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First Report of the Peach Fruit Fly, *Bactrocera zonata* (Saunders) Infesting Date Palm (*Phoenix dactylifera* L.) from North Gujarat, India

U. R. Karnavat[1*], R. D. Dodiya[2], N. P. Pathan[3], V. J. Joshi[1] and P. S. Patel[4]

[1]Dep. of Entomology, C. P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar, Gujarat (385 506), India
[2]Date Palm Research Station, Sardarkrushinagar Dantiwada Agricultural University, Mundra-Kachchh, Gujarat (370 421), India
[3]Dep. of Plant Protection, College of Horticulture, S. D. Agricultural University, Jagudan, Gujarat (384 460), India
[4]Directorate of Extension Education, S. D. Agricultural University, Sardarkrushinagar, Gujarat (385 506), India

Corresponding Author

U. R. Karnavat
e-mail: urvikarnavat@gmail.com

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Abstract

Field surveys were conducted during the peak fruiting season May-July, 2024–25 at different districts of North Gujarat. Significant fruit fly damage was reported in date palmat Dantiwada and Mundra(Kutch district). Adult flies were collected using methyl eugenol-baited parapheromone trapsand infested fruits were examined under laboratory conditions.Morphological and morphometric analyses identified the pest species as *Bactrocera zonata* (Saunders) (Diptera: Tephritidae). Females oviposited beneath the fruit epidermis and larval feeding caused extensive internal damage, resulting in fruit decay and rot. Molecular characterization using mitochondrial cytochrome oxidase subunit I (mtCOI) gene sequences confirmed species identity. Neighbor-Joining phylogenetic analysis showed that Gujarat populations clustered with *B. zonata* populations from India and Nepal, indicating close genetic relatedness, while showing moderate divergence from Iranian and Chinese populations.Protein structure prediction of the COI gene using the SWISS-MODEL server revealed monomeric, transmembrane-rich, predominantly α-helical protein structures with high confidence (GMQE=0.98). Ramachandran plot analysis confirmed excellent stereochemical quality of the predicted models. The integrated morphological, molecular and structural analyses confirm *B. zonata* as a serious pest of date palm in North Gujarat and provide baseline data for future monitoring and management programs.

Keywords: *Bactrocera zonata,* mtCOI, molecular, phylogenetic, North Gujarat

1. Introduction

The date palm (*Phoenix dactylifera* L.), a key crop in the Arecaceae family, is a vital source of food, nutrition and income for millions of people living in arid and marginal regions worldwide (Al-Khayri et al., 2021; Rashid et al., 2022). While India stands as the world's largest importer of dates, accounting for approximately 38% of the global market, its domestic production is largely confined to a narrow belt along its western border, particularly in the states of Gujarat, Rajasthan and Punjab. About 90% of India's date palm cultivated area is concentrated in the Kutch district of Gujarat, which enjoys near-monopoly status in commercial date cultivation within the country (Singh et al., 2025). However, production and productivity in India remain concerningly low compared to the world average, mainly due to the combined effects of biotic and abiotic stresses and the non-availability of quality planting material (Ahmed et al., 2023). Among the more common biotic stresses, insects, mites, bird pests and diseases cause considerable damage to the palms at both vegetative and fruiting stages, often compromising both yield and fruit quality (Hussain et al., 2020). More than 50 species of insects and mites have been reported as pests of date palms worldwide (Wakil et al., 2015). In India specifically, several insect pest species are known to be associated with date palm, causing significant yield losses each season (Haldhar et al., 2017). Major date palm pests reported worldwide include the red palm weevil, *Rhynchophorus ferrugineus* (Olivier), date dust mite, *Oligonychus afrasiaticus* (McGregor) (Al-Dhafer et al., 2022), lesser date moth, *Batrachedra amydraula* Meyrick, longhorn stem borer, *Jebusaea hammerschmidti* (Reiche) and almond moth, *Cadra cautella* (Walker), each contributing to substantial pre and post-harvest losses.

