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Integrative Morphometric and Molecular Characterization of Diarrhegma modestum (Fabricius, 1805) (Diptera: Tephritidae) Demonstrates Evolutionary Cohesion and Implications for Sustainable Pest Management

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Integrative Morphometric and Molecular Characterization of *Diarrhegma modestum* (Fabricius, 1805) (Diptera: Tephritidae) Demonstrates Evolutionary Cohesion and Implications for Sustainable Pest Management

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Abstract

Fruit fly infestations pose a persistent threat to tropical fruit production, yet integrative morphometric and molecular studies remain limited for emerging Tephritid pests in South Asia. *Diarrhegma modestum* (Fabricius, 1805) (Diptera: Tephritidae) is a recently re-emerging species attacking *Cucurbita maxima*, *Trichosanthes cucumerina*, *Luffa acutangular*, *Mangifera indica*, *Psidium guajav*a and other hosts, but its morphological variability, genetic stability, and evolutionary position have not been comprehensively characterized. This study bridges that knowledge gap by integrating morphometric, multivariate, and molecular phylogenetic analyses to define intraspecific structure and evolutionary relationships of *D. modestum* populations from South India. Morphometric evaluation revealed pronounced sexual dimorphism, with females exhibiting the largest size difference in whole-body length (6.41 mm) compared to males (5.16 mm). Principal Component Analysis (PCA) explained 56.95 % of total variance, identifying head width, thorax width, wing width, and antenna length as positively correlated traits forming a dominant "size-breadth" axis associated with flight and reproductive performance. The mitochondrial COI sequence analysis (682 bp) confirmed 97.8-99.2 % identity with reference *D. modestum* accessions, while Bayesian and chronogram analyses dated the *Diarrhegma–Carpomya* divergence to 9-11 Mya, reflecting moderate substitution rates (0.1567 subs/site/unit time) and neutral evolution. By linking morphometric differentiation with genetic stability, this study establishes a diagnostic and evolutionary framework for sustainable pest management. The integrative characterization enables oviposition-targeted surveillance, female-biased baiting, and molecularly validated monitoring. Together, these insights provide a foundation for precision-based, eco-adaptive strategies that strengthen fruit-fly management and support resilient tropical agroecosystems.

Keywords: *Diarrhegma modestum*; Morphometric analysis; Principal Component Analysis (PCA); COI barcoding; Bayesian phylogeny; Evolutionary divergence and Sustainable pest management

1. Introduction

Fruit flies (Diptera: Tephritidae) are among the most destructive insect pests of horticultural crops worldwide, causing direct damage to fruits and vegetables and imposing stringent quarantine restrictions on trade. Their economic importance in tropical and subtropical regions is well documented, particularly for genera such as *Bactrocera*, *Ceratitis*, *Anastrepha* and *Rhagoletis* that attack high-value fruit crops and significantly reduce marketability and export potential (Smit et al. 2013; Leblanc et al. 2019). In the Indian subcontinent, more than 120 fruit fly species are known, distributed across several subfamilies and tribes, with multiple species co-occurring on the same host plants (Agarwal and Sueyoshi 2005; David and Ramani 2011). This species richness complicates field diagnosis and makes accurate identification a prerequisite for designing effective pest-management strategies. Within Tephritidae, *Diarrhegma modestum* (Fabricius 1805) is a member of the subfamily Tephritinae, tribe Acanthonevrini, historically recorded from parts of India but largely overlooked compared with major Dacini pests. Early taxonomic treatments and catalogues listed *D. modestum* from Karnataka, Tamil Nadu and West Bengal, and noted its complex synonymy under genera such as *Dacus*, *Ptilona*, *Tephritis* and *Rioxa* (Bezzi 1913; Agarwal and Sueyoshi 2005). More recent work by David and Ramani (2011) provided an illustrated key and checklist for Indian fruit flies and confirmed *D. modestum* as a distinct species within Tephritinae, characterized by its patterned wings, brownish body, and distinctive female aculeus.

