Global Journal of Agricultural Innovation, Research & Development, 2025, 39-47

Published by Avanti Publishers

Global Journal of Agricultural Innovation, Research & Development

ISSN (online): 2409-9813

Molecular Identification of Some African and Asian Tephritid Fruit Flies (Diptera) Using Mitochondrial Cytochrome Oxidase 1

Mohammed E.E. Mahmoud[1,5], Nur A.B. Adam[2], Sumaia M.A. Abukashwa[3], Mohamed A.O. Kambal[3], Alia A. Rizki[4], Samira A. Mohamed[5] and Sunday Ekesi[5]

[1]Agricultural Research Corporation, Sudan, [2]University Putra Malaysia (UPM), [3]University of Khartoum, Sudan, [4]National University of Malaysia UKM and [5]International Center of Insect Physiology and Ecology, Nairobi, Kenya

ARTICLE INFO

Article Type: Research Article

Academic Editor: Shubhajyoti Das

Keywords:
IPM
CO1
Sudan
Tephritid
Phylogenetic

Timeline:
Received: August 28, 2025
Accepted: October 20, 2025
Published: November 14, 2025

Citation: Mahmoud MEE, Adam NAB, Abukashwa SMA, Kambal MAO, Rizki AA, Mohamed SA, Ekesi S. Molecular identification of some African and Asian tephritid fruit flies (Diptera) using mitochondrial cytochrome oxidase 1. Glob J Agric Innov Res Dev. 2025; 11: 39-47.

DOI: https://doi.org/10.15377/2409-9813.2025.12.4

ABSTRACT

Fruit flies of the family Tephritidae are among the most economically significant pests of horticultural crops worldwide, causing substantial losses in fruit production and trade. Accurate identification of species is crucial for effective pest management, quarantine, and biosecurity programs. In this study, mitochondrial cytochrome oxidase 1 (CO1) sequences were used to identify 15 fruit fly species from three genera (*Bactrocera, Ceratitis*, and *Dacus*). Complete DNA and amino acid sequences were determined, and phylogenetic relationships were assessed using sequences from both African and Asian populations. The CO1 marker proved effective for precise species identification and phylogenetic analysis, providing a valuable tool for both quarantine inspections and population genetics studies. This study provides the first molecular characterization of economically important fruit flies species from Sudan including some of *Bactrocera* species using mitochondrial COI gene sequencing. Previous work in the region focused mainly on morphological identification, which can be unreliable due to overlapping features among closely related species. The inclusion of molecular tools in this research enhances diagnostic precision, supports early detection of invasive fruit flies, and strengthens the foundation for integrated pest management (IPM) programs in tropical Africa. The generated COI sequence data will serve as a reference for future phylogenetic and biogeographical analyses of tephritid flies.

---

*Corresponding Author
Email: nazeerazo@yahoo.com
Tel: +(96) 6552652759

1. Introduction

Fruit flies (Diptera: Tephritidae) are among the most economically important pests of horticultural crops worldwide, particularly in tropical and subtropical regions. They infest a wide range of fruits and vegetables, causing both direct damage through larval feeding and indirect losses due to quarantine restrictions imposed on infested produce [1-3]. Globally, fruit fly infestations are estimated to cause billions of dollars in losses annually, emphasizing the urgent need for accurate identification, monitoring, and management.

Taxonomic classification of fruit flies has historically relied on morphological characteristics such as wing patterns, coloration, and the structure of male genitalia. However, the presence of cryptic species, sibling species complexes, and significant intraspecific variation often complicates morphological identification [1, 2, 4]. For example, the *Bactrocera dorsalis* complex comprises morphologically similar species that are difficult to differentiate without molecular tools. Similarly, African *Ceratitis* species exhibit overlapping morphological traits, making species-level identification challenging [1, 2, 5].

Molecular genetic techniques have emerged as powerful tools to complement traditional taxonomy. Approaches such as PCR-RFLP, amplified fragment length polymorphisms (AFLPs), microsatellite analysis, and DNA sequencing enable precise species identification and provide insights into genetic diversity, population structure, and phylogeography [5-8]. Among these, the mitochondrial cytochrome oxidase I (CO1) gene is widely used as a “DNA barcode” due to its high rate of evolution, maternal inheritance, and sequence variability sufficient to distinguish closely related species [9-11].

