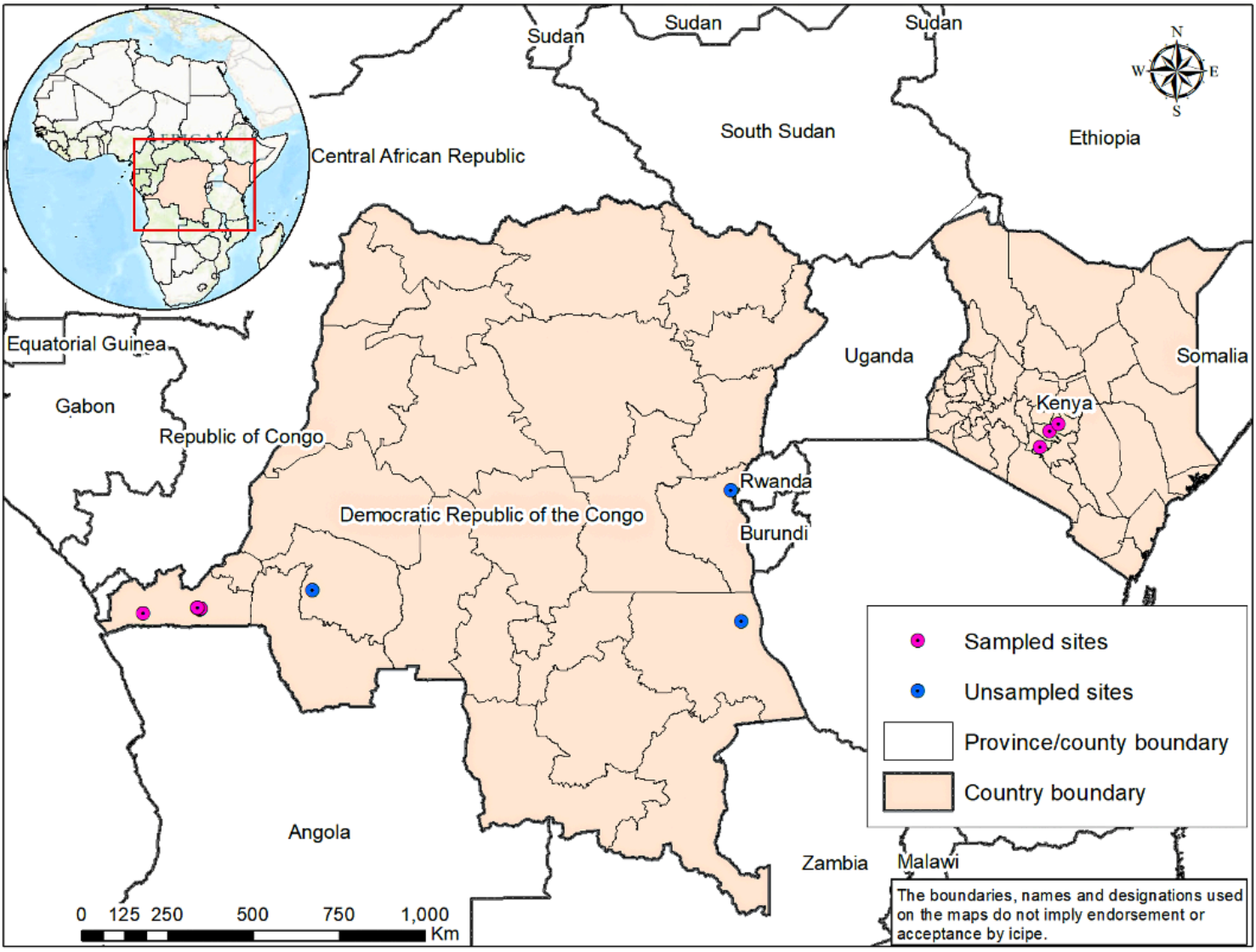

Figure 2

Map of the Democratic Republic of Congo (DRC) and the Republic of Kenya showing the locations where samples were collected and where the presence of the insect pest was confirmed in the DRC.