Fruit flies are among the most destructive agricultural pests worldwide, posing significant threats to horticultural crops,

including both fruits and vegetables across tropical and subtropical regions (Hasyim et al., 2008; Clarke et al., 2011). Belonging to the family Tephritidae, one of the largest dipteran families, fruit flies comprise more than 5,000 described species across approximately 500 genera, with around 250 species recognized as being of major economic importance (Scolari et al., 2021; Dias et al., 2022). Among these, *Bactrocera zonata* (Saunders) has emerged as a particularly major pest in recent years, causing significant infestations in date palm orchards across its invaded range. *B. zonata*, commonly known as the peach fruit fly or guava fruit fly, is native to South and Southeast Asia (Mudavanhu et al., 2021) and is now established in more than 20 countries worldwide, spanning parts of Africa, the Middle East and several Indian Ocean islands (Zingore et al., 2020). Recognized as one of the world's most destructive fruit flies, it causes significant damage to fruit yield and quality, and has been classified as an A1 quarantine pest by the European and Mediterranean Plant Protection Organization (EPPO), thereby restricting access to lucrative export markets (Imran et al., 2022). Infestation rates can range from 25% to as high as 90%, particularly during the peak summer season (Shinwari et al., 2015). This highly polyphagous pest attacks over 50 host plant species, including guava, mango, peach, sapota, apricot, fig and citrus (Ahmad et al., 2022). Female flies lay eggs inside developing fruits and the emerging larvae feed voraciously on the pulp, often leading to secondary infections by bacterial and fungal pathogens. Infested fruits frequently drop prematurely, rendering them unfit for both local markets and export (Jaleel et al., 2021).

2. Materials and Methods

2.1. Sampling

The survey was conducted during the peak fruiting season of date palm (*Phoenix dactylifera* L.) from late May to late July during 2024–2025 at North Gujarat, India. During survey, fruit fly infestation on date palm fruits were noticed at the Horticulture Farm of Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University (24°19'35.8"N 72°18'19.0"E) and Mundra in the Kutch district (22°49'28.1"N 69°43'13.5"E) (Figure 1). Adult fruit flies were monitored using parapheromone traps baited with methyl eugenol (1 ml) along with malathion 50 EC (0.5 ml) as a killing agent. The traps were installed at a height of approximately 1.5 m on date palm trees and were recharged at weekly intervals throughout the study period. Specimens collected from the traps, along with infested and damaged date palm fruits (Figure 2), were brought to the laboratory for further observations, including adult emergence analysis. Emerged and field-collected specimens were preserved and processed for accurate taxonomic identification. For confirmatory identification, representative specimens were sent to renowned fruit fly taxonomist at the ICAR–National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, Karnataka.

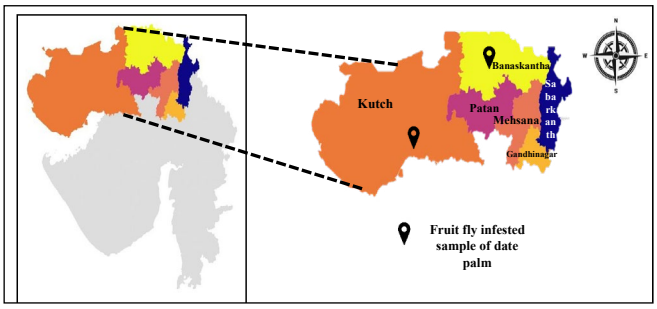

Figure 1: Map representing surveyed districts for fruit fly occurrence in North Gujarat