Over the last decade, *D. modestum* has attracted renewed attention due to its apparent geographic expansion and broadened host range. It was first recorded from Bangladesh as a fruit fly associated with moringa (*Moringa oleifera*) and recognised as a potential emerging pest (Leblanc et al. 2019). Subsequent reports documented first records from mango and guava orchards of Bihar (Leblanc et al. 2019) and Odisha (Sahoo and Tripathy 2021), indicating a wider distribution than previously recognised and suggesting that *D. modestum* may become an important pest of horticultural commodities in eastern and southern India. In addition to cucurbits, mango and guava, *D. modestum* has been reported on other hosts in Indian agro-ecosystems, and its adult morphology and mt-COI barcode (e.g. accession MK559395) are now documented in the ICAR-NBAIR reference collections (ICAR-NBAIR 2020).

Despite these taxonomic and distributional advances, integrative studies combining fine-scale morphometrics, multivariate (PCA) analysis and molecular phylogenetics are still lacking for *D. modestum*. Existing work has largely focused on first records, host association and basic

diagnostic features (Sahoo and Tripathy 2021), while broader Tephritid research has shown that detailed morphometric and geometric morphometric analyses can reveal subtle patterns of sexual dimorphism, population structure and host-induced shape change in species such as *Bactrocera dorsalis* and *Ceratitis capitata* (Van Cann et al. 2015; Pieterse et al. 2017). Similarly, DNA barcoding and COI-based phylogenies have been widely used to clarify species limits and support identification across Tephritidae, with large-scale efforts demonstrating the feasibility of COI for routine diagnostics (Smit et al. 2013).

Furthermore, although several global and regional studies have modelled Tephritid evolution and diversification(Han and Ro 2009; He et al. 2024), the evolutionary placement, divergence time and substitution dynamics of *D. modestum* remain poorly quantified. This limits our ability to interpret its apparent range expansion in the context of evolutionary history, host shifts and climate-driven changes in agroecosystems. At the same time, sustainable management of fruit flies increasingly relies on integrative taxonomic frameworks that combine morphology, DNA barcoding and phylogenomics to guide surveillance, quarantine decisions and Integrated Pest Management (IPM) strategies (Smit et al. 2013).

Given these gaps, there is a clear need to characterize *D. modestum* using a combined morphometric and molecular approach. Specifically, detailed linear morphometrics and PCA can quantify sexual dimorphism and intraspecific variability; mt-COI sequencing and Bayesian phylogenetic analysis can validate species identity and infer evolutionary relationships; and time-calibrated trees and rate analyses can place *D. modestum* within the broader Tephritid diversification timeline.

In this study, *D. modestum* populations saprophytes in decayed ber tree trunks in South India were analysed through an integrated morphometric and molecular framework to address these knowledge gaps. Detailed morphometric measurements and Principal Component Analysis (PCA) were employed to quantify sexual dimorphism, identify the most discriminating body traits, and assess population-level variation. Concurrently, mt-COI gene sequencing and Bayesian phylogenetic analysis were performed to validate species identity, infer evolutionary relationships within Tephritinae, and estimate divergence times and substitution rates. This combined approach provides the first comprehensive insight into the morphological stability and evolutionary dynamics of *D. modestum*. By linking morphometric differentiation with molecular homogeneity, the study establishes a robust diagnostic foundation and evolutionary context that can inform precision-based, sustainable pest management strategies. Such

integration of phenotypic and genotypic data enhances early detection, species confirmation, and the development of targeted, eco-adaptive interventions for managing *D. modestum* and related Tephritid pests in tropical agroecosystems.

2. Materials and methods

2.1 Sample Collection

Adult flies samples were captured nearby decayed tree trunk of jujube (ber) orchards from Regional Research Station, Aruppukottai (9.5567°N, 78.0910°E), Tamil Nadu. Taxonomical identification and morphologically identified by using taxonomical key of David and Ramani, (David and Ramani 2011; Hancock 2015) and also specimen was identified based on keys of true fruit flies of India organized by Dr. K. J. David, Senior Scientist from ICAR-National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Hebbal (Bengaluru). Morphometric measurements of various body parts were carried out from ten insect samples. It was performed under LEICA M205A, Software LAS v4.12 stereoscopic microscope fitted with camera at Centre of Innovation at Tamil Nadu Agricultural University, Madurai.

