Pakistan J. Zool., pp 1-8, 2026. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20241211055707

Identification of Major *Tephritidae* Pests of Kinnow in Pakistan and DNA Barcoding of *Bactrocera scutellaris* in Gilgit-Baltistan

Anbareen Gul, Muhammad Bilal Ur Ramzan, Syed Hamid Jalal Shah and Javaria Qazi*

Molecular Virology and Epidemiology Laboratory, Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

ABSTRACT

In Pakistan, the *Bactrocera* species presents significant threat to horticultural crops due to its extensive host range. Particularly, *Bactrocera dorsalis, Bactrocera zonata,* and *Bactrocera cucurbitae* are globally recognized as important polyphagous insects. *B. dorsalis* and *B. zonata* mainly infest fruits while *B. cucurbitae* infest Cucurbitaceae crops. As citrus is a major fruit crop in Pakistan contributing an important role in the country's agricultural economy. However, its export is being declining due to fruit fly infestation. Among citrus fruits, Kinnow mandarin is particularly important. Therefore, this study focuses on the identification of fruit fly species affecting citrus and cucurbit crops in Pakistan. For this purpose, fruit fly infested Kinnow mandarin samples were collected from major citrus producing district of Punjab and pheromone traps were installed in Danyor city of Gilgit Baltistan. A total of 313 flies were collected, consisting of 238 individuals reared from infested Kinnow and 75 adult males' flies were captured in pheromone traps. Morphological identification revealed five distinct species namely, *B. dorsalis*, *B. zonata*, *B. scutellaris*, *B. cucurbitae*, and *B. tau*. Laboratory rearing of infested Kinnow yielded *B. dorsalis* and *B. zonata*, with approximately equal emergence rates and sex ratio. Pheromone traps predominantly captured *Bactrocera scutellaris* (65.33%) and *B. cucurbitae* (28%). The identification of *B. scutellaris* using morphological key was further validated through DNA barcoding technique using mt-COI gene sequencing marking its first recorded presence and dominance in the Gilgit-Baltistan region. The findings from this study, highlights *B. dorsalis* and *B. zonata* as major pests of Kinnow in Pakistan and also document the growing threat of spread of *B. scutellaris* as major pest of cucurbit crops in Gilgit Baltistan. This study also represents the first molecular confirmation of *B. scutellaris* in Pakistan.

Article Information
Received 11 December 2024
Revised 25 July 2025
Accepted 20 August 2025
Available online 20 February 2026
(early access)

Authors' Contribution
AG: Original drafting, sample processing, data collection, result compilation. MBR: Sampling and result compilation. SHJS: Data and result compilation. JQ: Idea of the study, original drafting, result compilation.

Key words
Bactrocera, Cucurbitaceae, *mt-COI*, Barcoding, Emergence, Infestation

INTRODUCTION

Tephritid fruit flies, particularly those belonging to the *Bactrocera* species pose significant threat to both fruits and vegetables worldwide. Their infestation not only reduces crop yields but also adversely affect the quality of fruits, leading to unprofitable farming endeavors (Dhillon *et al*., 2005). Pakistan has diverse agroclimatic conditions, making it more suitable to grow wide variety of fruits and vegetables. Notably, Pakistan ranks 12th globally in the production of citrus (Sikandar *et al*., 2017) and second in guava production, following India. The country stands 6th in mango and apricot, 18th in pumpkins, squashes and gourds in the world (Jaskani and Khan, 2021; Khan *et al*., 2020). No doubt, Pakistan's ranking in world fruit and vegetable production is significant but the volume of exports remains relatively low, mainly due to fruit fly attack on fruits. Almost 20-90% losses has been reported in different fruit orchards in Pakistan (Khan *et al*., 2005; Stonehouse *et al*., 2002).

Among the citrus fruits, Kinnow mandarin which is also called as king of all varieties of citrus and is dominating the citrus markets due to its best taste and flavor are majorly cultivated in the Punjab province. Punjab covers almost 91% of the nation's citrus growing area (Ghafoor and Ch, 2008). However, only 10-12% of its total production is being exported, with the majority being consumed domestically (Nawaz *et al*., 2019). The decline in its export is attributed to infestation by tephritid fruit flies.

* Corresponding author: javariaqazi@qau.edu.pk
0030-9923/2026/0001-0001 $ 9.00/0

Similarly, cucurbitaceous crops such as cucumbers, melons, and pumpkins also suffer due to damage cause by fruit flies. Various species of *Bactrocera* have been identified as pests affecting Cucurbitaceae including *B. caudata* and *B. diversa* (Maula *et al*., 2023). However, the most significant impact on the cucurbitaceous family is attributed to *B. tau* (Jaleel *et al*., 2018) and *B. cucurbitae* (Somegowda *et al*., 2021). More recently, a new specie, *B. scutellaris* has been observed affecting the Cucurbitaceae, as reported by (Choudhary *et al*., 2014).

In Pakistan, cucurbitaceous crops suffered considerable damage due to fruit fly infestations, predominantly attributed to *B. cucurbitae* (Khan *et al*., 2020) and *B. tau*. However, recent observations indicate the emergence of *B. scutellaris* as a new fruit fly species infesting cucurbit vegetables, particularly in the Khyber Pakhtunkhwa (KPK) (Kausar *et al*., 2022) and Kashmir regions (Zubair *et al*., 2019). However, during our survey *B. scutellaris* was found to be the dominant species in Gilgit Baltistan in bottle traps, surpassing *B. cucurbitae*, with only one species of *B. tau* observed in one of the traps, marking its first recorded presence in the region. Previous studies have highlighted *B. scutellaris* as a significant pest of cucumbers in the mid hill regions of Himachal Pradesh, with more pronounced damage observed during the early stages of plant growth (Sunandita, 2007).

Therefor the current study was designed to identify the tephritid fruit fly species infesting kinnow mandarins in Sargodha, the major kinnow-producing district of Punjab, through laboratory rearing and morphological identification and assess the diversity and relative abundance of fruit fly species in the Danyor region of Gilgit-Baltistan using pheromone traps.

MATERIALS AND METHODS

Sample collection

Sample collection was carried out using two primary approaches during the autumn season of 2023. Two pheromone traps were installed in September 2023 in a cherry orchard located in Danyor city, Gilgit-Baltistan. The orchard was surrounded with fields of cucumber, long melon, loofah, musk melon, pumpkin, and watermelon. These traps remained active for one month. The trap was made up of plastic bottle measuring 20 cm in length and 12 cm in diameter, with two entry holes on opposite sides. Traps contained two cotton wicks, one soaked with 2 ml of methyl eugenol (ME) and the other soaked with cue lure (CL), each combined with a few drops of insecticide (Bifenthrin 10% EC). Male adult flies drawn towards the lures, were promptly exterminated by the insecticide on the cotton wick. The traps were strategically positioned two meters above ground level and suspended from a tree. The second approach involved the collection of infested kinnow fruits from six locations of Sillanwali Tehsil of Sargodha district on October, 2023. The complete sampling detail including GPS coordinates are mentioned in Table I. All the samples collected from both pheromone traps and infested kinnow fruits were brought in laboratory for further processing.