Mitochondrial DNA markers have proven particularly effective in quarantine and biosecurity contexts. They allow rapid identification of pest species from immature stages, eggs, or damaged specimens where morphological characters are absent. This capability is crucial for early detection of invasive species and for preventing their spread across borders [10, 12, 13].

In Sudan, fruit fly infestations significantly impact the production of mango, citrus, guava, and other fruit crops. Despite their importance, the genetic structure and diversity of Sudanese fruit fly populations remain largely unexplored. This study aims to fill this knowledge gap by using CO1 markers to identify major African and Asian Tephritid species, assess their phylogenetic relationships, and provide baseline genetic data that can inform pest management, quarantine, and biosecurity programs.

2. Materials and Methods

2.1. Sample Collection

Fruit flies were collected from multiple orchards and farms in Sudan and Malaysia using a combination of male pheromone lures and Torula yeast traps. Collection was carried out during peak fruiting seasons to ensure the presence of target species. All specimens were morphologically identified following A simplified key to common fruit fly species prepared by Billah [14] and then preserved in 96% ethanol to prevent DNA degradation during transport and storage**.**

2.2. DNA Extraction

Genomic DNA was extracted from individual adult flies; Table **1**, using the Qiagen DNeasy Blood and Tissue Kit, following the manufacturer’s protocol with minor modifications to optimize yield. The quality and quantity of DNA were assessed using spectrophotometry and agarose gel electrophoresis to ensure suitability for PCR amplification.

2.3. Polymerase Chain Reaction (PCR)

PCR was performed using Maxime PCR PreMix Kits (iNtRon Biotechnology, Inc.), which contained 2.5 U i-Taq DNA polymerase, 2.5 mM dNTPs, 1X reaction buffer, and 1X gel loading buffer. Each 20 µL reaction contained 2 µL of extracted DNA, 1 µL of forward primer, 1 µL of reverse primer, and 16 µL of nuclease-free water. Two primer sets were used: CO1F/CO1R (targeting a ~1300 bp region of the CO1 gene) and UEA7/UEA10 (targeting a ~700 bp region) Table **2** [6, 12, 15].

Table 1: Fruit fly species and sampling locations.

Species	Location	Situation of Preservation	Sampling Date
C. capitata	Khartoum, Sudan	96% ethanol	2008
B. invadens-1	Khartoum, Sudan	96% ethanol	2008
B. invadens-2	Khartoum, Sudan	96% ethanol	2008
B. invadens-3	Khartoum, Sudan	96% ethanol	2008
B. invadens-4	Khartoum, Sudan	96% ethanol	2008
B. invadens-5	Khartoum, Sudan	96% ethanol	2008
B. longistylus	Khartoum, Sudan	96% ethanol	2008
D. vertibratus	Khartoum, Sudan	96% ethanol	2008
D. ciliatus	Khartoum, Sudan	96% ethanol	2008
B. cucurbitae	Aboukarshoula, Sudan	96% ethanol	2008
B. latifrons	UPM Farm,Malaysia	96% ethanol	2009
B. carambolae	Selangor, Malaysia	96% ethanol	2009
B. papayae-1	UPM Farm,Malaysia	Fresh	2010
B. papayae-2	Selangor, Malaysia	96% ethanol	2010
B. umbrousa	Selangor, Malaysia	96% ethanol	2010
C. quinaria	Khartoum, Sudan	96% ethanol	2008
C. cosyra	Khartoum, Sudan	96% ethanol	2008
C. incompleta	Khartoum, Sudan	96% ethanol	2008

Table 2: Oligonucleotides used for DNA amplification and sequencing of fruit fly spp.

Primer	Sequence	Reference
1st set	COIF5′TACAATTTATCGCCTAAACTTCAGCC-3′ COIR 5′ CATTTCAAGTTGTGTAAGCATC-3′	Han and Ro , 2005
2nd set	UEA10 5′TCCAATGCACTAATCTGCCATATTA-3′ UEA7 5′ TCCAATGCACTAATCTGCCATATTA-3′	Lunt *et al*; Jumnongluk *et al*. 2003

PCR conditions were optimized according to Han and Ro [12], with initial denaturation at 94°C, followed by 30–35 cycles of denaturation, annealing, and extension, and a final extension at 72°C. PCR products were electrophoresed on 1% agarose gels with 1 kb DNA markers (Promega, USA) to verify expected fragment sizes.