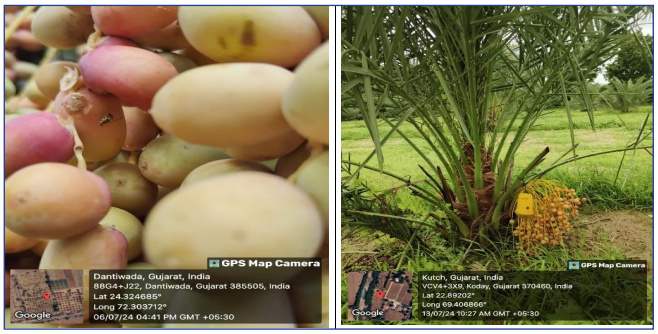

Figure 2: Infestation of fruit fly in date palm orchard at Dantiwada and Kutch

2.2. Morphological study

For the morphological study, adult fruit fly specimens collected from methyl eugenol traps and emerged from infested date palm fruits were examined in detail. Identification was based on external diagnostic characters such as body coloration, wing pattern, scutellum markings, facial spots, thoracic vittae and abdominal banding. Morphological identification was carried out using standard taxonomic keys and descriptions available for tephritid fruit flies (White and Elson-Harris, 1992; Drew and Romig, 2013). Detailed observations and measurements were made using a stereo-zoom binocular microscope (Nikon SMZ800N) fitted with a MIchrome 20 MP digital camera at the Department of Entomology, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. Diagnostic structures were photographed for documentation.

2.3. Molecular study

The molecular characterization of the fruit fly species was carried out using adult specimens preserved in 100% ethanol and stored at -20 °C until further processing. Genomic DNA was extracted from individual adult flies using the DNeasy Blood and Tissue Kit (QIAGEN), following the manufacturer's instructions. The mitochondrial cytochrome oxidase subunit I (mtCOI) gene was amplified using universal primers LCO1490F (5-GGTCAACAAATCATAAAGATATTGG-3) and HCO2198R (5-TAAACTTCAGGGTGACCAAAAAATCA-3). Polymerase chain reaction (PCR) amplification was performed with an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 45 s and

extension at 72°C for 1 min, with a final extension at 72°C for 10 min, following standard DNA barcoding protocols for tephritid fruit flies (Hebert et al., 2003; Armstrong and Ball, 2005; Dodiya et al., 2024). The amplified PCR products were verified by electrophoresis on a 1% agarose gel using a 100 bp - 1 kb DNA ladder and visualized under a gel documentation system. Successfully amplified products were commercially sequenced using the Sanger dideoxy sequencing method. The obtained chromatograms were edited to remove ambiguous bases and the consensus sequences were compared with available sequences in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) for species confirmation. The validated sequences were submitted to GenBank and accession numbers were obtained. The sequenced data were submitted to NCBI and accession numbers were obtained. For phylogenetic analysis, 16 COI sequences of tephritid fruit fly's species available in the GenBank database (NCBI) were retrieved. Phylogenetic relationships were reconstructed using MEGA XI with the Tamura 3-parameter distance model and the Neighbor-Joining method. One thousand bootstrap replicates were performed to assess branch support in the given tree (Felsenstein, 1985; Saitou and Nei, 1987; Tamura, 1992; Tamura et al., 2021). The predicted protein structure of tephritid fruit fly's (Sardarkrushinagar and Kutch locations) was predicted using SWISS-MODEL homology modeling server (https://swissmodel.expasy.org/). The aminoacid sequence, derived from the translated mtCOI gene, was submitted to identify suitable templates from the Protein Data Bank (PDB). The model was built based on the best matching template with high GMQE and QMEAN scores. Structural validation was performed using Ramachandran plot analysis to assess stereochemical quality. The final predicted protein structures (3D models) were visualized using PyMOL in ribbon format, highlighting α-helices and loop regions.

3. Results and Discussion

3.1. Morphological study

3.1.1. Diagnosis

Adult fruit fly specimens were identified as *Bactrocera zonata* (Saunders) (Diptera: Tephritidae: Dacinae) based on diagnostic morphological characters described in standard taxonomic keys (White and Elson-Harris, 1992; Drew and Romig, 2013). The described species was predominantly pale orange-brown, with small circular to oval-shaped spots in the antennal furrow (Figure 3 A and B).The face was fulvous and bears a pair of moderately sized oval black spots (Figure 3 C). The scutum was red-brown with pale fuscous patterning on the posterior region, while the postpronotal lobes and notopleura were yellow. The mesopleural stripe extended dorsally to, or nearly to, the anterior notopleural seta. The lateral postsutural vittae were of medium width, parallel-sided and terminate at or just behind the intra-alar seta, whereas the medial postsutural vitta is absent. The scutellum was entirely yellow (Figure