Laboratory rearing of Kinnow *for 1st generation fruit flies*

The infested kinnow were brought in laboratory rearing facility in molecular virology lab (MVL) of biotechnology department of Quaid-i-Azam University and kept under conditions of 28 ± 2°C, 50 ± 10% RH, and a 12:12 (L:D) photoperiod. The fruit fly metamorphosis took 12–16 days and flies were observed daily for the hatching

Table I. Fruit fly sampling details from both pheromone traps and infested Kinnow.

Sample	Area	Date	Farmer/Field	GPS	
				Latitude	Longitude
Gilgit Baltistan					
GBL1	Danyor city	03-09-23	Cherry orchard	35.9196714	74.3880699
Sargodha					
SGL1	Chak 20 NB, Sillawali	29-10-23	Kinnow	32.232011	72.831502
SGL2	Chak 21 NB, Sillawali	29-10-23	Kinnow	32.232467	72.830585
SGL3	Chak 22 NB, Sillawali	27-10-23	Kinnow	32.231107	72.860762
SGL4	Chak 25 NB, Sillawali	26-10-23	Kinnow	32.211265	72.806190
SGL5	Chak 26 NB, Sillawali	26-10-23	Kinnow	32.201436	72.826964
SGL6	Chak 34 NB, Sillawali	29-10-23	Kinnow and Guava	32.150065	72.722242

and pupation. Upon hatching, mature larvae freely left the fruit for pupation into a 10-15cm deep layer of moist (5–8% water) sand (Susanto *et al*., 2022). After pupation, the puparium was subsequently separated from the sand substrate by sieving method and shifted on 2-5 cm deep layer of sterilized sand till emergence of first generation of adult fruit flies.

Morphological identification of flies

All the first-generation adult flies obtained from rearing of infested kinnow and trap collected flies were identified using the morphological key of (David and Ramani, 2011; Zubair *et al*., 2019). Subsequently all the adult flies were counted and recorded. There was a single species of fly collected from the pheromone trap. However, its identification using morphological key was unclear, requiring further verification through DNA barcoding by amplifying the *mt-COI* gene.

Isolation of DNA from individual fly

For the molecular confirmation of uncertain fly, two specimens underwent DNA extraction using the Cetyl trimethyl ammonium bromide (CTAB) method as previously described by Amad *et al*. (2019) with minor modifications. The abdomen portion of the fly was separated from the rest of the body and homogenized in 1.5 mL centrifuge tube containing 150 µL of lysis buffer containing 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB (Sigma-Aldrich, Darmstadt, Germany), and 7 µL of proteinase K (Fermentas). The resulting lysate was incubated at 55 °C for 1 h. Further, 150µL of chloroform: isoamyl-alcohol (24:1) was added. The emulsion was then separated by centrifugation at 14,000 rpm for 10 min. Supernatant is then shifted to separate tubes. For DNA precipitation, 150 µL of 100% ethanol and 30 µL of sodium acetate was added, followed by -20 °C frozen for 2 h. The resulting pellet was collected by centrifugation at 14,000 rpm for 10 min, washed with 150 µL of 70% ethanol and centrifuged again at 14,000 rpm for 10 min After air drying, the pellet was dissolved in 20 µL of ddH2O and stored at−20 °C.

Amplification and sequencing of mt-COI *gene region*

Isolated DNA from the fly were subjected to polymerase chain reaction (PCR) using universal primers LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') targeting the 680 bp mitochondrial cytochrome c oxidase subunit I (*mt-COI*) gene barcode region, as described by Sharma and Kobayashi (2014). The PCR procedure initiated with an initial denaturation step at 94°C for 10 min, proceeding 35 cycles comprising denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and extension at 72°C for 45 sec. The process concluded with a final extension step at 72°C for 10 min. The total PCR reaction mixture was 25 µl, containing 2.5 µl of template DNA, 0.25 µl (5 U/µl) Taq polymerase (Fermentas, USA), 2.5 µl (10 X) Taq buffer (Fermentas, USA), 1.5 µl (25 mM) MgCl2 (Fermentas, USA), 2.5 µl (2 mM) dNTPs (Fermentas, USA), 0.5 µl (10 pMol) of both forward and reverse primers, and 14.75 µl ddH2O. The final amplified products were separated using electrophoresis in a 1% agarose gel, using a 1 Kb ladder, with a gel run time of 30-35 min at 80V. DNA bands were visualized under UV light in an ultraviolet (UV) transilluminator, and the results were compared with the ladder to determine the size of the DNA samples. The PCR products were then subjected to sanger DNA sequencing using the forward primer by Macrogen (South Korea).

Analysis of mt-COI sequences for barcoding and construction of phylogenetic tree

To identify the sequence, the sequence was refined and aligned using nBLAST tool which is available at National Center for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Subsequently, the sequence was submitted to NCBI for its accession number. After assigning accession number to our sequence, phylogenetic analysis was performed, for that closely related sequences were retrieved from the database in FASTA format and aligned using Muscle executed in MEGA version XI (Edgar, 2004). The maximum composite likelihood method was used to construct a phylogenetic tree (Tamura *et al*., 2021) with bootstrap values of 1000.

Matrix analysis of sequences

For the validation of phylogenetic analysis, matrix analysis of the sequences was also performed using the sequence demarcation tool (SDTv1.2) (Muhire *et al*., 2014). The SDT tool classify our sequences by comparing each sequence in pairs. This software takes sequences in FASTA format, aligns every pair of sequences and calculates pairwise similarity scores, and finally display these scores in color-coded matrix form. It displays results in both pairwise identity scores and text files containing analysis results. The identity scores are calculated as 1-(M/N) where M is the number of mismatching nucleotides and N the total number of positions along the alignment.

RESULTS

Morphological identification of fruit flies

During the study, a total of 313 flies were collected. Among these, 238 first generation flies were obtained

from the rearing of infested kinnow while 75 adult male flies were captured from pheromone traps. Morphological identification of flies from both infested kinnow and pheromone trap resulted in five distinct species namely, *B. scutellaris*, *B. cucurbitae*, *B. tau, B. zonata* and *B. dorsalis*. Rearing of infested kinnow in the laboratory conditions yielded two species, *B. dorsalis* and *B. zonata* while four species were identified from the pheromone traps, *B. scutellaris*, *B. cucurbitae*, *B. tau* and *B. zonata*. The representative image of the five species are presented in Supplementary Figures 1-5.

