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Molecular Identification Confirms Different Species of Cotton Jassids (Hemiptera: Cicadellidae) in Tanzania

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Abstract

Cotton (*Gossypium hirsutum* L.) is a strategic cash crop in Tanzania, supporting the livelihoods of approximately two million people and contributing substantially to the national agricultural economy through lint production and the textile industry. However, cotton productivity is severely constrained by sap-sucking insect pests, particularly cotton jassids (Hemiptera: Cicadellidae), which can cause yield losses of up to 50%. Pest management strategies have largely been implemented with limited knowledge of the actual species composition of jassids infesting cotton fields. This study aimed to establish the molecular identity of cotton-associated sucking pests, with an emphasis on jassids, in major cotton-growing areas of Tanzania. A total of 25 specimens were collected from cotton fields in Bunda, Meatu, Misungwi, and Kilosa districts. Genomic DNA was extracted using the Quick-DNA™ Mini Prep Plus Kit, and the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using COI 1-3b and COI 2-4b primer pairs. PCR amplicons were visualized on 1.2% agarose gels, and successfully amplified products were sequenced. Approximately 650-bp COI sequences were analyzed using Maximum Likelihood methods implemented in MEGA v.12 and aligned with global reference datasets to infer phylogenetic relationships. Phylogenetic reconstruction resolved well-supported clades representing *Amrasca, Jacobiasca, Ghauriana,* and *Empoasca species*. Most specimens clustered strongly with authenticated reference sequences of *Amrasca biguttula* (bootstrap support 92%–100%), confirming its presence as a dominant cotton pest. Additional samples grouped with *Jacobiasca lybica, Jacobiasca formosana, Ghauriana sinensis, Empoasca distinguenda,* and *E. flavescens*. Findings reveal considerable species diversity among cotton jassids in Tanzania and underscore the importance of integrating molecular diagnostics into routine pest surveillance to support species-specific and sustainable integrated pest management strategies.

Keywords: Cicadellidae, COI gene, DNA barcoding, phylogenetic analysis, sap-sucking pests, species diversity

Introduction

Cotton (*Gossypium hirsutum* L.) is a vital fiber crop, cultivated commercially in over 50 countries worldwide (Rahman et al., 2023). In Tanzania, it plays a significant role, being grown in 17 regions and providing employment and income to more than 500,000 households on the mainland (Ram et al., 2020). The lives of an estimated 2 million people have been reported to depend on the cotton crop. It makes a substantial contribution to export revenues and employment, particularly in the Western Cotton Growing Area (WCGA) and the Eastern Cotton Growing Area (ECGA) (Kabissa & Rwegasira, 2022). Cotton also contributes to the nation's gross domestic product (GDP), generating earnings of USD 243.07 million in 2019/20, USD 79.25 million in 2020/21, USD 125.79 million in 2021/22, USD 237.38 million in 2022/23, USD

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276.82 million in 2023/24, and USD 117.23 million in 2024/25 (Tanzania Cotton Board, 2025). The WCGA encompasses administrative regions of Shinyanga, Simiyu, Mwanza, Mara, Geita, Tabora, Kigoma, Katavi, Kagera, Singida, Manyara, and parts of Dodoma, and accounts for 97%–99% of total cotton production. The ECGA includes regions like Morogoro, Coast, Kilimanjaro, Iringa and Tanga, which collectively contribute to 1–5% of cotton production (International Cotton Advisory Committee, 2023; Kabissa & Rwegasira, 2022).