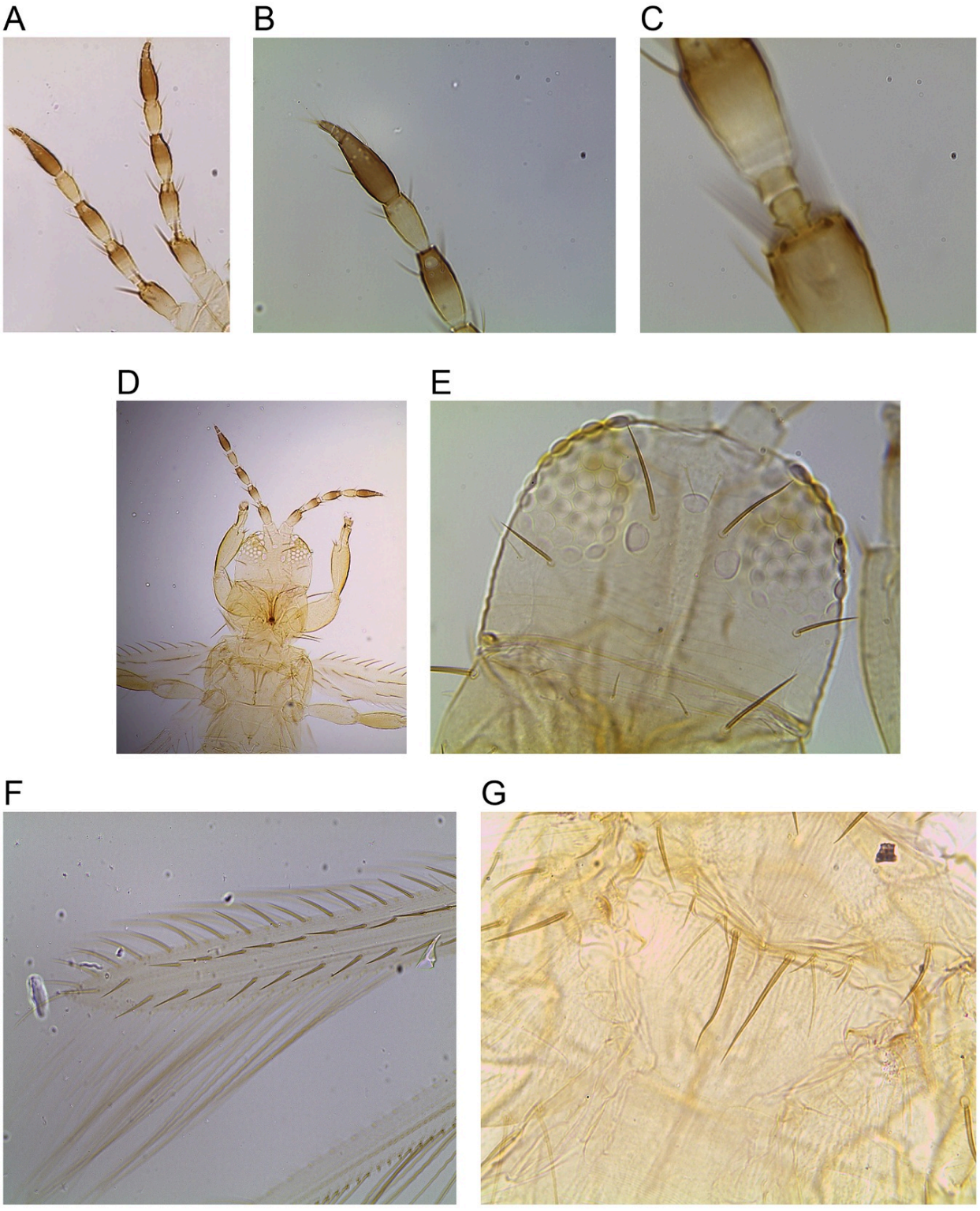

Figure 3

Antenna, scape, pedicel, and the first segment of flagellum (A). Segment VIII is equal in length to or shorter than segment VII (B). Special pedicel shape of segment III (C). Body colour is nearly uniformly pale yellow, deepest in the pterothorax (D). Head dorsal with an ocellar triangle (E). Forewing, mid and distal region (F), median setae longer than lateral setae and arising at the anterior margin (G). Pictures were taken with a Leica microscope DM 2000 LED fitted with a Leica EC3 camera (Leica Microsystems, Glattbrugg, Switzerland).