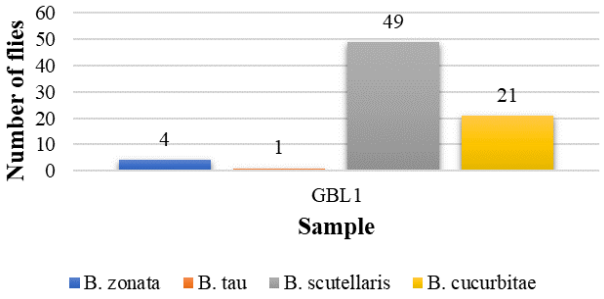


Fig. 1. Fruit fly species count from pheromone trap. The figure demonstrates the number of fruit flies captured in the pheromone trap from the sampling site (GBL1). Different species are represented by distinct colors like *B. zonata* (blue), *B. tau* (orange), *B. scutellaris* (gray), and *B. cucurbitae* (yellow). The data highlights the predominance of *B. scutellaris* and *B. cucurbitae* among the captured flies.

The number of *B. dorsalis* and *B. zonata* emerged from the rearing of infested kinnow were almost same and no significant difference was found in the predominance of either specie. The male-to-female ratio of flies was also approximately equal. Additionally, pupal count and pupal weight were measured and are presented in Table II. Among the flies captured in pheromone traps, *B. scutellaris* emerged as the predominant species, accounting for 65.33% of the total catch, followed by *B. cucurbitae* at 28%, *B. zonata* at 5.33%, and *B. tau* at 1%. The distribution of these species is illustrated in Figure 1. The identification of *B. scutellaris* were further verified through insect barcoding using *mt-COI* gene amplification.

Mt-COI amplification and phylogenetic analysis

For the molecular confirmation, the two specimens were subjected to DNA extraction for molecular analysis in our study. BLAST searches on the amplified region of mt-COI indicated a 99% similarity between our sequence S3(PP396898) and *B. scutellaris* Nepal (OP804513), while our sequence S4(PP396899) exhibited a 98% similarity with the same reference sequence. For the phylogenetic analysis, we downloaded the most similar four sequences of *B. scutellaris* (OP804513, OK103986, KT588389, KM024429), two sequence of *B. atrifacies* (KF659981, KF659968), two sequence of *B. diversa* (MN255899, MN629746) and two sequence of *B. cucurbitae* (KP851001, JQ692821) as reference sequence while *Drosophila melanogaster* (KP730938.1) was used as an outgroup. The evolutionary relationship among these sequences is displayed in phylogenetic tree in Figure 2 clearly depicting the clustering of S3 and S4 with *B. scutellaris*. This analysis involved 15 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 733 positions in the final dataset.

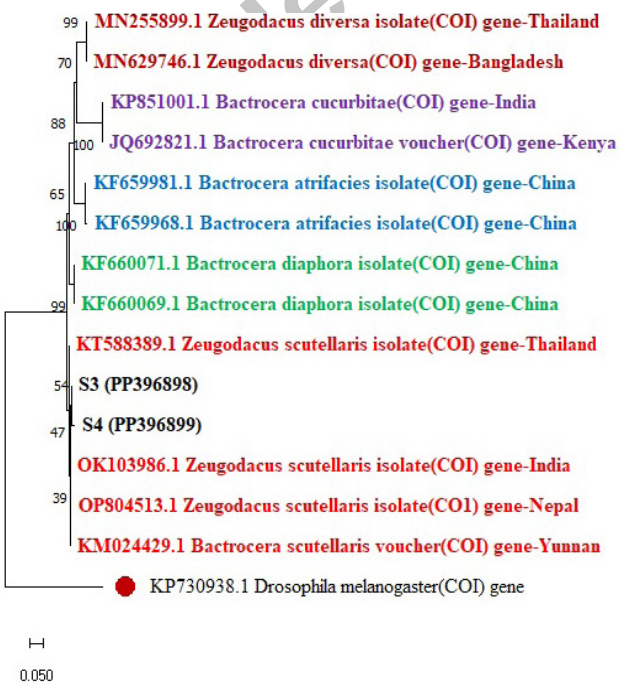


Fig. 2. Phylogenetic analysis of mt-COI sequence of *B. scutellaris*. The phylogenetic tree was constructed using the amplified sequence of mt-COI region of S3(PP396898) and S4(PP396899) showing its similarity with *B. scutellaris*. While the rest of the sequences are used as reference sequences. In the tree the bootstrap values are shown at nodes.

Matrix analysis of sequences

Our phylogenetic analysis is validated using the sequence demarcation tool (SDTv1.2) as depicted in Figure 3 revealing five distinct clusters. A colored key is also given in the right of side of matrix which indicates the percentage similarity between pairwise identities. In the percentage pairwise identity matrix, the most similar

Table II. Rearing details of fruit fly infested Kinnow under laboratory conditions.

Sample	Pupation period/day	Pupal count	Pupal weight (mg)	*B. dorsalis* emergence (Male/Female)	*B. zonata* emergence (Male/Female)
SGL1	15	66	15	44 (26/18)	5 (2/3)
SGL2	15	15	13	11 (6/5)	-
SGL3	13	42	13	20 (11/9)	20 (11/9)
SGL4	12	100	13	65 (28/37)	14 (4/10)
SGL5	12	65	15	31 (20/11)	28 (15/13)
SGL6	15	93	14	66 (33/33)	10 (5/5)