A) Female dorsal habitus B) Male dorsal habitus

C) Head D) Thorax

E) Female abdomen F) Male abdomen

G) Fore leg H) Middle leg I) Hind leg

J) Fore wing

Figure 3: Morphological identification of *B. zonata*

3 D). Abdominal terga III–V were red-brown and display a characteristic 'T' pattern, comprising a narrow transverse black band along the anterior margin of tergum III, often medially interrupted and a narrow medial longitudinal black band extending across all three terga, frequently reduced on parts of terga IV and V. Narrow anterolateral fuscous markings were present on terga IV and V and a pair of oval, red-brown, shining spots occurs on tergum V. The posterior lobe of the male surstylus was short and in females the apex of the aculeus was needle-shaped (Figure 3 E and F). All leg segments are fulvous, except for the apices of the femora, which were red-brown and the hind tibiae, which vary from pale fuscous to fuscous (Figure 3 G, H and I). The wings have colourless cells bc and c and are completely devoid of microtrichia; a narrow fuscous costal band was present, confluent with vein R_{2+3} and ending at the apex of this vein, along with a small oval fuscous spot across the apex of vein R_{4+5}. The anal streak was reduced to a faint tint within cell cup and the supernumerary lobe was moderately developed (Figure 3 J).

3.2. Morphometric analysis

Observations on the body length ofmales of adult *B. zonata* ranged from 7.04 mm to 7.60 mm, while in females it ranged from 7.83 mm to 8.30 mm. The mean body length was recorded as 7.45±0.21 mm for males and 8.06±0.15 mm for females. The body width of adult *B. zonata* males ranged from 11.03 mm to 11.77 mm, while in females it varied from 11.03 mm to 11.90 mm. The mean body width was recorded as 11.41±0.25 mm for males and 11.98±0.26 mm for females. The head dimensions of *B. zonata* revealed that the head length and width in males were 1.51±0.06 mm and 1.78±0.07 mm, respectively, while in females these measurements were 1.52±0.08 mm and 1.79±0.08 mm, respectively. Thorax measurements indicated a length of 2.78±0.06 mm and a width of 2.02±0.10 mm in males, whereas in females, the thorax length and width were 2.80±0.06 mm and 2.03±0.08 mm, respectively. More prominent differences were observed in the abdominal dimensions, with males exhibiting an abdominal length of 3.09±0.07 mm and a width of 2.17±0.05 mm, while females displayed an abdominal length of 3.68±0.08 mm and a width of 2.18±0.05 mm. The observations on wing dimensions showed that the total wing length for male was 4.75±0.08 mm, while for female, it measured 4.77±0.09 mm. The wing width was recorded at 1.77±0.08 mm for male and 1.78±0.10 mm for female. Regarding leg measurements, the average length for fore leg both male and female fruit flies was 5.26±0.07 mm and 5.26±0.09 mm, respectively. The mid-leg length was observed at 5.87±0.12 mm in male and 5.86±0.11 mm in female. For hind legs, male had an average length of 5.48±0.10 mm, while female exhibited a slightly longer measurement of 5.50±0.12 mm. It has a reddish-yellow body with a faint yellow stripe. The black band along the anterior margin of the wing was incomplete and the posterior margin lacks bands. Additionally, the abdomen does not feature the characteristic T-shaped marking.