2.4. DNA Purification and Sequencing

PCR products displaying clear single bands were purified using the MEGAquick-spin™ Kit (iNtRon Biotechnology, Inc.) and sequenced at NHK Company, Malaysia. Both forward and reverse primers were used to obtain high-quality sequence data.

2.5. Sequence Analysis

Raw nucleotide sequences were edited and assembled into contigs using CLC Workbench software. Sequences were aligned using ClustalW and compared against the NCBI GenBank database using BLAST to confirm species identity. Phylogenetic analyses were performed using MEGA 5 and CLC Main Workbench, employing neighbor-joining and UPGMA methods. *Musca domestica* was used as an out-group to root the trees.

2.6. Nucleotide Composition and Percent Identity

The length, molecular weight, and nucleotide composition (A+T and C+G content) of CO1 sequences were calculated for each species. Percent identity matrices were generated to quantify genetic similarity among samples, and comparisons were made with sequences obtained from NCBI to assess global genetic relationships.

3. Results

3.1. DNA Extraction and PCR Amplification

Genomic DNA was successfully extracted from all 15 fruit fly species collected from Sudan and Malaysia (Table **1**). The DNA showed high purity and integrity, as verified by spectrophotometry and agarose gel electrophoresis. PCR amplification using the two primer sets (CO1F/CO1R and UEA7/UEA10) produced clear bands of expected sizes for all target species. The CO1F/CO1R primers amplified ~1300 bp fragments, while UEA7/UEA10 primers amplified ~700 bp fragments (Table **2**), confirming the suitability of these primers for molecular identification across geographically distinct populations.

3.2. Nucleotide Composition

Analysis of nucleotide composition revealed that the (A+T) content of *Bactrocera* species ranged from 778 bp in *B. umbrousa* to 866 bp in *B. invadens-3*, while (C+G) content ranged from 464 bp in *B. carambolae* to 484 bp in *B. papayae* (Table **3**). For *Ceratitis* species, (A+T) content varied between 867 bp in *C. cosyra* and 904 bp in *C. incompleta*, with (C+G) content ranging from 409 bp (*C. capitata*) to 467 bp (*C. cosyra*). *Dacus vertibratus* showed 844 bp (A+T) and 491 bp (C+G), indicating a slightly higher GC content compared to most *Bactrocera* species.

Table 3: Nucleotide composition of *Bactrocera* species (CO1F/CO1R).

Species	DNA Length (bp)	Molecular Weight (kilodalton) (kDa)	A+T Content	C+G Content
B. invadens-1	1300	425	860	440
B. invadens-2	1300	425	858	442
B. umbrousa	1300	425	778	464
B. papayae-2	1300	425	860	484

For the second primer set (UEA7/UEA10), *B. invadens* samples displayed 454–457 bp (A+T) and 239–243 bp (C+G), while *D. ciliatus* and *B. cucurbitae* showed 479/464 bp (A+T) and 216/228 bp (C+G), respectively. These results indicate a consistent nucleotide composition pattern among conspecifics, supporting their identification and highlighting the reliability of the CO1 gene as a species-level marker.

3.3. Phylogenetic Analysis

3.3.1. Bactrocera Species

Alignment of CO1 sequences revealed that five *B. invadens* samples had high sequence identity (96.5–99.4%) with each other (Table **3**, Fig. **1**), indicating low intraspecific variation. Comparisons with other *Bactrocera* species showed percent identities ranging from 80.5% (*B. longistylus*) to 95.3% (*B. carambolae*), reflecting moderate interspecific divergence. Notably, *B. invadens-3* and *B. papayae-2* shared only 30% identity, while both showed ~84.8% similarity to the remaining species, suggesting possible misidentification or high divergence in these samples.