Production of cotton has for a long time been affected by several factors, including diseases, insect pests, ineffective inputs like pesticides, fertilizers, and seeds, as well as labor availability, transportation of inputs, unreliable market pricing, and climate change (Sengupta & Thangavel 2023). Worldwide, the cotton plant hosts 1,326 insect pest species, including cotton jassid, that damage the crop, leading to average yield losses of up to 60% (Kabissa & Rwegasira 2022; Patel, 2018). Cotton jassid is a polyphagous pest that feeds on various plant species, including cotton, okra, and hibiscus, and has a wide geographical distribution (Azrag et al., 2025). Adult jassids are active and can hop and fly. The eggs hatch within 4 to 15 days, and the nymph takes about 7 to 21 days to reach the adult stage, completing 7 to 12 generations in a year (Omkar, 2020). The pest causes damage by piercing plants and sucking sap, introducing toxins that inhibit photosynthesis (Jacques et al., 2024).

The associated signs include burns, cupping, and falls, which leads to stunted plant growth and reduced cotton productivity (Bhoge et al., 2019; Karim et al., 2022). Apart from Tanzania, jassid infestation has been reported in many cotton-growing countries, including India, Pakistan, China, Egypt, Thailand, Bangladesh, Côte d'Ivoire, and the United States (Bhoge et al., 2019; Lopez & Sword, 2015). Their infestation begins early in the crop season and persists throughout, with the highest population recorded during the vegetative phase due to the succulence of plant tissues (Kouadio et al., 2024). The pest is known to cause yield losses ranging from 30% to 50%, depending on the severity of the infestation (Chossterfield et al., 2024; Jindal et al., 2022). Environmental factors such as temperature, humidity, and rainfall play a crucial role in jassid population dynamics (Faheem et al., 2021). Warm temperatures and moderate humidity levels favor rapid reproduction, while heavy rainfall suppresses their population by washing away nymphs and adults. Additionally, dry conditions can enhance jassid survival and feeding activity, leading to greater crop damage (Madankar et al., 2017; Patel, 2018).

Other sucking pests include thrips (*Thrips tabaci*), cotton aphids (*Aphis gossypii*), cotton mealybugs (*Phenacoccus solenopsis*), and *Dysdercus* spp. Cotton jassid were rarely reported in Tanzania from the 1960s till recent years (Kabissa & Rwegasira, 2022; Mrosso, 2013), which suggested that the pest could have been eradicated. Recently, jassid has been reported as a ferocious and the most destructive pest in cotton production in the WCGA (International Cotton Advisory Committee, 2023). This forced changes in insecticide spray regimes from the previous three to four times to the recent four to eight times as farmers strive to overcome damages mainly inflicted by the pest on cotton in both ECGAs and WCGAs (International Cotton Advisory Committee, 2023; Kabissa & Rwegasira, 2022).

Increased insecticide spray regimes have raised cotton production costs, rendering the crop less profitable for growers. Although the government continues to subsidize insecticides and sprayers to help offset these costs, overall cotton yields have declined due to persistent pest pressure. As noted by Singh et al. (2020), the increased insecticide spraying regime puts the farm profit, goodwill, and country revenue at stake. With all these facts in mind, useful knowledge of the correct identities of jassid species affecting cotton in Tanzania was lacking. The current study investigated the molecular diversity of cotton jassid species in Tanzania's cotton-growing areas.