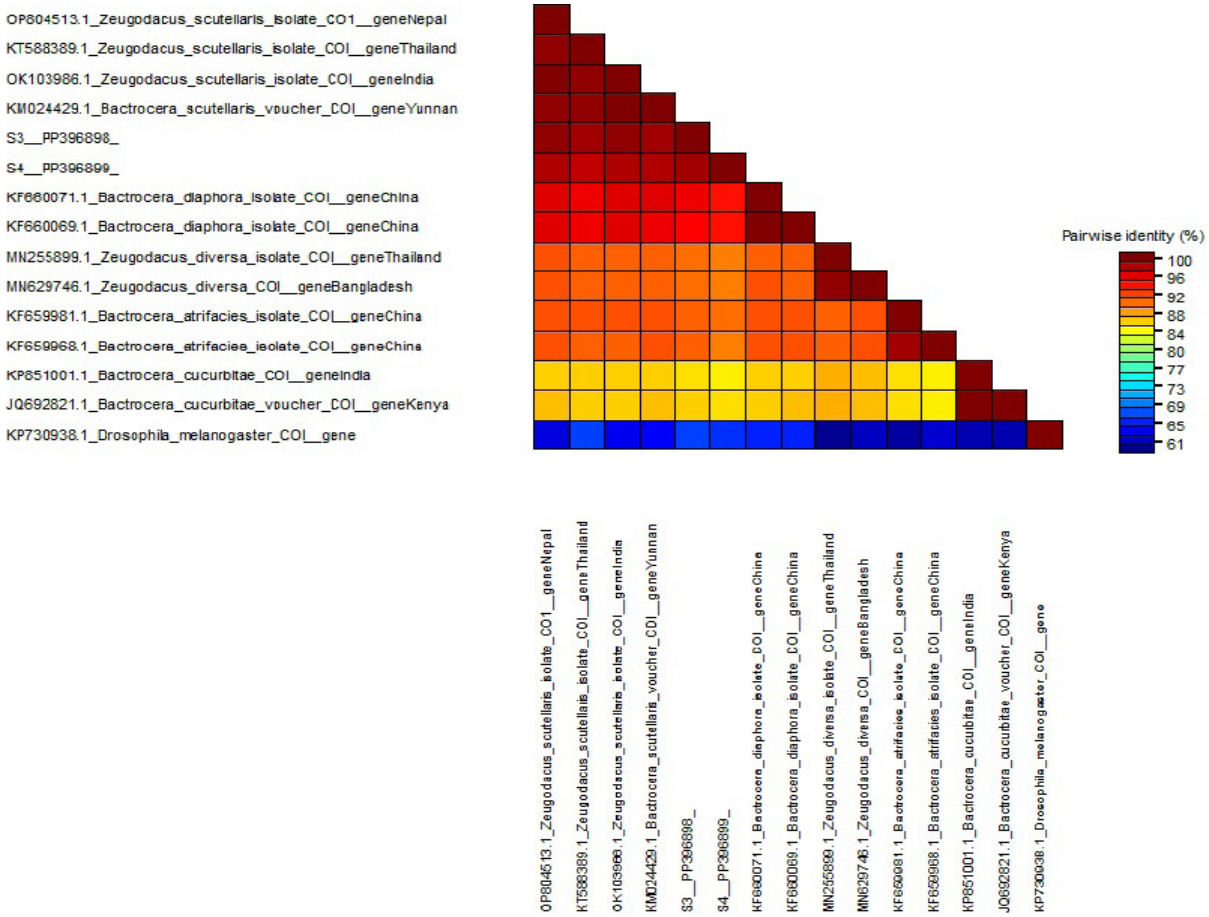


Fig. 3. Color coded pairwise identity matrix of *mt-COI* gene of fruit flies. The color coded pairwise identity matrix was generated from 15 mt-COI sequences of fruit flies. Each colored cell represents a share identity score between 2 sequences (one indicated horizontally to the left and other vertically at the bottom).

sequences are highlighted in dark brown while least similar sequences are in dark blue color. The matrix exhibits five triangles of dark brown color, aligning with the clustering observed in our phylogenetic tree.

DISCUSSION

Pakistan is an agriculture country, is undergoing rapid population growth, surpassing many Asian countries. In 1947, Pakistan, population along with former East Pakistan (now Bangladesh), ranked as the 13th most populous country globally, with a combined population of 32.5 million. By 1996, its rank became seventh with population of 140 million. Projections for 2050 anticipate that Pakistan's rank will ascend to fifth place in terms of global population ranking (Afzal, 2009). According to the growth in population, its agriculture growth is not up to the standard due to many reasons among which the fruit flies

infestation in fruit and vegetable fields are major.

In Pakistan, the *Bactrocera* species presents a significant threat to horticultural crops due to its extensive host range and dominant traits (Clarke *et al*., 2005). Particularly, *B. dorsalis, B. zonata,* and *B. cucurbitae* are globally recognized as important polyphagous insects (Clarke *et al*., 2005). *B. dorsalis* and *B. zonata* mainly infest fruits. According to a study, *B. dorsalis* alone caused a 30% loss in mango fruit production (Noman *et al*., 2021), whereas along with *B. zonata*, it led to a 35% loss (Mohyuddin and Mahmood, 1992). According to studies of Noman *et al*. (2021), fruit flies were responsible for 50-55% losses in guava and 74.66% losses in mango orchards. Field assessments conducted by Stonehouse *et al*. (2002) revealed an infestation rate of 23% for *B. dorsalis* in plum orchards of Peshawar, 80% for *B. zonata* in guava orchards of DI Khan, and 50% for *B. cucurbitae* in melon orchards of DI Khan (Stonehouse *et al*., 2002). Despite of economic losses due to fruit fly species, very limited studies have been conducted on fruit fly infestations in citrus fruits in Pakistan and most of these studies focuses on the efficacy of different types of traps conducted on small scale (Abbas *et al*., 2021; Noman *et al*., 2021). Currently, no studies have been reported on the rearing of fruit flies from infested citrus fruit or the identification of first-generation fruit flies emerging from these infestations.

The aim of the current study is to address this critical research gap as citrus is a major fruit crop in Pakistan, plays an important role in the country's agricultural economy (Ashraf *et al*., 2014). However, its export is being declining due to infestation caused by fruit flies. Among citrus fruits, kinnow mandarin is particularly important, often referred to as the king of citrus fruits. According to previous studies, kinnow fields were 85% infested with *B. dorsalis* and *B. zonata* (Sandeep and Sharma, 2016). These two species were also identified in our study, confirming their role as primary pests of citrus. Similarly, presence of these two species in bottle traps placed in citrus orchards of Sargodha was documented by (Sikandar *et al*., 2017). Furthermore, in our neighboring country China, *B. dorsalis* as major pest of same fruit has been reported (Li *et al*., 2023).

Furthermore, by focusing on the rearing and identification of first-generation fruit flies from infested citrus fruits, this study provides insights into the biology of fruit fly species. The findings from this study not only enhance our understanding of the species involved but also contribute to the development of effective pest management strategies to safeguard citrus production and improving its export potential. In our study we selected Sillanwali tehsil of Sargodha district of Punjab because this district is one of the dominant citruses producing area. During the peak Kinnow production season in October, infested Kinnow samples were collected, and after laboratory rearing, two fruit fly species, *B. dorsalis* and *B. zonata* were identified.

In the same way melon fly inflicts significant damage to all cucurbit crops wherever it is present. Reports from Pakistan indicate that *B. cucurbitae* typically causes damage ranging from 20% to 75% in melon production (Kafi, 1986). There are various species of *B.* that have been identified as pests affecting Cucurbitaceae in Pakistan, including *B. caudata* and *B. diversa* (Maula *et al*., 2023). However, the most significant impact on the cucurbitaceous family is attributed to *B. tau* (Jaleel *et al*., 2018) and *B. cucurbitae* (Somegowda *et al*., 2021). More recently, a new species, *B. scutellaris*, has been observed affecting the Cucurbitaceae family, as reported by Prabhakar *et al*. (2013). This specie was also recognized in Pakistan as a new fruit fly species infesting cucurbit vegetables, particularly in the Khyber Pakhtunkhwa (Kausar *et al*., 2022) and Kashmir regions (Zubair *et al*., 2019). However, in Gilgit Baltistan this species was identified for the first time during our survey and was found to be the dominant species in pheromone bottle traps, surpassing *B. cucurbitae*, with only one species of *B. tau* observed, marking its first recorded presence in the region. Previous studies have highlighted *B. scutellaris* as a significant pest of cucumbers in the mid hill regions of Himachal Pradesh, with more pronounced damage observed during the early stages of plant growth (Sunandita, 2007). Our study highlights the presence and dominance of *B. scutellaris* in Gilgit Baltistan for the first time and also first molecular confirmation of this specie from the country. For the molecular confirmation the two specimens were subjected to DNA extraction and amplification of mt-COI region. After sequencing of the amplified region when these two specimens were subjected to BLAST searches then 99% similarity found between sequence S3 (PP396898) and *B. scutellaris* Nepal (OP804513), while sequence S4 (PP396899) exhibited a 98% similarity with the same reference sequence. Phylogenetic analysis of our sequence shows clustering of S3 and S4 sequence with *B. scutellaris* group (OP804513, OK103986, KT588389, KM024429) which were downloaded as reference sequence from NCBI. The evolutionary relationship among these sequences is displayed in phylogenetic tree in Supplementary Figure 2. Our results were further verified through sequence demarcation tool (SDT). In the SDT matrix the most similar sequences are highlighted in dark brown while least similar sequences are in dark blue color. The matrix exhibits five triangles of dark brown color, aligning with the clustering observed in our phylogenetic tree.