2.2 Molecular Identification

One adult insect was taken for molecular identification from RRS, Aruppukottai, Tamil Nadu. This work was performed at the National Professor Lab at ICAR- Indian Institute of Horticultural Research, Bengaluru. The DNA extraction was done by using the Cetyltrimethylammonium bromide (CTAB) method. Samples were crushed with a pestle in a mortar by adding a sufficient amount of liquid nitrogen. Crushed samples were transferred into 2ml of microcentrifuge tube and 700µl of CTAB buffer was added to that. Samples were incubated at 60ºC for 30-45 mins. Then, 700µl of each tube mix was added gently and kept it for centrifugation at 13,000 rpm for 10minutes. The clear upper solution was taken in a new 2ml tube and the lower debris were discarded. Phenol: Chloroform: Isoamyl alcohol (P:C:I) (25:24:1) equal volume mix was added gently and kept for centrifugation at 13,000 rpm for 10minutes. The supernatant was pipetted in a 1.5ml microcentrifuge tube, to that sodium acetate (3M 5.7P_H), and an equal volume of iso propanol was added and kept overnight for incubation for the duration of 30minutes. Centrifuged at 13,000 rpm for 15minutes and the solution was discarded without disturbing the pellets. Washed with ethanol (70%) and kept it for drying. The pellets were dissolved in nuclease free water. 3µl of RNAse was added and kept for 15minutes at room temperature. Incubation was carried out at 37ºC and then 55ºC for

30seconds and stored at -80° C (Sudhagar et al. 2014). The primers used in this study include LCO1490 (5′GGTCAACAAATCATAAAGATATTGG-3′) with an expected amplicon size of approximately 710 bp and HC02198 (5′- TAAACTTCAGGGTGACCAAAAAATCA-3′) (Vrijenhoek 1994). The PCR amplification was carried out using the primers LCO1490 and HC02198 following the protocol described by Vrijenhoek (Vrijenhoek 1994). The process included an initial denaturation, followed by denaturation, annealing, and extension. A final extension was performed, and the samples were subsequently stored. PCR was carried out to amplify the sequence by using the universal primer COXI-COXII to specify the region in mitochondrial genome. The primers were prepared at concentration of 10 picomoles. microlitre^{-1}, according to manufacturer's instruction. The PCR reaction was carried out using reaction mixture of 50 µl of the reaction components, 25µl of the prepared reaction mixture (TaqDNA Polymerase Master Mix), 0.25µl of each forward and reverse primer, water 3.5µl. Amplification was done by using a thermal cycler (Techne TC-3000X ThermalCycler, UK) according to the thermal cycling program mentioned by Vrijenhoek (Vrijenhoek, 1994). After confirming the success of the DNA amplification of the targeted gene segment by electrophoresis, the PCR products were sent to the sequencing company (Eurofins Genomics India Pvt Ltd.) for reading the sequences of nitrogenous bases for each sample.

The sequences processing was performed using BioEdit 7.0 (Informer Technologies, Inc.,). The processed sequences were compared with the deposited sequences at the US National Centre for Biotechnology Information (NCBI, USA) using the Basic Local Alignment Search Tool (BLAST) to determine identification, which was based on the maximum score and query cover, and identify percentage provided by the research tool; highest rates of the mentioned criteria were adopted to confirm the identification to species level. After confirming the identification, the confirmed sample sequences were deposited and registered in NCBI GenBank®(https://www.ncbi.nlm.nih.gov/). The sequence homology was interpreted by comparing other *Tephritidae* sequence within NCBI Gen Bank database.

2.3 Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown in the branches (Felsenstein 1985). The evolutionary distances were computed using the maximum composite likelihood method

(Tamura et al. 2004) and they were in the units of the number of base substitutions per site. This analysis involved 12 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 711 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 software (Stecher et al. 2020, Tamura et al. 2021). Phylotree were constructed by using iToL (Interactive Tree of Life) (https://itol.embl.de/) version 7.2.1.

2.4 Statistical analysis

2.4.1 Morphometrics analysis

All the statistical analyses to determine the impact of different replications and different individual characteristics on the morphometric variations and graphical representation were performed in open source “R” software (http://www.rstudio.com). Principal Component Analysis (PCA) for both male and female fruit flies was performed by using "Factoextra" (Kassambara and Mundt 2016) and "Factominer" (Husson et al. 2013a, Husson et al. 2013b) packages. The biplot of PCA was also obtained by using "ggplot2" packages.