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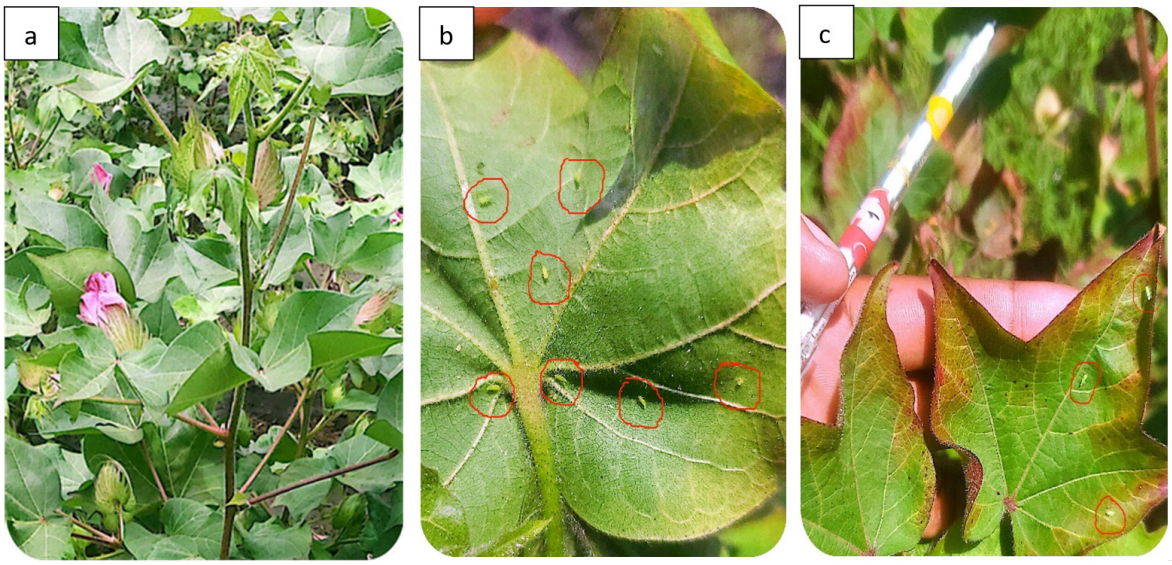
Figure 1

Stages of Cotton Jassid Infestation: (a) Uninfested Plant, (b) Jassids Present Beneath the Leaf Surface, and (c) Early Signs of Infestation with Jassids



Materials and Methods

Sample Collection for DNA Extraction

Cotton jassid samples were collected from four districts of Tanzania with varied conditions: Misungwi (2°51'3.6" S, 33°10'2.6" E), Bunda (1°58'51.3" S, 34°5'51.4" E) and Meatu (3°32′ S, 34°19′ E), located in the WCGA, and Kilosa (6°50'55.4" S, 37°39'30.8" E) located in the ECGA (Figure 2). Five fields were randomly selected in each district, with 10 infected plants selected along X-shaped transects. Ten (10) live cotton jassids were collected from each selected cotton plant using a hand net. The samples were preserved in 70% ethanol and stored in a cooler, then transferred to the laboratories of the Department of Crop Science and Horticulture at Sokoine University of Agriculture for molecular analysis.

DNA Extraction

Genomic DNA was extracted from the collected cotton jassid samples using the Quick-DNA™ Miniprep Plus Kit (Zymo Research, Irvine, CA, USA) following the manufacturer's instructions. A 200µl sample was added to a micro centrifuge tube, followed by adding 200µl BioFluid & Cell buffer and 20µl of Proteinase K. The mixture was vortexed for 15 s and then incubated at 55 °C for 10 min. 1 volume of genomic binding buffer was added to the digested sample, and the mixture was vortexed for 10-15 s. Thereafter, the mixture was transferred to a Zymo-Spin™ IIC-XLR Column in a collection tube. Centrifuge at 12,000 × g for 1 min and discard the flow. 400 µl of DNA Pre-Wash Buffer was added to the spin column in a new collection tube, and the mixture was centrifuged at ≥ 12,000 × g for 1 min; the collection tube was then emptied. Then 700 µl of g-DNA Wash Buffer was added to the spin column, followed by centrifugation at 12,000 × g for 1 min and emptying the collection tube. 200 µl of g-DNA Wash Buffer was then added to the spin column, followed by centrifugation at 12,000 × g for 1 min and discarding the flow-through from the collection tube. The spin column was transferred to a clean microcentrifuge tube, and 50 µl DNA Elution Buffer was added to the matrix. Finally, samples are incubated for 5 min at room temperature, then centrifuged at 12,000 x g for 1 min to elute the DNA. The eluted DNA was ready for PCR.