CONCLUSION

Our research represents significant milestone in

Pakistan identifying *Bactrocera* species as primary pests in both citrus and cucurbit crops in Pakistan. The findings confirm the prevalence of *Bactrocera dorsalis* and *Bactrocera zonata* as major pests in citrus orchards, particularly Kinnow mandarins. Moreover, this study also documents the first report of presence and dominance of *B. scutellaris* in Gilgit Baltistan. Given the emergence of this species and its displacement of *B. cucurbitae* as the primary pest of Cucurbitaceae in this region is alarming. Therefore, further continued efforts are required for monitoring, surveillance, and collaborative research to develop effective control strategies to lessen the spread of this pest on agricultural productivity. By developing interdisciplinary collaborations, we can better understand the ecological dynamics of this specie populations and implement targeted interventions to safeguard agricultural system in Pakistan.

DECLARATIONS

Acknowledgement

We would like to extend our gratitude to the farmers for granting us the permission to access their field for sample collection.

Funding

This research did not receive any external funding.

Ethical statement

This study did not involve any kind of endangered species or environmental risk; thus, no ethical approval was required.

Generative AI and AI-assisted technology statement

No AI assisted technology were used during the conduct of this research or in the manuscript preparation.

Data availability statement

Data generated or analyzed during this study are provided in full within this article.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20241211055707

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abbas, M., Hussain, D., Saleem, M., Ghaffar, A., Abbas, S., Hussain, N. and Ghaffar, A., 2021. Integrated pest management of guava, citrus and mango fruitflies at three districts of Punjab. *Pakistan J. Zool.*, **53**: 995. https://doi.org/10.17582/journal.pjz/20190115070156

Afzal, M., 2009. Population growth and economic development in Pakistan. *Open Demogr. J.*, **2**. https://doi.org/10.2174/1874918600902010001

Amad, I., Hafeez, F., Khan, M.A., Nahid, N., Javed, M.R., Shaheen, S., Farooq, M., Khan, A.Z. and Hussain, K., 2019. Mitochondrial gene cytochrome c oxidase I (CO1) used for molecular identification of *Bactrocera zonata* in Pakistan. *Cell. mol. Biol.*, **65**: 82–84. https://doi.org/10.14715/cmb/2019.65.2.13

Ashraf, S., Khan, G.A., Ali, S., Iftikhar, M. and Mehmood, N., 2014. Managing insect pests and diseases of citrus: On farm analysis from Pakistan. *Pak. J. Phytopathol.*, **26**: 301–307.

Choudhary, J.S., Naaz, N., Prabhakar, C.S., Das, B., Maurya, S. and Kumar, S., 2014. Field guide for identification of Fruit fly species of Genus *Bactrocera* prevalent in and around mango orchards. *Technical Booklets* No, 1–16.

Clarke, A.R., Armstrong, K.F., Carmichael, A.E., Milne, J.R., Raghu, S., Roderick, G.K. and Yeates, D.K., 2005. Invasive phytophagous pests arising through a recent tropical evolutionary radiation: the *Bactrocera dorsalis* complex of fruit flies. *Annu. Rev. Ent.*, **50**: 293–319. https://doi.org/10.1146/annurev.ento.50.071803.130428

David, K.J. and Ramani, S., 2011. An illustrated key to fruit flies (Diptera: Tephritidae) from Peninsular India and the Andaman and Nicobar Islands. *Zootaxa*, **3021**: 1–31. https://doi.org/10.11646/zootaxa.3021.1.1

Dhillon, M.K., Singh, R., Naresh, J.S. and Sharma, H.C., 2005. The melon fruit fly, *Bactrocera cucurbitae*: A review of its biology and management. *J. Insect Sci.*, **5**: 40. https://doi.org/10.1093/jis/5.1.40

Edgar, R.C., 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res.*, **32**: 1792–1797. https://doi.org/10.1093/nar/gkh340

Ghafoor, U.S.M. and Ch, K.M., 2008. Constrains in availability of inputs and information to citrus (Kinnow) growers of tehsil Toba Tek Singh. *Pak. J. agric. Sci.*, **45**: 4.

Jaleel, W., Tao, X., Wang, D., Lu, L. and He, Y., 2018. Using two-sex life table traits to assess the fruit preference and fitness of *Bactrocera dorsalis* (Diptera: Tephritidae). *J. econ. Ent.*, **111**: 2936–2945. https://doi.org/10.1093/jee/toy243

Jaskani, M.J. and Khan, I.A., 2021. *Horticulture: An overview.* University of Agriculture, Faisalabad,

pp. 3–22.

Kafi, A., 1986. *Progress and problems in controlling fruit flies infestation*. FAO, Rapa, Bangkock, 16–19.

Kausar, A., Ullah, F., Jahan, F., Khan, K., Wahid, S., Tanzila, G. and Khan, N.H., 2022. Bionomics of *Bactrocera* fruit flies (Diptera: Tephritidae) in Khyber Pakhtunkhwa, Pakistan; exploring performance of various trap types and their characteristics. *Florida Entomol.*, **105**: 231–242. https://doi.org/10.1653/024.105.0309

Khan, D., Ullah, A., Bibi, Z., Ullah, I., Zulfiqar, M. and Khan, Z.U., 2020. Forecasting area and production of guava in Pakistan: An econometric analysis. *Sarhad J. Agric.*, **36**: 272–281. https://doi.org/10.17582/journal.sja/2020/36.1.272.281

Khan, M.A., Ashfaq, M., Akram, W. and Lee, J., 2005. Management of fruit flies (Diptera: Tephritidae) of the most perishable fruits. *Ent. Res.*, **35**: 79–84. https://doi.org/10.1111/j.1748-5967.2005.tb00140.x

Li, D., Long, J., Tang, Z., Han, L., Gong, Z., Wen, L., Peng, H. and Wen, T., 2023. Detection and classification of citrus fruit infestation by *Bactrocera dorsalis* (Hendel) using a multi-path Vis/NIR spectroscopy system. *Agriculture*, **13**: 1642. https://doi.org/10.3390/agriculture13081642

Maula, F., Ali, A., Inamullah, K., Aa, A., Bari, A., Drew, M. and Drew, D., 2023. A survey of fruit flies (Tephritidae: Dacini) of District Swat, Khyber Pakhtunkhwa Province, Pakistan. *J. Xi'an Shiyou Univ. Natl. Sci. Ed.*, **19**: 789–803.