2.4.2 Mr. Bayes Analysis

Bayes analysed by packages of "Biostring" used to read FASTA files into R for alignment and analysis and "msa" for mulitple DNA sequence alignement converts to matrices for phylogenetic inference and "ape" for evolutionary distance calculation and chronograms and "phangorn" for build maximum likelihood (ML) phylogenetic trees and run optimizations (GTR+G+I model). and "ggplot2" used for plots of rates, clade comparisons, density distributions, regressions and "dplyr" for cleaning and summarizing rate tables, clade mappings, and test results and "tidyr" for reshape rate data into a tidy format for plotting and summaries and "patchwork" for multi-panel figures (density panels, clade vs regression plots, background diagnostics, selection trees) and "ggtree" used publication quality tree visualizations (ML tree, selection gradient tree) and "viridis" for nice consistent color palettes across clades/partitions and "scales" for axes are readable (percentages, scientific notation, etc.) (Ahmad et al. 2015).

3. Results

3.1 Morphometrics measurements of *D. modestum*

The species were morphologically identified as *D. modestum* (Fab.). The results for morphometrics measurements of male and female *D. modestum* were given in Table 2 and 3. With regards to comparative morphometric analysis of male and female adults of *D. modestum* (Fab.) revealed distinct differences between the sexes. The adults exhibits and distinguished by its brilliant coloration, including a reddish-brown scutum, a broad yellow prescutellar patch, yellow scutellum, and wings patterned with dark markings interspersed with hyaline indentations (Fig 1). Morphological features such as short antennae with a plumose arista, six scutellar bristles, and setose radial veins further aids to its identification.

Females consistently exhibited larger body dimensions than males across most traits. The mean female and male antenna were exhibited longer 0.62mm compared to the male 0.46 mm, suggesting a higher sensory adaptation in females. Head showed that difference, with females recorded 1.54 mm in length and 1.71 mm in width, whereas males measured 1.36 mm and 1.51 mm, respectively. Similarly, the mean length and width of thorax for females revealed with 1.59 mm length and 1.43 mm width and males recorded 1.42 mm and 1.23 mm.

Leg measurements also indicated subtle sexual variation. Females showed longer fore 3.21 mm and mid legs recorded 3.49 mm, while hind leg length was nearly similar between sexes, with males measured 3.51 mm and females 3.56 mm. Wing patterned with dark markings interspersed with hyaline indentations and size clearly differentiated the sexes, with mean length and wing expanse females exhibited 4.02 mm and 1.67 mm compared to males 3.87 mm and 1.43 mm, contributing to a broader wing expanse in females with 5.41 mm than in males recorded 4.95 mm.

The overall mean length and width of female and male abdomen displayed a marked difference with 1.92 mm and 1.90 mm than males recorded with 1.83 mm and 1.80 mm, reflecting reproductive adaptation. The mean length of whole body length of females recorded 6.41 mm compared to 5.16 mm in males, confirmed that overall larger size of females. An ovipositor, exclusively present in females, measured 0.31 mm and served as a distinctive morphological trait differentiating the sexes.

Taken together, these observations highlight that females of *D. modestum* are morphologically larger, with more developed thoracic, abdominal, and wing structures, while males remain relatively smaller and more slender. These differences provide reliable diagnostic characters for distinguishing sexes and may have ecological and reproductive significance.

3.2 PCA analysis of morphometrics of *D. modestum*

Principal Component Analysis (PCA) of morphometric traits in male and female adults of *D. modestum* (Fab.) revealed sex-specific patterns of trait contribution to overall variation. In males, the first three PCs accounted for 61.85% of the total variance, while in females the same components explained a slightly higher cumulative variance of 64.47%, indicating greater morphological variation among females given in Table 4 and 5 (Fig 2).