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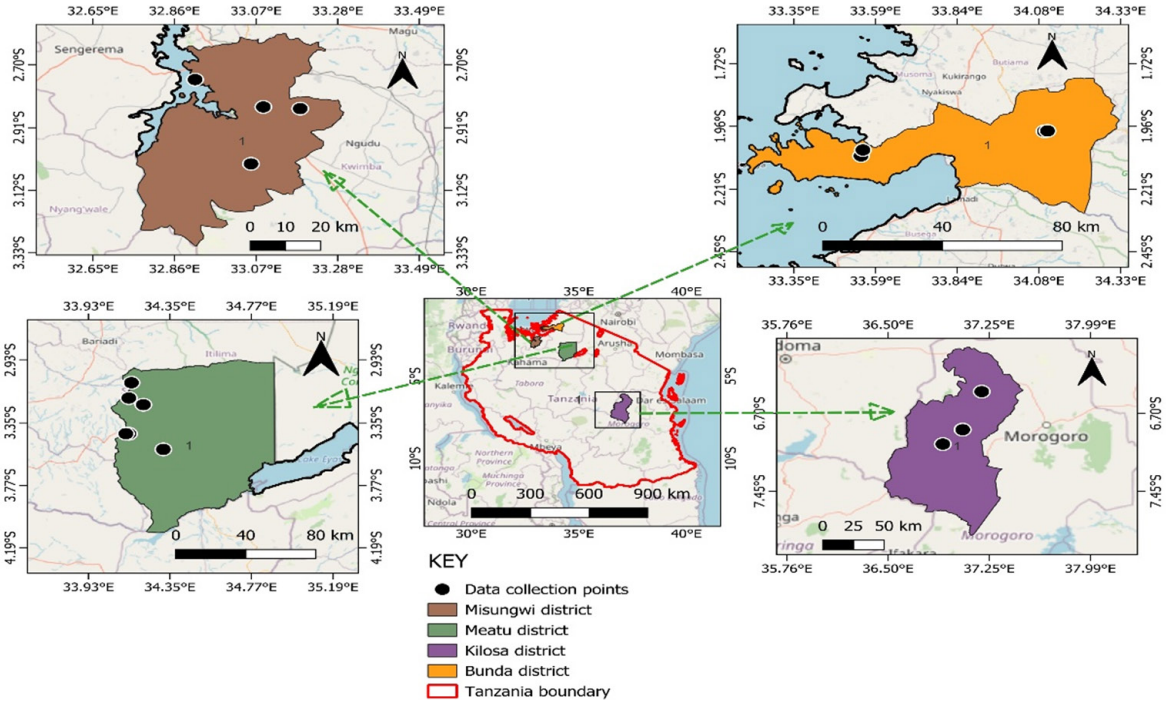
Figure 2

Geographical Locations of the Four Selected Districts and Within the Cotton-growing Regions of Tanzania, where Samples for DNA Extraction were Collected



Polymerase Chain Reaction (PCR) and Gel Electrophoresis

Amplification of isolated DNA was performed by polymerase chain reaction (PCR) targeting the mitochondrial cytochrome oxidase subunit I (COI) gene. The mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using primer pairs CO1 1-3b and CO1 2-4b, previously described by Rahman et al. (2023), which are widely used for insect DNA barcoding (Table 1). The PCR conditions consisted of an initial denaturation at 94 °C for 30 s, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 46.7 °C for 45 s, and extension at 68 °C for 59 s, with a final extension at 68 °C for 5 min. Gel electrophoresis was performed on a 1.2% agarose gel to confirm band size prior to sequencing. The gel was stained with ethidium bromide and visualized under ultraviolet (UV) light.