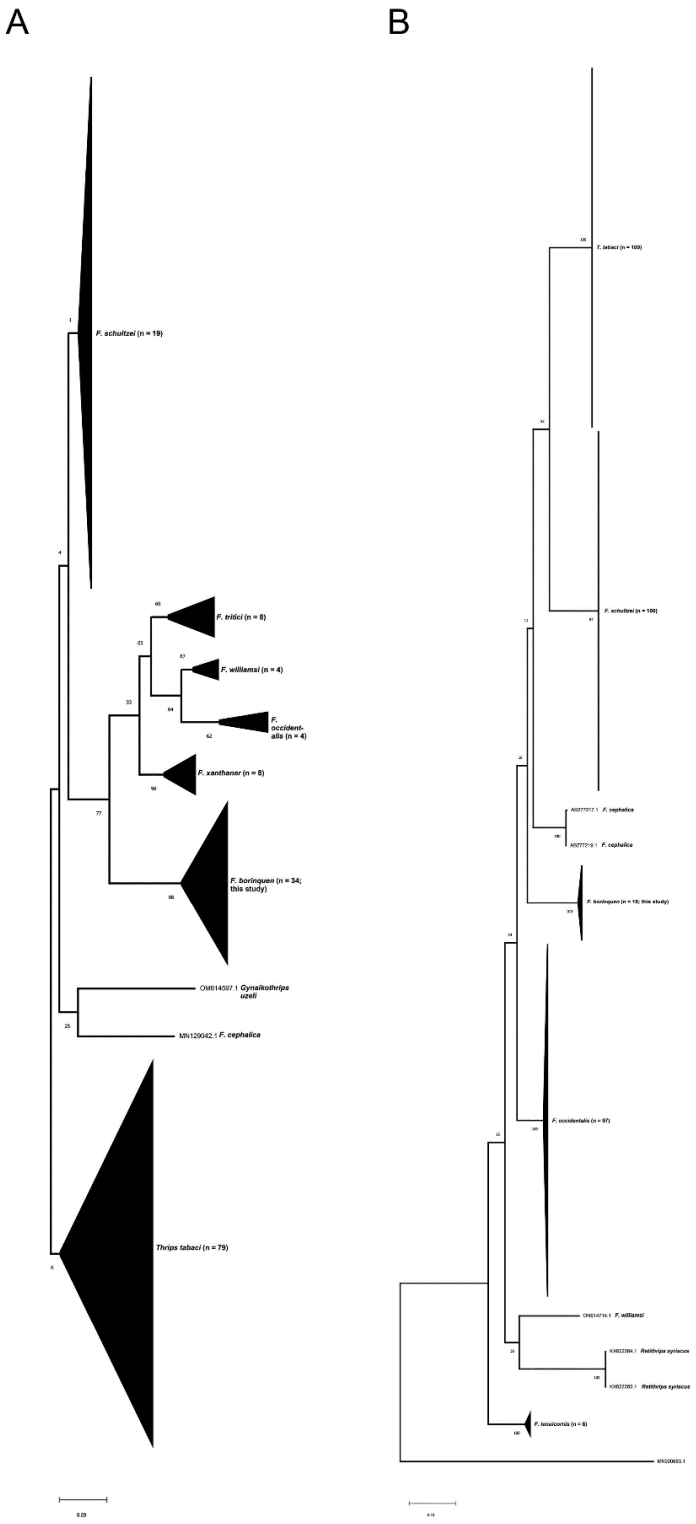

Figure 4

Phylogenetic tree analysis inferred by using the Maximum Likelihood method and the Kimura 2-parameter model. The percentage of the associated taxa clustered together is shown next to the branches. **(A)** ITS2 data set. **(B)** COI-5P dataset. The trees are drawn to scale, with branch lengths measured in the number of substitutions per site.

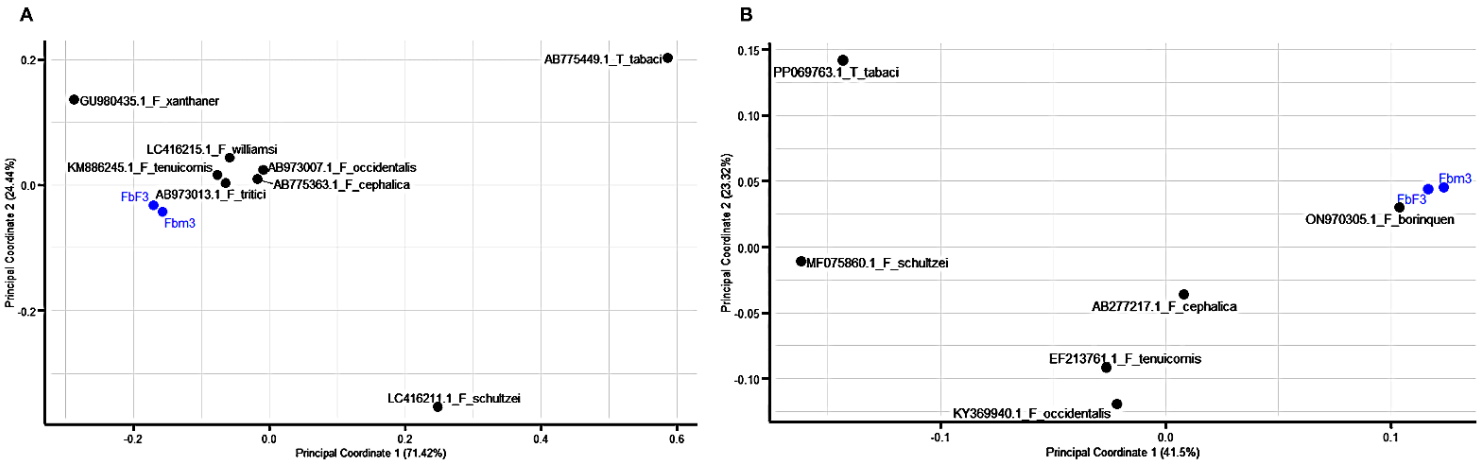

Figure 5

Principal Coordinates Analysis (PCA) of thrips genetic distances. **(A)** ITS2 data set. **(B)** COI-5P dataset.