For males, PCA1 (25.87%) was strongly influenced by thorax width, head length, antenna length, and hind leg length, highlighting the predominance of thoracic and antennal dimensions in explaining primary variation (Fig 3). In contrast, female PCA1 (26.69%) was dominated by mid leg length, antenna length, hind leg length, and fore leg length, suggesting that leg traits were the major contributors to variation in females provided in Table 5 and 6 (Fig 4). PCA2 in males (20.06%) was largely defined by wing width, thorax length, mid leg length, and hind leg length, whereas in females (21.77%), wing width, thorax width, head length, and mid leg length were the key contributors, showing stronger wing and thoracic influences in females (Table 4 and 5) (Fig 5). PCA3 in males (15.92%) highlighted contributions from mid leg length and wing length with negative associations of antenna and fore leg length, while in females (16.22%), head width and hind leg length were the major determinants of variation.

Thus, the PCA clearly indicates that in males, thoracic and antennal traits play a dominant role, while in females, leg and wing traits contribute more strongly to morphological structuring (Fig 6). The higher cumulative variance in females further suggests that sexual dimorphism in *D. modestum* is not only expressed through overall body size but also through distinct trait groupings influencing each sex.

3.3 Comparative PCA and K-Means clustering for morphometrics of *D. modestum*

Unsupervised K-means clustering resolved the *D. modestum* morphometric dataset into three discrete groups, which were strongly supported by the underlying multivariate structure. Trait-wise distributions showed that Cluster 1 comprised individuals with the largest thoracic, wing, and overall body dimensions; Cluster 2 represented intermediate phenotypes; and Cluster 3 contained uniformly smaller individuals across most traits. PCA corroborated this grouping pattern. PC1 (25.9% variance) was driven primarily by wing metrics and whole-body size, while PC2 (20.2% variance) was dominated by leg lengths and thoracic measurements. These high-loading traits were the same variables that differentiated the three K-means clusters in the univariate distributions (Fig 7). The congruence between PCA loadings and cluster-wise trait profiles confirms that body size, wing architecture, and appendage length form the principal

axes of morphometric divergence within the population. Collectively, the analyses identify three robust phenotypic classes large-bodied, intermediate, and small-bodied morphotypes indicating clear intra-population morphometric structuring in *D. modestum*.

3.4 Hierarchical clustering for grouping categorical morphometric data

Hierarchical clustering of the standardized morphometric dataset resolved the traits into three distinct groups, reflecting clear structural differentiation in measurement magnitude (Fig. 8). Traits with the lowest absolute values (0.256-1.94) formed a tightly associated cluster, indicating high covariance among early-stage measurements. A second cluster comprised intermediate-scale traits (4.84-5.44), which exhibited uniform positive loadings and minimal within-cluster variance. The largest traits (6.35-7.17) segregated into a separate terminal branch, demonstrating strong divergence from the smaller morphometric variables. Column clustering showed that all replicates grouped into two primary branches with minor sub-structuring, confirming overall measurement consistency across samples. The heatmap pattern demonstrates that trait variance is strongly size-structured, with early, mid and late-stage metrics forming coherent clusters driven by shared morphological scaling. These results indicate that morphometric traits exhibit stable covariation patterns suitable for downstream multivariate analyses (Fig 8).

3.5 Molecular characterisation

The results of PCR product showed two clear bands, ladded with size of about 682 basepairs, confirmed that amplification of the mitochondrial-COI COXI – COXII targeted region and again molecular based analyses are confirmed the species belongs to has mentioned keys of David and Ramani, (David and Ramani 2011, Hancock 2015). the nucleotide sequences of *D. modestum* from Tamil Nadu was deposited in GenBank as PQ198000.1 which had nearly 99.24% genetic similarity with sequences PV245016.1 from (Bangladesh, Dhaka);98.33% from PV245295.1 (Bangladesh, Natore) and 98.85% from MK559395.1 (India, Bengaluru) were showed in NCBI database (Table 1).

3.6 Phylogenetic analysis

The phylogenetic tree based on mt-COI sequences resolved distinct clades within Tephritidae and related Diptera, clearly separating species by geographic origin and genetic affinity. The first major cluster (Clade I) comprised *Rhagoletis* species. Within this group, *R. ramosae* from Mexico (Michoacán) formed a close branch with *R. zoqui* populations from Mexico, including El Madroño (Querétaro), Cuapiaxtla, and Veracruz. *R. completa* was represented by both Mexican sequences from San José Boquillas and European sequences from

Switzerland (Wallis and Basel), demonstrating geographic spread within this species. Closely allied within the clade, *R. berberis* was represented by multiple USA populations originating from Oregon, Oregon State University, and Cle Elum, forming a consistent sub-branch (Fig 9).