Molecular Identification and Phylogenetic Analysis of Cotton Jassids

After gel electrophoresis, PCR products were purified and sent for commercial sequencing. Raw sequence chromatograms (ABI files) were visually inspected for quality using Sequence Scanner Software v2.0 (Applied Biosystems). Forward and reverse sequence reads were assembled into consensus contigs for each sample, and ambiguous base calls were manually checked and corrected. Low-quality regions at the 5′ and 3′ ends were trimmed to improve sequence accuracy prior to downstream analysis. The resulting nucleotide sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) against the NCBI GenBank database to determine sequence similarity with reference taxa. Accession numbers for closely related sequences were retrieved from GenBank for comparative analysis (O'Leary et al., 2024). Multiple sequence alignment was performed using MUSCLE implemented in MEGA version 12 (Kumar et al., 2024), and alignments were

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Table 1

Primer Name and the Sequence

Region	Name	Direction	Sequence (5'-3') a	Annealing temperature (°C)
Mitochondrion Cytochrome oxidase Subunit -1	CO1 1-3b	Forward	ATAATTTTTTTTATAGTTATACC	46.7
	CO1 2-4b	Reverse	TCCTAAAAAATGTTGAGGAAA	

manually inspected to ensure accuracy. A Maximum Likelihood (ML) phylogenetic tree (Figure 3) was inferred in MEGA version 12 using the General Time Reversible model with gamma-distributed rate variation (GTR+G), selected as the best-fit nucleotide substitution model based on the Bayesian Information Criterion. Branch support was assessed using bootstrap analysis with 1,000 replicates.

Results

Species identification was achieved through mitochondrial DNA analysis, specifically targeting the cytochrome oxidase subunit I (COI) gene region. Resulting nucleotide sequences were submitted to the NCBI GenBank and assigned official accession numbers. Table 2 presents the identified species, their localities, taxonomy, and associated GenBank accession numbers. All identified jassids belonged to the order Hemiptera, family Cicadellidae, and subfamily Typhlocybinae.

Molecular Phylogeny of Cotton Jassids in Tanzania

Phylogenetic analysis resolved several well-supported clades corresponding to recognized genera and species within the family Cicadellidae. Confirmation of *Amrasca biguttula* in Tanzania: Multiple Tanzanian specimens (MS2Y and BD1) clustered tightly with reference sequences of *Amrasca biguttula* (KU684376.1; MZ427334.1; KU948694.1), forming a strongly supported monophyletic clade with bootstrap values ranging from 92%–100%. This robust relationship confirms the presence and widespread occurrence of *A. biguttula*, the principal cotton jassid pest in the surveyed regions of Tanzania.

Presence of *Jacobiasca lybica:* Several field samples, KL3Y, MSS2Y, ME3Y, BD5 (G), and MS3Y, grouped firmly within the *Jacobiasca lybica* lineage, clustering alongside established haplotypes (OQ381276.1; OQ381275.1) and an additional isolate (HM026802.1). The clade displayed strong bootstrap support (87%–100%), indicating that *J. lybica* occurs sympatrically with *A. biguttula* in Tanzanian cotton fields. Detection of *Jacobiacasa formosana*-like lineages: A distinct cluster composed of MW429482.1, MW429482.1, and MZ673803.1 represented *J. formosana*, with several Tanzanian specimens positioned adjacent to this clade. Moderate to high bootstrap support (54%–74%) suggests that populations genetically like *J. formosana* are also present in Tanzania.

Occurrence of *Ghauriana sinensis*: A separate, well-defined clade (MN698874.1; MN698874.1; MN698874.1) represented *G. sinensis*. The Tanzanian specimen ME2Y clustered within this group with 34%–54% bootstrap support, confirming the presence of this species in the regional jassid complex. Detection of *Empoasca Species:* A highly supported cluster (63%–100%) contained multiple Empoasca species, including *E. distinguenda* (PP783601.1; PP758465.1), *E. flavescens* (MK211224.1; NC024883.1) and E. vitis (NC024838.1). Several Tanzanian sequences (MS4Y, BD4(Y), BD2(Y), ME1Y, MS1 (G) and ME2Y) aligned confidently within this genus. Although the tree included an *Empoasca fabae* reference clade, none of the field samples grouped within it, indicating that *E. fabae* is absent from the Tanzanian jassid community.