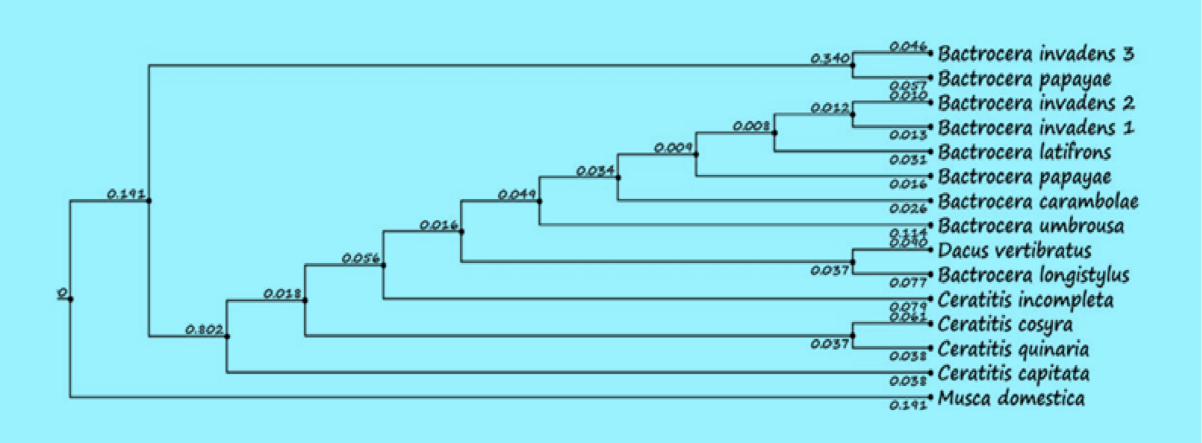

Figure 1: Neighbor joining topology tree percent identity comparison between fruit fly species from Sudan and Malaysia (CO1 R/F primers).

3.3.2. Ceratitis Species

Within *Ceratitis*, the species collected from Sudan exhibited more than 80% identity among themselves. *C. quinaria* was most closely related to *C. cosyra* (89.8% identity) and less closely related to *C. capitata* (86.9%). Cross-comparison with NCBI accessions of other *Ceratitis* species revealed low percent identities (35–38%), highlighting substantial genetic divergence between Sudanese populations and previously reported African species (Table **4**, Fig. **2**).

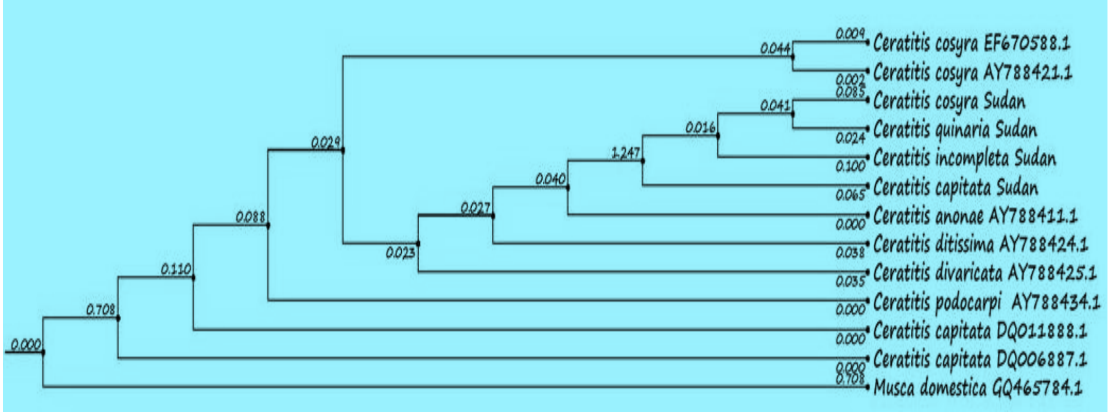

Figure 2: Neighbor joining topology phylogenetic tree of *Ceratitis* spp. from Sudan and other *Ceratitis* spp. accessions from NCBI.

Table 4: Percent identity among *Ceratitis* species (CO1F/CO1R).