3.3. Damage

The female fly punctured the fruit with its stout and hard ovipositor and laid eggs below the epidermis (Figure 4 A). The female fly prefers to oviposit on soft area of the fruit rather than hard surface; this was observed under field conditions when fruit fly was found ovipositing on Date palm. After egg hatching, the maggots begin to develop inside the pulp and a brown soft rotten patch became visible on the surface of the fruit. The developing larvae fed internally, causing fruit decay characterized by a foul odour and transformation of the fruit pulp into a discolored, semi-liquid mass (Figure 4 B). The maggots remained inside the fruit till they become fully mature. The full-grown maggots (Figure 4 C) were found coming out of the fruit through the exit hole made by its sharp oral hooks and pupate in the soil till the emergence of adults.

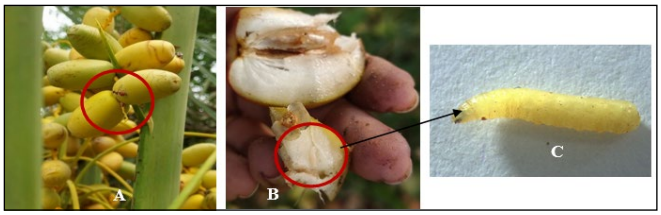

Figure 4: (A) *B. zonata* oviposition on date plam fruit; (B) Maggots feeding inside the fruit of date plam; (C) Maggot of *B. zonata*

3.4. Molecular study

The Neighbor-Joining (NJ) phylogenetic analysis based on the mitochondrial COI gene sequences clearly confirmed the identity of the collected specimens as *Bactrocera zonata*. The mtCOI sequences generated from the present study, including specimens from Kutch (PX506270.1) and Dantiwada, Gujarat (PX506246.1), clustered firmly within the *B. zonata* clade, along with reference sequences retrieved from GenBank originating from India, Pakistan, Nepal, Iran and China, thereby validating the morphological identification.The Gujarat populations grouped closely with *B. zonata* sequences from Nepal (PP569085.1) and India (PV211193.1; New Delhi), indicating a close genetic relationship among South Asian populations. The Pakistani specimen (MK296117.1) formed a nearby lineage, suggesting regional genetic continuity across the Indian subcontinent. In contrast, several Iranian populations (e.g., MG881756.1, MG881760.1, MG881699.1) and the Chinese specimen from Beijing (MK564011.1) appeared relatively more distant, though still within the same species clade, reflecting moderate intraspecific genetic divergence.Bootstrap support values across major nodes ranged from low to moderate (29–71), which was typical for intraspecific phylogenies based on mtCOI sequences. The placement of the Gujarat specimens within the main *B. zonata* cluster, without forming a distinct outgroup, suggested no cryptic species differentiation, but rather geographically structured genetic variation. Overall, the phylogenetic topology indicated that *B. zonata* populations from Gujarat were genetically closer to other South Asian

populations than to Middle Eastern or East Asian populations, supporting the hypothesis of regional population connectivity and recent dispersal (Figure 5).

Figure 5: Neighbor-Joining tree of COI sequences from 16 *B. zonata* specimens based on Tamura 3-parameter model with the 1000 bootstrap analysis. Numbers above/ below nodes represent bootstrap values

Protein structure prediction of *B. zonata* was carried out using the SWISS-MODEL server, employing AlphaFold v2–derived templates of cytochrome c oxidase subunit I (COI). The predicted structures for both analyzed samples were inferred to exist as monomeric proteins, with a high Global Model Quality Estimation (GMQE) score of 0.98, indicating excellent confidence in the generated homology models. In both cases, the models were predicted to contain a transmembrane segment, consistent with the known functional role of COI proteins in mitochondrial electron transport.For the Dantiwada sequence, the best template identified was A0A3G2WJU1.1. A, corresponding to the AlphaFold DB model of cytochrome c oxidase subunit 1 from *Bactrocera zonata* (guava fruit fly). This model showed a high sequence identity of 98.48%, with complete sequence coverage (1–197 amino acids) and a sequence similarity score of 0.60. The biounit oligo-state was predicted as monomeric, further supporting the structural consistency of the model (Figure 6A). The Kutch sample predicted structure utilized A0A4D6DKH2.1. A as the best template, representing the AlphaFold DB model of cytochrome c oxidase subunit 1 from *Dacus ciliatus* (lesser pumpkin fly). This model exhibited 100% sequence identity, full coverage across 1–229 amino acidsand a slightly higher sequence similarity score of 0.61. As with the first model, the predicted oligo-state was monomeric and the structural confidence was reinforced by the high GMQE value (Figure 6B). The ribbon representations of the predicted protein structures (Figure. 6 A and B) revealed that both models were predominantly α-helical, arranged in a compact and elongated fold typical of mitochondrial membrane-associated COI proteins. The helices formed a tightly packed core with minimal loop regions and the rainbow coloring clearly illustrated the N- to C-terminal orientation, indicating orderly folding and structural