Mohyuddin, A.I. and Mahmood, R., 1992. Integrated control of mango pests in Pakistan. *IV Int. Mango Symp.* **341**: 467–483. https://doi.org/10.17660/ActaHortic.1993.341.51

Muhire, B.M., Varsani, A. and Martin, D.P., 2014. SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. *PLoS One*, **9**: e108277. https://doi.org/10.1371/journal.pone.0108277

Nawaz, R., Abbasi, N.A., Hafiz, I.A., Khalid, A., Ahmad, T. and Aftab, M., 2019. Impact of climate change on Kinnow fruit industry of Pakistan. *Agrotechnology*, **8**: 186. https://doi.org/10.35248/2168-9881.19.8.186

Noman, Q.M., Shah, F.M., Mahmood, K. and Razaq, M., 2021. Population dynamics of Tephritid fruit flies in citrus and mango orchards of Multan, Southern Punjab, Pakistan. *Pakistan J. Zool.*, **54**: 325–330. https://doi.org/10.17582/journal.pjz/20191021181023

Prabhakar, C.S., Sood, P., Mehta, P.K. and Sharma, P.N., 2013. Population genetic structure of the pumpkin fruit fly, *Bactrocera tau* (Walker)(Diptera: Tephritidae) in Himachal Pradesh, India. *Biochem. Syst. Ecol.*, **51**: 291–296. https://doi.org/10.1016/j.bse.2013.09.008

Sandeep, S. and Sharma, D., 2016. *Integrated pest management for* Bactrocera dorsalis *(Hendel) and* Bactrocera zonata *(Saunders) on* Kinnow *mandarin in the Indian Punjab.*

Sharma, P. and Kobayashi, T., 2014. Are "universal" DNA primers really universal? *J. appl. Genet.*, **55**: 485–496. https://doi.org/10.1007/s13353-014-0218-9

Sikandar, Z., Afzal, M.B.S., Qasim, M.U., Banazeer, A., Aziz, A., Khan, M.N., Mughal, K.M. and Tariq, H., 2017. Color preferences of fruit flies to methyl eugenol traps, population trend and dominance of fruit fly species in citrus orchards of Sargodha, Pakistan. *J. Ent. Zool. Stud.*, **5**: 2190–2194.

Somegowda, M., Raghavendra, S., Sridhara, S., Rajeshwara, A.N., Pramod, N.S., Shivashankar, S., Lin, F., El-Abedin, T.K.Z., Wani, S.H. and Elansary, H.O., 2021. Defensive mechanisms in cucurbits against melon fly (*Bactrocera cucurbitae*) infestation through excessive production of defensive enzymes and antioxidants. *Molecules*, **26**: 6345. https://doi.org/10.3390/molecules26216345

Stonehouse, J., Mahmood, R., Poswal, A., Mumford, J., Baloch, K.N., Chaudhary, Z.M., Makhdum, A.H., Mustafa, G. and Huggett, D., 2002. Farm field assessments of fruit flies (Diptera: Tephritidae) in Pakistan: Distribution, damage and control. *Crop Prot.*, **21**: 661–669. https://doi.org/10.1016/S0261-2194(02)00018-2

Sunandita, G.D., 2007. A note on host range of fruit fly species infesting summer vegetable crops in mid hills of Himachal Pradsesh. *J. Insect Sci.*, **20**: 106–107.

Susanto, A., Faradilla, M.G., Sumekar, Y., Yudistira, D.H., Murdita, W., Permana, A.D., Djaya, L. and Subakti Putri, S.N., 2022. Effect of various depths of pupation on adult emergence of interspecific hybrid of *Bactrocera carambolae* and *Bactrocera dorsalis*. *Sci. Rep.*, **12**: 4235. https://doi.org/10.1038/s41598-022-08295-w

Tamura, K., Stecher, G. and Kumar, S., 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.*, **38**: 3022–3027. https://doi.org/10.1093/molbev/msab120

Zubair, U., Shehzad, A., Mastoi, M.I. and Mahmood, K., 2019. New record of fruit flies (Diptera: Tephritidae) from poonch division of Azad Jammu and Kashmir. *Pak. J. agric. Res.*, **32**: 466. https://doi.org/10.17582/journal.pjar/2019/32.3.466.473

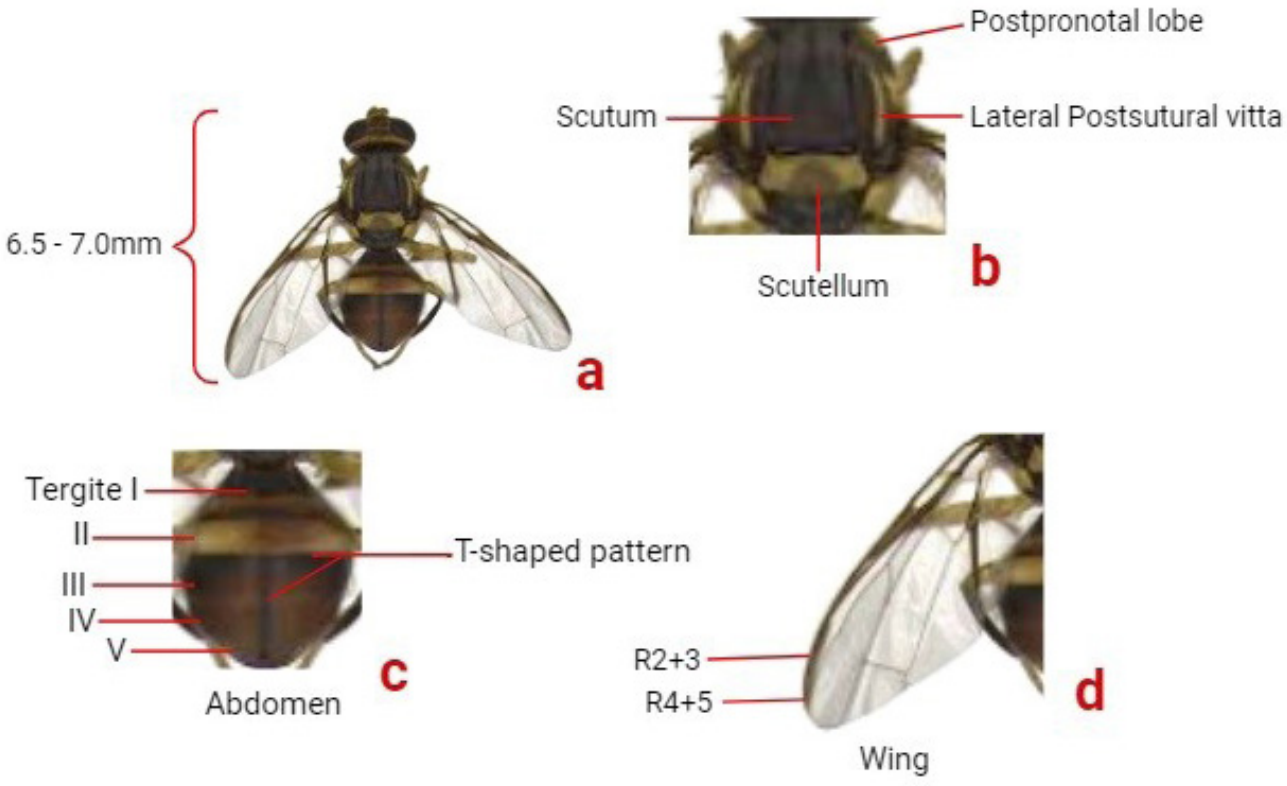
Pakistan J. Zool., pp 1-8, 2026. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20241211055707