The second cluster (Clade II) contained *Carpomya* and allied lineages. *C. vesuviana* from India (Tamil Nadu and Punjab) grouped together, while another *C. vesuviana* sequence from Iraq (Baghdad) branched separately, suggesting regional divergence. *C. incompleta* from Iraq (Basrah and Baghdad) formed an independent subclade, while *C. schineri* was represented by populations from Ukraine (Lukyanivka) and Kyrgyzstan (Bishkek and Boom), indicating a wide Eurasian distribution. Additionally, *R. turanica* from Kyrgyzstan (Bishkek and Kashka Suu) formed a neighboring branch within this clade (Fig 9).

The third major cluster (Clade III) included *D. modestum*, which grouped tightly across Indian and Bangladeshi sequences. Indian isolates originated from Tamil Nadu and Karnataka, while Bangladeshi populations included samples from Dhaka, Savar, Natore, and Atomic Energy Research Establishment (AERE), forming a compact and well-supported lineage with strong regional representation.

A distinct clade (Clade IV) included *Anastrepha* species, distributed across the Americas. *A. fraterculus* was represented by samples from Mexico, Belize, Texas, and Bolivia, while *A. suspensa* included sequences from Peru and the Dominican Republic. *A. distincta* was found in Guatemala and Peru (Cusco), along with additional Peruvian representatives. *A. bahiensis* grouped populations from French Guiana and Suriname. Whereas, *A. obliqua* formed a broad cluster containing sequences from Peru, Florida, Edinburg, Pennsylvania, and Texas. Other related species within this clade included *A. conjuncta* from Peru and *A. alveata* from Panama (Fig 9).

Beyond these three focal clades, several additional lineages were placed distinctly. *Chaetolonchaea pallipennis* from Germany (Hesse) and Finland (Ålandia), *Spelobia ochripes* from Canada (Elizabethtown and Ontario), *Cochliomyia macellaria* from Brazil (São Paulo), *Acanthonevra vaga* from Korea (Gangwon-do), and *Paramyiolia atra* from Korea (Gangwon-do) were separated as independent branches. *Thaumatomyia trifasciata* was represented by sequences from Canada (Ontario and Guelph), while *T. pulla* occurred in the USA (New York), forming distinct lineages outside the main tephritid clusters. The tree was rooted using *M. dorsatus* from Tamil Nadu, India, ensuring separation of tephritid and non-tephritid taxa. Overall, the topology demonstrated clear species-level clustering, with *D. modestum*

populations showing strong genetic similarity within India and Bangladesh, while *Carpomya*, *Rhagoletis*, and *Anastrepha* species formed geographically distinct but genetically cohesive clades (Fig 9).

3.7 Phylogenetic structure and divergence of *D. modestum*

Bayesian phylogenetic reconstruction of the mitochondrial COI gene dataset generated a highly supported topology (posterior probabilities > 0.90) that distinctly separated Tephritid lineages from outgroup Diptera. Within this framework, *D. modestum* formed a well-defined, monophyletic clade consisting of accessions from Bangladesh (AERE, Natore, Dhaka, Savar) and southern India (Karnataka, Tamil Nadu). The terminal branches of this clade were extremely short (< 0.02 chronos units), demonstrating very low sequence divergence and strong mitochondrial conservation across the South Asian isolates. The species of *Diarrhegma* grouped closest to *Carpomya vesuviana* and *Rhagoletis completa*, while remaining clearly distinct from the rapidly evolving non-Tephritid lineages such as *M. dorsatus* and *Cochliomyia macellaria*. The inclusion of *Myllocerus* as a basal outgroup provided a rooted framework for rate comparisons, confirming that *D. modestum* occupies an intermediate evolutionary position faster than the slow-evolving Neotropical *Anastrepha* complex yet slower than Palearctic dipterans.