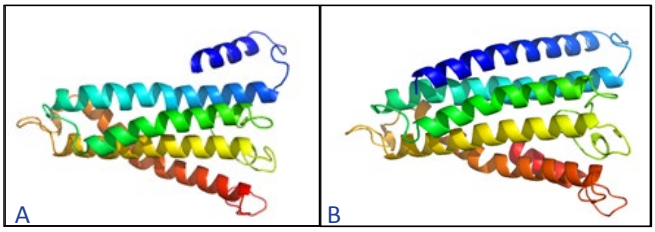

Figure 6: Predicted protein structure of *B. zonata* coloured from N-terminus (blue) to C-terminus (red) collected from a) Dantiwada, showing a compact α-helical fold b) Kutch, showing a stable, though slightly more open, α-helical fold

integrity. The overall architecture supports the presence of functionally conserved transmembrane helices essential for electron transport activity. Stereochemical validation using Ramachandran plot analysis (Figure 7 A and B) demonstrated that the majority of amino acid residues were located in the most favoured regions, particularly within the right-handed α-helix domain. Only a small proportion of residues occupied additionally allowed regions and very few or no residues were observed in disallowed regions, confirming that the backbone dihedral angles (Φ and Ψ) fall within energetically permissible limits. These results validated the high stereochemical quality and reliability of both predicted models. Overall, the high sequence identity, complete coverage, excellent GMQE scores and favourable Ramachandran statistics confirm that the predicted protein structures of *B. zonata* are robust, accurate and biologically meaningful. The dominance of α-helical transmembrane architecture reflects strong functional conservation of COI proteins among hymenopteran and dipteran taxa. These validated models were therefore suitable for downstream computational analyses, including molecular docking, functional annotation, phylogenetic inference and ecotypic differentiation of closely related or cryptic parasitoid species (Dodiya et al., 2025).

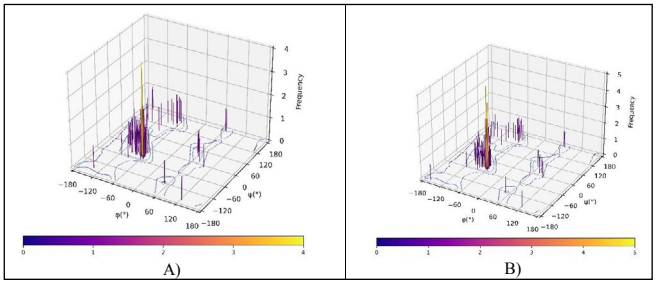

Figure 7: Ramachandran plot of the predicted protein structure of B. zonatacollected from a) Dantiwada, showing most residues within the favored regions b) Kutch, displaying favorable stereochemical geometry; bar represents frequency of torsion angles

The present study provides an integrative assessment of fruit fly infestation in date palm orchards of North Gujarat and conclusively identifies *Bactrocera zonata* as the dominant pest species associated with *Phoenix dactylifera*. The combined use of field surveillance, morphological taxonomy, molecular

barcoding, phylogenetic analysis and protein structure prediction offers a robust framework for pest identification and population assessment, which is essential for effective management strategies. The detection of *B. zonata* infestation during the peak fruiting season corroborates earlier reports describing the species as a highly polyphagous and economically important pest in tropical and subtropical regions (White and Elson-Harris, 1992; Drew and Romig, 2013). Although *B. zonata* is primarily known for damaging fruits such as mango, guava, peachand citrus, its infestation on date palm observed in the present study highlights its expanding host range and adaptive potential. Oviposition beneath the fruit epidermis and subsequent larval feeding within the pulp resulted in fruit decay, foul odour and tissue breakdown, symptoms consistent with previous descriptions of tephritid fruit fly damage (Garcia et al., 2020). Such concealed larval feeding not only reduces marketable yield but also complicates early detection and control under field conditions.