Supplementary Material

Identification of Major *Tephritidae* Pest Species of *Kinnow* in Pakistan and First DNA Barcoding of *Bactrocera scutellaris* in Gilgit-Baltistan

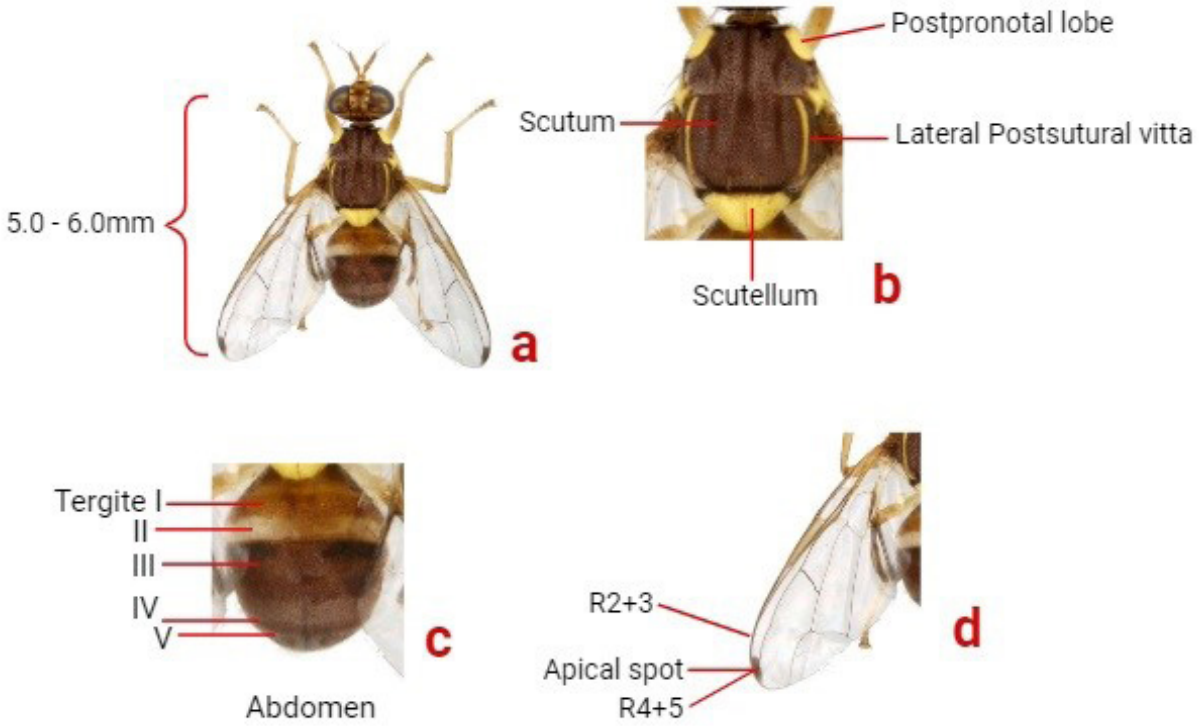
Anbareen Gul, Muhammad Bilal Ur Ramzan, Syed Hamid Jalal Shah and Javaria Qazi*

Molecular Virology and Epidemiology Laboratory, Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

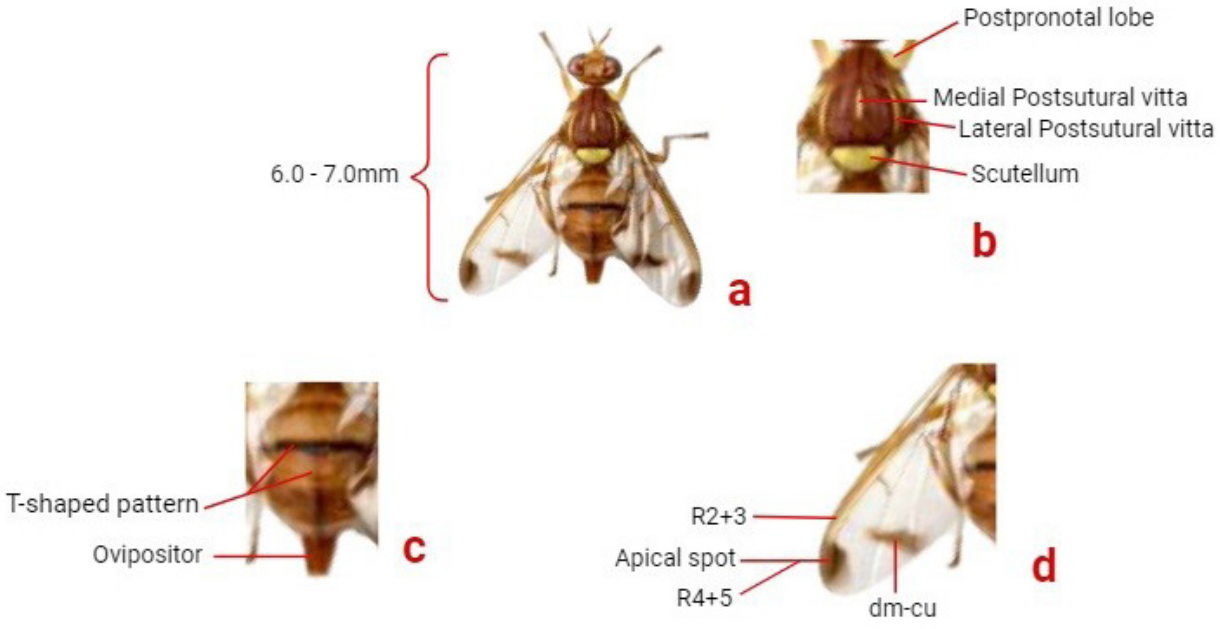


Supplementary Fig. 1. Image of *B. dorsalis*: (a) represents the size of the fly, (b) highlights the scutum, (c) shows the abdomen with a T-shaped pattern, and (d) indicates the wing with a dark costal band.