3.8 Evolutionary timeline and substitution-rate pattern

The time-calibrated chronogram was ultrametric (root-to-tip = 1.0 chronos units) with internal branches distributed from 4.7×10^{-8} to 1.0 chronos units (median = 0.029). The basal divergence of the Tephritidae family corresponded to the early–mid Miocene, approximately 22-26 Millions of years ago (Mya), while the split between *Diarrhegma* and *Carpomya* occurred later, around 9-11 Mya (0.15-0.25 chronos units). In contrast, the Neotropical *Anastrepha* species diversified between 8-10 Mya, and *Rhagoletis* underwent more recent radiation during the Pliocene (~5–6 Mya). Terminal nodes within *D. modestum* had extremely short durations (< 0.03 chronos units), indicating very recent intraspecific differentiation and probable contemporary expansion within South Asia.

Across all 107 branches analyzed, substitution rates spanned 1.0×10^{-8} -0.6373 subs/site/unit time (mean = 0.1271). The *D. modestum* lineage exhibited a mean evolutionary rate of 0.1567, very close to *Carpomya* (0.1590) and *Rhagoletis* (0.1434). This moderate rate was substantially

higher than that of *Anastrepha* (0.0328) but lower than fast-evolving outgroups (*Cochliomyia* 0.2279; *Chaetolonchaea* 0.2244; *Myllocerus* 0.6373). The internal variability within *D. modestum* (range = 4.2×10^{-7}-0.4655) reflected balanced evolutionary pressure neither completely conserved nor undergoing rapid mutation.

Pairwise *t*-tests identified the strongest rate differences between *Anastrepha* and the rest of the Tephritids, with $\Delta = 0.124$ ($p_a dj = 5.9 \times 10^{-3}$) when compared to *Diarrhegma*. However, *Diarrhegma* did not differ significantly from *Carpomya* ($p_a dj > 0.05$), confirming that both genera share a comparable mid-level substitution rate. Overall, *D. modestum* occupies a pivotal position in the Tephritid evolutionary spectrum faster than the conservative *Anastrepha* group but slower than Palearctic outgroups implying stable mitochondrial evolution within a relatively ancient lineage.

3.9 Selection dynamics and evolutionary interpretation

Branch-wise selection profiling revealed that the majority of nodes evolve under neutral selection. Using a ± 1 SD threshold (0.1271 ± 0.1165), ≈ 89 % of branches were neutral and ≈ 11 % exhibited stabilizing selection. When the threshold was increased to ± 3 SD, ≈ 97 % remained neutral and only ≈ 3 % (including internal nodes of *Diarrhegma* and *Carpomya*) showed stabilizing tendencies. These constrained branches corresponded to mid-depth divergence nodes (0.1-0.3 chronos units), indicating that stabilizing selection acted during periods of adaptive fine-tuning rather than at the earliest or most recent stages of evolution.

Integrating phylogeny, molecular dating, and rate partitioning reveals that *D. modestum* represents a genetically coherent, moderately evolving Tephritid lineage. It diverged from *Carpomya* around 9-11 Mya, maintaining an average substitution rate near 0.1567 subs/site/unit time and evolving primarily under neutral to weak stabilizing selection. Compared with high-rate outgroups (*Myllocerus*, 0.6373) and the slow Neotropical *Anastrepha* (0.0328), *D. modestum* exemplifies an evolutionarily stable intermediate, reflecting long-term mitochondrial stability accompanied by recent regional expansion in South Asia following a Miocene-era divergence from the ancestral Tephritid stem.

4 Discussion

4.1 Morphometric and multivariate characterization of *D. modestum*

The morphometric data of *D. modestum* collected from decayed ber tree trunks revealed a distinct pattern of sexual dimorphism, where females were larger in most body dimensions than males. The female body length averaged 6.4 mm compared to 5.2 mm in males, and similar differences were observed in wing length, head width, and abdomen width. These observations correspond with general patterns reported in Dipteran insects, where females exhibit greater body size and wing expansion due to their reproductive role and the need for enhanced dispersal and host-finding efficiency (Zhou et al. 2020).

The Principal Component Analysis (PCA) further clarified the structure of variation. In males, the first three principal components explained 61.85% of total variance. The first axis (PC1) showed strong positive loadings for thorax width, head length, and antenna length, while wing length and fore leg length contributed negatively. This suggests a morphological trade-off where males with robust thoracic regions possess relatively shorter wings, a pattern linked to mating performance and flight manoeuvrability, similar to findings in *Ceratitis capitata* (Rodriguero et al. 2002).