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Table 2

Taxonomic Identification of Cotton Jassid Species Collected from Cotton Growing Areas of Tanzania, with Assigned GenBank Accession Numbers

Species name	ACCESSION No.	Percentage of Identity	Cover query	E-value	Sample ID	Locality
Jacobiasca formosana	MW429482.1	86.47%	99%	0.0	KL3Y	Kilosa
Ghauriana sinensis	MN699874.1	86.55%	99%	0.0		
Empoasca fabae	PQ351619.1	85.39%	98%	0.0	KL4Y	
Empoasca decipiens	OZ184581.1	85.96%	98%	0.0		
Jacobiasca formosana	MZ673803.1	85.68%	100%	2e-114	MS2Y	Misungwi
Amrasca_biguttula_biguttula	KU684376.1	98.27%	59%	1e-112		
Jacobiasca lybica	HM026602.1	94.91%	64%	0.0	MS3Y	
Empoasca fabae	PQ351619.1	86.00%	99%	0.0		
Empoasca_distinguenda	PP783601.1	95.18%	74%	1e-160	MS4Y	
Empoasca flavescens	MK211224.	86.63%	99%	0.0	MS1 G	
Empoasca vitis	NC_024838.1	86.63%	99%	0.0		
Amrasca_biguttula	MZ427334.1	99.50%	43%	0.0	BD1	Bunda
Amrasca_biguttula_biguttula	KU948694.1	98.74%	43%	0.0		
Ghauriana sinensis	MN699874.1	85.68%	97%	0.0	BD2Y	
Ghauriana sinensis	MN699874.1	85.68%	98%	0.0	BD4Y	
Empoasca flavescens	MK211224.1	84.16%	100%	0.0		
Empoasca_fabae	PQ351619.1	85.74%	100%	0.0	BD5G	
Empoasca distinguenda	PP758465.1	96.87%	83%	0.0	ME1Y	Meatu
Jacobiasca_formosana	MW429482.1	85.53%	97%	0.0	ME2Y	
Empoasca flavescens	MK211224.1	86.51%	95%	0.0		
Empoasca_fabae	PQ351619.1	85.31%	100%	0.0	ME3Y	
Jacobiasca_lybica	OQ381276.1	95.74%	75%	0.0		

The phylogenetic findings reveal that cotton-growing areas in Tanzania harbor a broad assemblage of jassid species. Among these, *Amrasca biguttula* stands out as the principal pest, accompanied by several other taxa, including *Jacobiasca lybica, J. formosana, Ghauriana sinensis,* and various *Empoasca species*, all contributing to feeding damage on cotton crops. The well-defined molecular groupings, supported by high bootstrap values at major nodes, clearly indicate the coexistence of multiple jassid taxa on cotton, with *A. biguttula and J. lybica* forming the predominant components. The COI-based phylogenetic reconstruction also substantiates the genetic identity of the jassid species present in Tanzania, most notably *Amrasca biguttula*, as well as *Empoasca and Jacobiasca species*. Clear clustering and strong bootstrap support confirm the genetic distinctiveness of these groups. Importantly, this work represents the first molecular verification of *A. biguttula* in Tanzanian cotton agroecosystems, closing a significant gap in national pest diagnostic records.

Discussion

This study constitutes the first molecular confirmation of *Amrasca biguttula biguttula* and several other jassid species (*Empoasca distinguenda, E. flavescens, Jacobiasca formosana, J. lybica, and Ghauriana sinensis*) in Tanzanian cotton agro-ecosystems using