Species 1	Species 2	Percent Identity (%)	Notes
C. quinaria	*C. cosyra*	89.8	Highest similarity
C. quinaria	*C. capitata*	86.9	—
C. cosyra	*C. capitata*	84.5	—
C. quinaria	*NCBI C. rosa*	35	Low similarity

3.3.3. Dacus Species

D. vertibratus and *B. longistylus* shared 83% sequence identity, suggesting a closer relationship than with other genera. Comparisons using UEA7/UEA10 primers showed that *D. ciliatus* and *B. cucurbitae* shared moderate similarity (83%) with *B. invadens* sequences, confirming that the second primer set is effective for detecting broader phylogenetic relationships among Tephritid species (Table **5**, Fig. **3**).

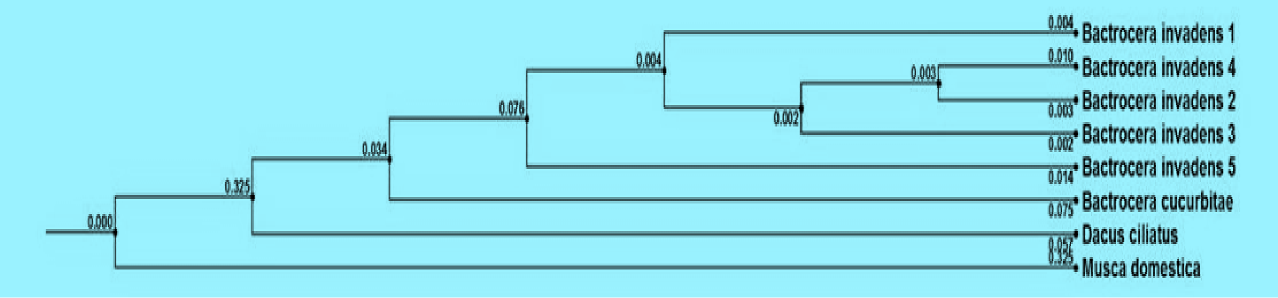

Figure 3: Neighbor joining topology tree percent identity comparison between fruit fly species from Sudan (Second set of primers).

Table 5: Percent identity among *Tephritid* species (UEA7/UEA10).

Species 1	Species 2	Percent Identity (%)
B. invadens-1	*B. invadens-2*	99.4
B. invadens-4	*B. invadens-5*	96.7
B. invadens	*D. ciliatus*	83
B. invadens	*B. cucurbitae*	83

3.4. Global Comparisons

Comparison of *B. invadens* sequences with NCBI accessions revealed the highest similarity with *B. papayae* (94.7–95.8%) and moderate similarity with *B. dorsalis* accessions (91–95%). Lower percent identities were observed with other accessions such as EU076695.1 (63%) and GQ458045.1 (41%) (Table **6**, Fig. **4**). These results indicate that *B. invadens* is genetically closer to Asian *Bactrocera* species than to other African accessions, supporting hypotheses of an Asian origin or introduction into Africa.

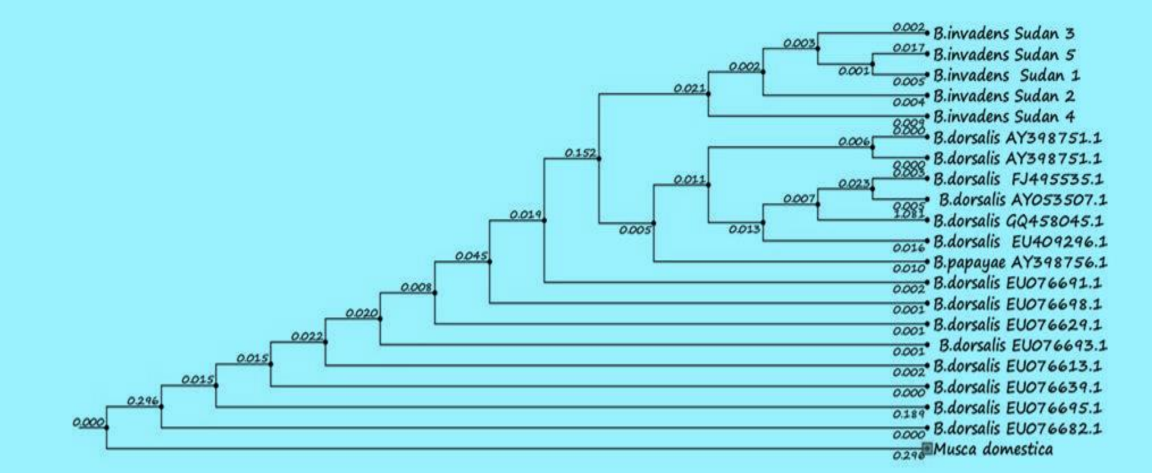

Figure 4: Neighbour joining topology tree percent identity comparison between B. invadens from Sudan and accessions of B. dorsalis and B. papayae from NCBI (UAE 7/10 primers).