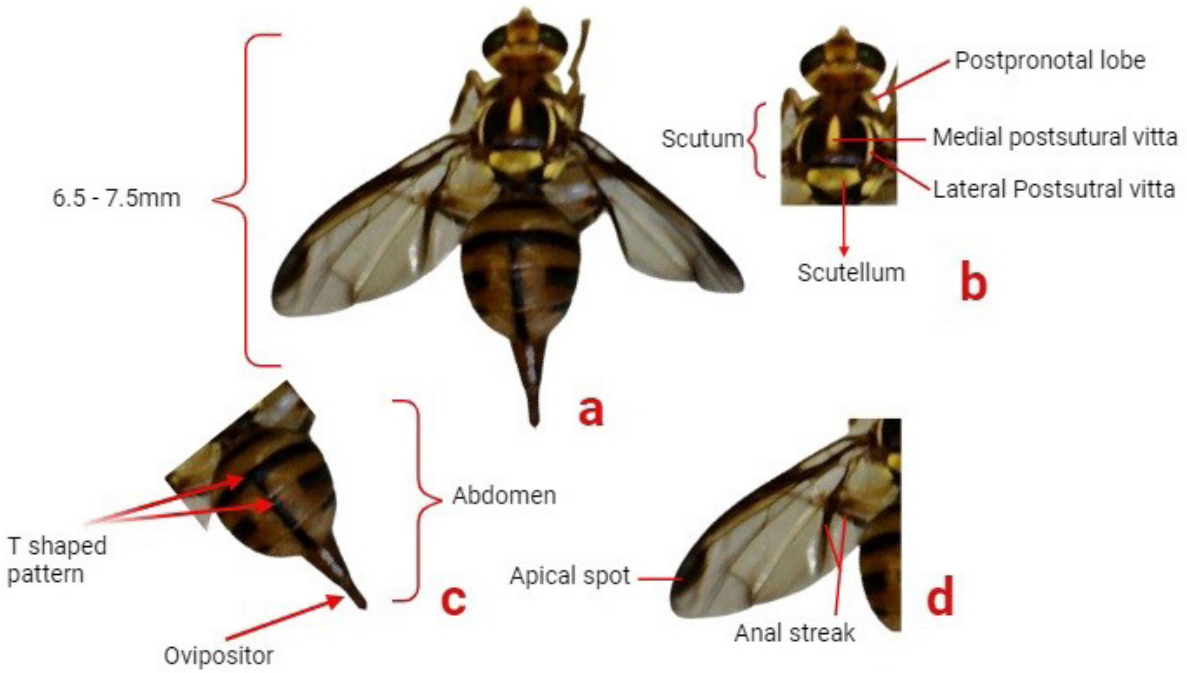
* Corresponding author: javariaqazi@qau.edu.pk
0030-9923/2026/0001-0001 $ 9.00/0



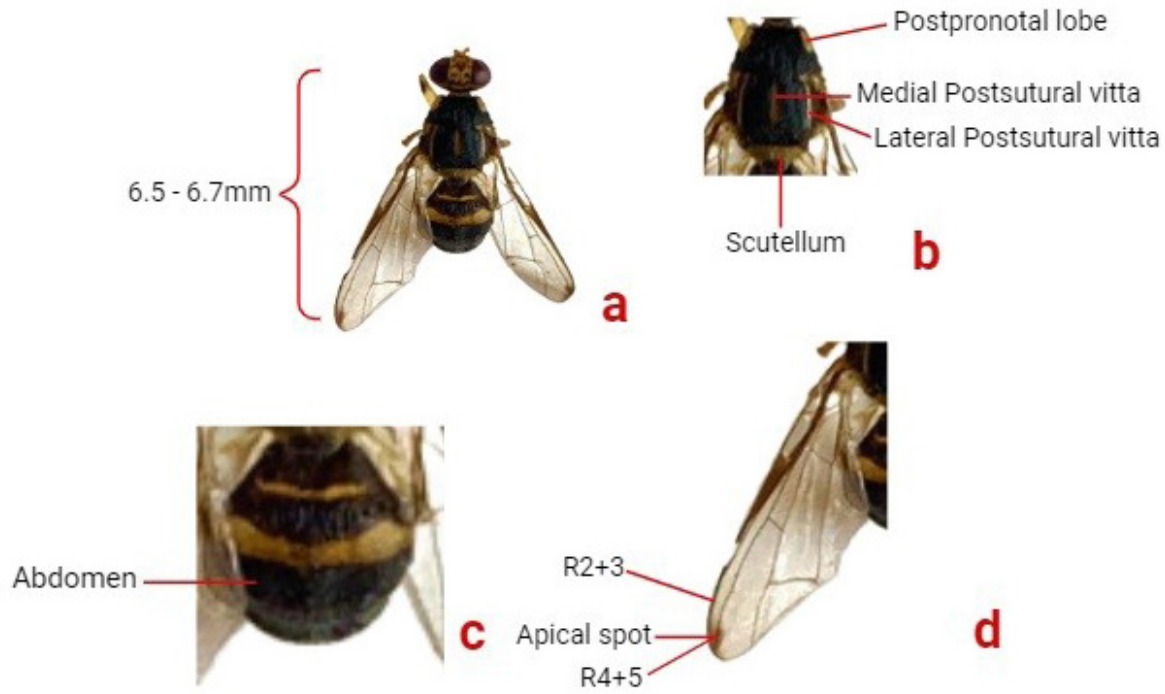
Supplementary Fig. 2. Image of *B. zonata*: (a) represents the size of the fly, (b) highlights the scutum, (c) shows the abdomen without T-shaped pattern, and (d) indicates the wing with small apical spot at R4+5 vein.



Supplementary Fig. 3. Image of *Z. cucurbitae*: (a) represents the size of the fly, (b) highlights the scutum having three yellow vitae, (c) shows the abdomen with T shaped pattern, (d) indicates the wing with dark costal band, big apical spot at R4+5 vein, spot at dm-cu and dark anal streak.



Supplementary Fig. 4. Image of *Z. tau*: (a) represents the size of the fly, (b) highlights the scutum having three yellow vitae, (c) shows the abdomen with T shaped pattern, (d) indicates the wing with dark costal band, dark anal streak and prominent apical spot at R4+5 vein.



Supplementary Fig. 5. Image of *Z. scutellaris*: (a) represents the size of the fly, (b) highlights the scutum having three narrow yellow vitae, (c) shows abdomen with fused tergites, (d) indicates the wing with apical spot at R4+5 vein.