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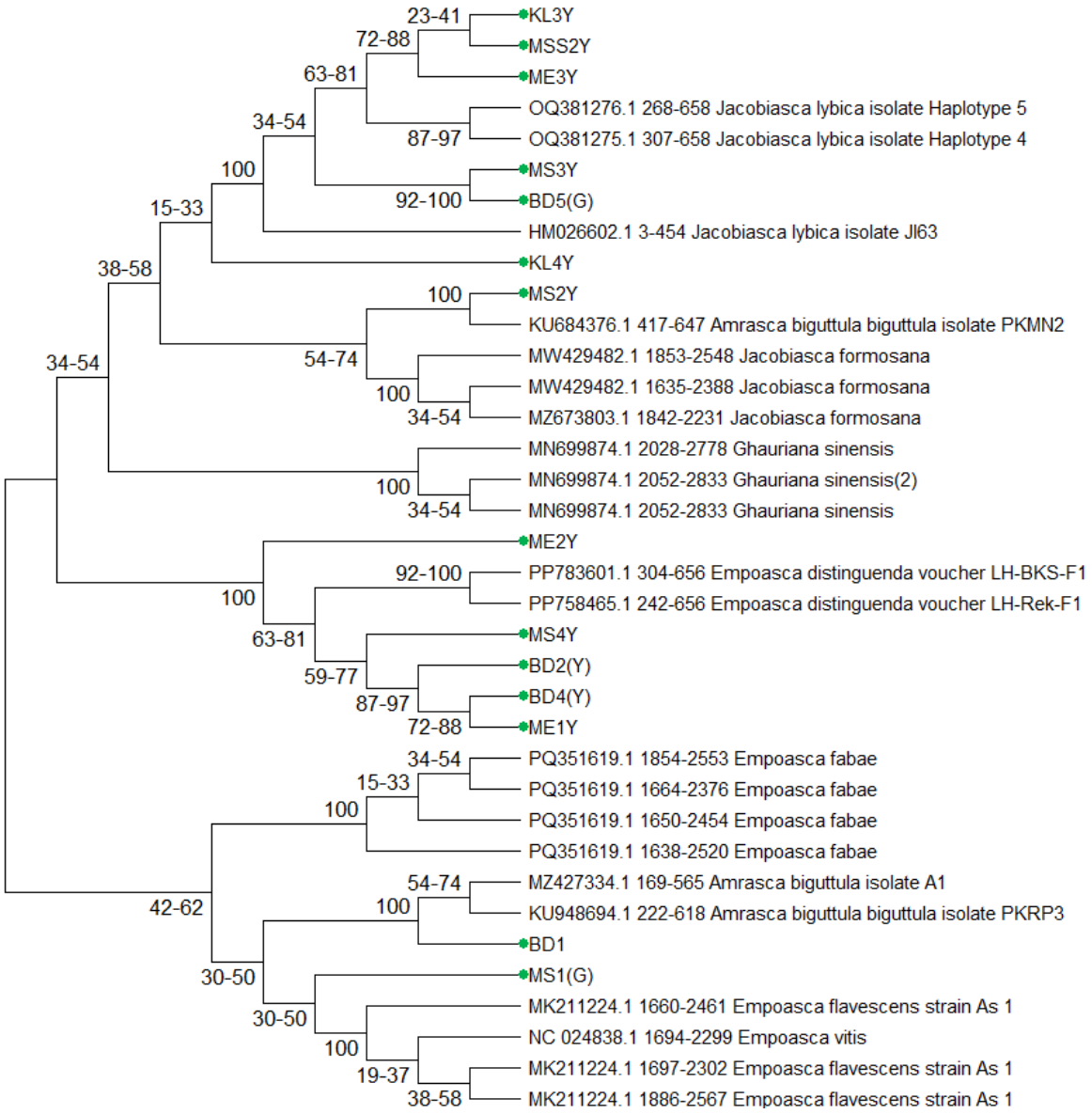
COI gene analysis. Phylogenetic reconstruction robustly grouped these species into well-supported clades (bootstrap 77%-100%), thus validating previous morphological identifications while addressing a significant knowledge gap in the region's pest biodiversity. The detection of *A. biguttula biguttula*, a major pest known to cause considerable cotton yield losses in South and Southeast Asia, is especially noteworthy and raises a wake-up call for cotton pest management in Tanzania. The co-occurrence of multiple jassid species mirrors findings from West Africa and South Asia, where complex polyphagous jassid assemblages dominate cotton fields, implying potential regional similarities in pest dynamics (Azrag et al., 2025; Esquivel et al., 2025).