Table 6: Percent identity of *B. invadens* with NCBI accessions.

Sample	NCBI Accession	Species	Percent Identity (%)	Notes
B. invadens-1	AY398756.1	*B. papayae*	95.8	Highest similarity
B. invadens-1	AY398751.1	*B. dorsalis*	91	Moderate similarity
B. invadens-1	EU076695.1	*B. dorsalis*	63	Low similarity
B. invadens-1	GQ458045.1	*B. dorsalis*	41	Lowest similarity

3.5. Summary of Percent Identities

1. Intraspecific identity for *B. invadens*: 96.5–99.4%
2. Interspecific identity among *Bactrocera*: 80.5–95.3%
3. Interspecific identity among *Ceratitis*: 80–89.8% (Sudanese species), 35–38% (global comparison)
4. Identity among *Dacus* and *Bactrocera* species: ~83%

Overall, the results demonstrate that CO1 sequences provide high-resolution species identification and reveal phylogenetic relationships that are consistent with both geographic origin and taxonomic classification. The data support the use of CO1 markers for biosecurity, pest monitoring, and studies of population genetics in both African and Asian Tephritid fruit flies.

4. Discussion

This study demonstrates the utility of CO1 mitochondrial markers in accurately identifying Tephritid fruit flies belonging to the genera *Bactrocera*, *Ceratitis*, and *Dacus*. The application of PCR-based molecular methods offers several advantages over traditional morphological identification, particularly for cryptic species or immature stages, which are often challenging to differentiate using conventional taxonomic keys [10, 12, 13, 16]. The clear amplification and sequencing of CO1 fragments from multiple species from Sudan and Malaysia indicate that these markers are robust across different geographic populations and ecological contexts.

4.1. Evolutionary Insights and Phylogenetic Relationships

Phylogenetic analyses revealed distinct clustering of species according to their respective genera, supporting previous studies on Tephritid systematics [2, 4, 12]. Within the genus *Bactrocera*, high percent identity among *B. invadens* samples (>96%) suggests low intraspecific genetic variation, which may reflect recent colonization events or strong gene flow within populations [5, 6]. Conversely, lower identity (80–95%) with other *Bactrocera* species highlights the divergence between closely related taxa, confirming the capacity of CO1 sequences to resolve interspecific relationships [1, 2].

Among *Ceratitis* species, *C. quinaria* showed higher similarity to *C. cosyra* (89.8%) than to *C. capitata* (86.9%), consistent with morphological and ecological data from Africa [1, 2]. Low percent identities (35–38%) with other African *Ceratitis* species indicate substantial genetic differentiation, reflecting historical biogeographic separation and adaptive diversification in response to different host fruits and environmental pressures [2, 5].

These findings provide insights into the evolutionary dynamics of Tephritid fruit flies, suggesting that both historical colonization and local adaptation contribute to the observed genetic structure. The use of mitochondrial markers enables the detection of cryptic speciation and the reconstruction of phylogeographic patterns, which are critical for understanding the evolutionary history of invasive pests [9, 16-18].

4.2. Implications for Pest Management

Accurate species identification is essential for the development of targeted integrated pest management (IPM) strategies. Misidentification of fruit flies can lead to ineffective quarantine measures, inappropriate pesticide applications, and the unintentional spread of invasive species [1, 4, 12]. The high resolution of CO1-based identification makes it particularly valuable for quarantine laboratories, especially at airports and national borders where rapid, reliable diagnostic tools are needed [10, 13, 19-21].