Morphological diagnosis based on external characters, including wing pattern, thoracic vittae, scutellar coloration, abdominal banding and genital structures, confirmed the identity of the species as *B. zonata*. These diagnostic traits are in agreement with standard taxonomic descriptions provided by White and Elson-Harris (1992) and Drew and Romig (2013). Morphometric analysis further supported species-level identification and revealed subtle sexual dimorphism, particularly in body size and abdominal dimensions, with females being larger than males. Similar morphometric trends have been reported in other *Bactrocera* species and are generally associated with reproductive adaptations in females (Clarke et al., 2005). Molecular characterization using the mitochondrial cytochrome oxidase subunit I (mtCOI) gene successfully validated the morphological identification of *B. zonata*. The clustering of Gujarat specimens within the main *B. zonata* clade in the Neighbor-Joining phylogeny confirms the reliability of COI barcoding for tephritid fruit fly identification, as widely demonstrated in earlier studies (Hebert et al., 2003; Armstrong and Ball, 2005). The close genetic affinity of Gujarat populations with those from India and Nepal suggests strong regional connectivity and possible gene flow across South Asia. In contrast, moderate divergence from Iranian and Chinese populations reflects geographic structuring, which may be influenced by ecological barriers, climatic variation, or historical dispersal events. Such patterns of intraspecific variation are common in widely distributed fruit fly species and have been documented in *B. zonata* and other members of the *Bactrocera dorsalis* complex (Majumder, 2019). The absence of a distinct outgroup or cryptic lineage among Gujarat populations indicates that the observed variation represents population-level differentiation rather than speciation.Protein structure prediction of the mtCOI-derived amino acid sequences provided additional molecular-level insights into *B. zonata*. The high GMQE values (0.98), complete sequence coverage and strong template similarity indicate that the predicted models are highly reliable. The predominance of α-helical transmembrane domains observed in both predicted structures is characteristic of cytochrome c oxidase subunit I, a key component of the mitochondrial electron transport chain (Tsukihara et al., 1996).

The excellent stereochemical quality of the models, as confirmed by Ramachandran plot analysis, further validates their structural accuracy. Minor conformational differences between the Dantiwada and Kutch samples may reflect geographic or genetic variation, although these differences do not appear to affect overall protein stability or functional integrity. Similar conservation of COI protein structure despite nucleotide-level variation has been reported in several insect taxa and underscores the functional constraints acting on mitochondrial proteins (Cameron, 2014).The confirmation of *B. zonata* as a pest of date palm in North Gujarat has important implications for regional pest management and quarantine programs. Accurate species identification is a prerequisite for implementing targeted control strategies such as male annihilation techniques, bait sprays and biological control (Dodiya et al*.,* 2023). The molecular and structural data generated in this study also provide valuable reference material for future population genetic studies, resistance monitoring and the development of molecular diagnostics (Dodiya et al., 2025). Overall, the integrative approach adopted in this study strengthens confidence in pest diagnosis and enhances understanding of the biology and population structure of *B. zonata*. Such multidisciplinary frameworks are increasingly essential for addressing complex pest management challenges under changing agricultural and climatic conditions.

4. Conclusion

Bactrocera zonata as a key date palm pest in Gujarat. Morphological, morphometric and mtCOI barcoding verified species identity, ruling out cryptic variation, while phylogenetic analysis revealed strong genetic links with other South Asian populations. COI protein modeling showed conserved, transmembrane-rich structures. Together, these findings support pest surveillance and quarantine management in date palm orchards.

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