The clustering of Tanzanian specimens with authenticated reference sequences

Figure 3

Maximum Likelihood Phylogenetic Tree of Cotton Jassid Species Based on COI Gene Sequences



Notes. The tree was constructed in MEGA v.12 using the GTR+G model with 1,000 bootstrap replicates. Bootstrap values (%) are indicated at the nodes. Green clades represent Tanzanian isolates, while uncolored clades denote reference sequences retrieved from GenBank.

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suggests that earlier morphological assessments underestimated species diversity, while moderate bootstrap values in some clusters may reflect haplotype diversity influenced by environmental stress and long-term insecticide selection (Jindal et al., 2022; Rahman et al., 2023). This resurgence of jassids amid increasing insecticide use in Tanzania highlights the urgent need for precise species identification and consistent resistance monitoring. Accurate molecular confirmation is essential because species such as *A. biguttula* and *J. formosana* differ in insecticide susceptibility, climatic tolerance, and ecological behavior (Omkar, 2020; Van Timmeren et al*.,* 2011). These results reinforce the utility of DNA barcoding as a complementary method to classical taxonomy for reliable pest diagnostics, enhancing decision-making in integrated pest management (Bahadur, 2012; Hebert et al., 2003). Furthermore, the study reveals that cotton jassid diversity in Tanzania is considerably underestimated, underscoring the indispensability of molecular tools in resolving cryptic or morphologically indistinguishable taxa (Phom, 2024; Podsiadło & Polz-Dacewicz, 2013).

These species are recognized pests of cotton and other crops throughout Africa, Asia, and Europe; their detection in Tanzanian cotton fields extends the known range of Cicadellidae in regional agro-ecosystems. For instance, *J. lybica* dominates West African jassid communities (Kouadio et al., 2024), while *E. flavescens and E. distinguenda* are established pests in Asia and North Africa (Bahadur, 2012; Khalafallah et al., 2006). Such documentation highlights the complex, dynamic composition of jassid assemblages, influenced by factors including trade, ecological shifts, and climate variability. The simultaneous presence of *A. biguttula biguttula* and newly confirmed jassid species poses significant challenges for Tanzanian pest management.

Current integrated pest Management (IPM) strategies must be reassessed and adapted to address this evolving pest complex marked by high species diversity. Such diversity likely contributes to variable outbreak patterns and damage severity reported by farmers (Benjamin & Rwegasira, 2024; International Cotton Advisory Committee, 2023) and may be exacerbated by climate change, altered agronomic practices, and human-mediated dispersal (Kouadio et al., 2024).

Conclusions

This study confirms the presence of *A. biguttula biguttula* in Tanzanian cotton fields and identifies a genetically distinct, undocumented jassid complex (*Empoasca distinguenda, E. flavescens, Jacobiasca formosana, J. lybica, and Ghauriana sinensis*), revealing the taxonomic complexity of jassid infestations and underscoring the need for enhanced molecular diagnostics. Despite the limited number of isolates analyzed, these baseline DNA barcoding data establish a foundation for understanding cotton jassid diversity in Tanzania. Given cotton's economic importance and reports of insecticide resistance in jassids elsewhere, future research should prioritize larger, seasonally stratified sampling; ecological characterization of species-specific life cycles and host associations; and systematic surveillance of insecticide susceptibility in Tanzanian systems, where such data are currently scarce. Integrating DNA barcoding and phylogenetic tools into routine IPM monitoring will enable early detection of cryptic or invasive species, support evidence-based control, and address population genetics and resistance mechanisms. These advancements promise sustainable cotton production and protection of farmer livelihoods against evolving pest pressures.

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