For Sudan, the findings have practical applications in monitoring major pests such as *B. invadens*, *C. capitata*, and *D. vertibratus*. Knowledge of species-specific distribution and genetic relationships allows for improved pest risk assessment and more efficient deployment of management practices, including biological control and sterile insect technique (SIT) programs [1, 2, 4]. Furthermore, understanding the genetic similarity between Sudanese and Asian populations of *Bactrocera* species helps anticipate potential pathways of invasion and implement proactive measures.

4.3. Comparison with Global Studies

The results align with previous molecular studies on Tephritid flies in Asia and Africa [1, 4, 5, 12, 22]. For example, Chua *et al*. [4] reported similar CO1 sequences in Malaysian *Bactrocera* species, confirming the utility of these markers in diverse geographic regions. Baliraine *et al*. [1] highlighted high microsatellite polymorphism in African *Ceratitis* species, supporting the observed genetic diversity in this study. These global comparisons reinforce the notion that mitochondrial markers are reliable tools for cross-continental studies and can be integrated into international biosecurity frameworks.

4.4. Limitations and Future Research

While CO1 sequences provide robust identification at the species level, mitochondrial markers alone may not fully resolve cases of recent speciation, hybridization, or introgression among closely related species [10, 12, 23]. Nuclear markers, such as ITS regions or microsatellites, could complement CO1 data and provide additional resolution for population-level studies.

Future research should focus on:

- Expanding sampling across more regions in Sudan and neighboring countries to assess population structure and migration patterns.
- Incorporating nuclear markers alongside CO1 to detect hybridization and resolve cryptic species complexes.
- Investigating the ecological and host-plant associations of different Tephritid species to better understand adaptive evolution and pest behavior.
- Applying molecular tools in real-time monitoring and early-warning systems for invasive species, which can support quarantine and IPM programs.

In conclusion, the use of mitochondrial CO1 markers is a highly effective approach for species identification and phylogenetic analysis of Tephritid fruit flies. This study not only confirms the genetic relationships among major African and Asian fruit fly species but also provides a framework for improved pest management strategies and biosecurity measures in Sudan and other affected regions.

Conflict of Interest

Authors would like to declare there is no any conflicts of interest or potential biases related to this research.

Funding

Authors would like to thank TWAS for its generous fund.

References

[1] Baliraine NF, Bonizzoni M, Guglielmino CR, Osir EO, Lux SA, Mulaa FJ, *et al*. Population genetics of the potentially invasive African fruit fly species, Ceratitis rosa and Ceratitis fasciventris (Diptera: Tephritidae). Mol Ecol. 2004; 13(3): 683-95. https://doi.org/10.1046/j.1365-294X.2004.02105.x

[2] Clarke RA, Armstrong KF, Carmichael AE, Milne JR, Raghu S, Roderick GK, *et al*. Invasive phytophagous pests arising through a recent tropical evolutionary radiation: the Bactrocera dorsalis complex of fruit flies. Annu Rev Entomol. 2005; 50: 293-319. https://doi.org/10.1146/annurev.ento.50.071803.130428

[3] White IM, Elson-Harris MM. Fruit flies of economic significance: their identification and bionomics. Wallingford (UK): CAB International; 1992. https://doi.org/10.1079/9780851987903.0000

[4] Chua THC, Vern Y, Lim SH. Species determination of Malaysian Bactrocera pests using PCR-RFLP analyses (Diptera: Tephritidae). Pest Sci. 2009; 65: 1886. https://doi.org/10.1002/ps.1886

[5] De Meyer M. On the identity of the Natal fruit fly Ceratitis rosa Karsch (Diptera: Tephritidae). Bull Inst Sci Nat Belg Entomol. 2000; 71: 55-62.

[6] De Meyer M, Freidberg A. Revision of the subgenus Ceratitis (Pterandrus) Bezzi (Diptera: Tephritidae). Isr J Entomol. 2006; 35-36: 179-315.

[7] Scheffer SJ. Molecular evidence of cryptic species within Liriomyza huidobrensis (Diptera: Agromyzidae). J Econ Entomol. 2000; 93: 1146-51. https://doi.org/10.1603/0022-0493-93.4.1146

[8] Simon C, Prati F, Beckenbach A, Crespi B, Liu H, Flook P. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved PCR primers. Ann Entomol Soc Am. 1994; 87: 651-701. https://doi.org/10.1093/aesa/87.6.651

[9] De Meyer M, Copeland SR. Description of new Ceratitis MacLeay (Diptera: Tephritidae) species from Africa. J Nat Hist. 2005; 39: 1279-83. https://doi.org/10.1080/00222930400004347

[10] Hardy DE. A reclassification of the Dacini (Tephritidae: Diptera). Ann Entomol Soc Am. 1955; 48: 425-37. https://doi.org/10.1093/aesa/48.6.425

[11] Scheffer SJ, Lewis ML. Two nuclear genes confirm mitochondrial evidence of cryptic species within Liriomyza huidobrensis (Diptera: Agromyzidae). Ann Entomol Soc Am. 2001; 94: 648-53. https://doi.org/10.1603/0013-8746(2001)094[0648:TNGCME]2.0.CO;2

[12] Han HY, Ro KE. Molecular phylogeny of the superfamily Tephritoidea (Insecta: Diptera): new evidence from the mitochondrial 12S, 16S and COII. Mol Phylogenet Evol. 2005; 34: 416-30. https://doi.org/10.1016/j.ympev.2004.10.017

[13] Roderick GK. Geographic structure of insect populations: gene flow, phylogeography, and their uses. Annu Rev Entomol. 1996; 41: 263-90. https://doi.org/10.1146/annurev.en.41.010196.001545

[14] Billah M. A simplified key to common fruit fly species. Nairobi: ICIPE; 2004.

[15] Portillo PD, Thomas MC, Martinez E, Maranon C, Valladares B, Patarroyo ME, *et al*. Multiprimer PCR system for differential identification of Mycobacteria in clinical samples. J Clin Microbiol. 1996; 34: 324-8. https://doi.org/10.1128/jcm.34.2.324-328.1996

[16] Bohonak AJ, Davies N, Villablanca FX, Roderick GK. Invasion genetics of New World medflies: testing alternative colonization scenarios. Biol Invasions. 2001; 3: 103-11. https://doi.org/10.1023/A:1014559617282

[17] Drew RAI. The generic and subgeneric classification of Dacini (Diptera: Tephritidae) from the South Pacific area. J Aust Entomol Soc. 1972; 11: 1-22. https://doi.org/10.1111/j.1440-6055.1972.tb01601.x

[18] Mun JH, Song YH, Heong KL, Roderick GK. Genetic variation among Asian populations of rice planthoppers, Nilaparvata lugens and Sogatella furcifera (Hemiptera: Delphacidae): mitochondrial DNA sequences. Bull Entomol Res. 1999; 89: 245-53. https://doi.org/10.1017/S000748539900036X

[19] Lunt DH, Zhang DX, Szymura JM, Hewitt GM. The insect cytochrome oxidase I gene: evolutionary profiles and conserved primers for phylogenetic studies. Insect Mol Biol. 1996; 5: 153-65. https://doi.org/10.1111/j.1365-2583.1996.tb00049.x

[20] Miura K, Tagami Y, Ohtaishi M, Iwasaki A. Application of molecular techniques to distinguish Liriomyza trifolii from L. sativae (Diptera: Agromyzidae) on tomato cultivation in Japan. J Econ Entomol. 2004; 97(3): 964-70. https://doi.org/10.1093/jee/97.3.964

[21] Roehrdanz RL. Multiplex polymerase chain reaction method for differentiation of western and northern corn rootworm larvae (Coleoptera: Chrysomelidae). J Econ Entomol. 2003; 96: 669-72. https://doi.org/10.1093/jee/96.3.669

[22] Gasparich GE, Silva JG, Han H, McPheron BA, Steck GJ, Sheppard WS. Population genetic structure of Mediterranean fruit fly (Diptera: Tephritidae) and implications for worldwide colonization patterns. Ann Entomol Soc Am. 1997; 90: 790-7. https://doi.org/10.1093/aesa/90.6.790

[23] Hardy DE. Resurrection of Bactrocera Macquart and clarification of the type-species longicornis Macquart (Diptera: Tephritidae). Proc Hawaii Entomol Soc. 1976; 22: 245-9.