In females, the first three PCs accounted for 64.47% of total variance. Here, PC2 emphasized positive contributions from wing length and width, contrasting negatively with head and thorax dimensions. Such patterns highlight that female variation primarily reflects differences in flight morphology, as individuals with broader wings are likely better adapted for dispersal and host localization paralleling results in *B. dorsalis* and *C. rosa* (Pieterse et al. 2017). When both sexes were analyzed together, PC1 explained 56.95% of total variance, mainly influenced by head width, thorax width, wing width, and antenna length. This confirms that size and wing breadth are key morphometric discriminators in *D. modestum*. Comparable results in *Bactrocera* and *Anastrepha* morphometrics show that overall body robustness and wing dimensions dominate shape variation among populations (Van Cann et al. 2015).

Hence, the morphometric analysis demonstrates that *D. modestum* exhibits structured dimorphism and functional differentiation, which are likely linked to reproductive roles and ecological behavior.

4.2 Concordance of morphometric traits with molecular and evolutionary data

The mt-COI barcoding analysis reinforced the morphometric evidence of species identity. The *D. modestum* population shared 96-99% query coverage and 97.8-99.2% identity

with NCBI reference accessions from India and Bangladesh. This level of genetic similarity fits within the accepted 95-99% threshold used in Tephritidae for definitive species-level confirmation (Smit et al. 2013). Historical records from India (David and Ramani 2011) and later discoveries from Bangladesh (Leblanc et al. 2019) confirm *D. modestum* as a South Asian species associated with drumstick (*M. oleifera*), cucurbits, and recently, decayed ber tree trunks. The congruence between morphometric clustering and mt-COI similarity strongly supports the taxonomic validity and population homogeneity of the sampled material.

The phylogenetic and evolutionary analysis further substantiated this pattern. Bayesian and chronogram-based reconstructions positioned *D. modestum* within Tephritinae, closely allied to *Carpomya vesuviana*, but distinct from Dacini and other subfamilies. The *Diarrhegma-Carpomya* divergence was estimated at 9-11 Mya, within a broader Miocene diversification of Tephritidae (~22-26 Mya). These estimates correspond with molecular phylogenies that place Tephritidae radiation within the Miocene and late Oligocene epochs (Han and Ro 2009). The evolutionary rate analysis indicated that *D. modestum* had a mean substitution rate of 0.1567 substitutions/site/unit time, which is moderate and comparable to *Carpomya* and *Rhagoletis*, but higher than *Anastrepha*. Approximately 89-97% of branches evolved neutrally, while 3-11% showed stabilizing selection. These results align with mitogenomic analyses in other fruit flies, where mitochondrial evolution is largely governed by neutral drift with occasional constraints (Castellanos et al. 2025).

Integrating these datasets morphometrics, PCA, barcoding, and rate evolution reveals that *D. modestum* is a morphologically and genetically cohesive lineage, moderately evolving and phenotypically stable. Its uniform COI profile and constrained morphometric variability imply that it represents an ancient but regionally expanding pest species, reflecting ecological rather than genetic drivers of its distribution. Similar integrative studies in *A. fraterculus* and *B. dorsalis* have shown comparable consistency between morphometric and genetic homogeneity across broad geographic ranges (Congrains et al. 2021).

4.3 Implications for sustainable pest management

The synthesis of morphometric, molecular, and evolutionary findings offers several implications for sustainable management of *D. modestum* in cucurbits, (*C. maxima*, *T. cucumerina*, *L. acutangula*), mango, *M. indica*, guava, *P. guajava* and other hosts (Leblanc et al. 2019). **Diagnostic reliability and surveillance:** The combination of diagnostic

morphometric traits (e.g., body length, wing span, thorax width, antenna length) with high COI identity provides a dependable diagnostic system for rapid identification. Such integrative taxonomy minimizes misidentification and enhances quarantine surveillance efficiency, as emphasized in recent regional barcoding initiatives for fruit flies (Bartolini et al. 2020).

4.3.1 Sex-based behavioral management: The strong sexual dimorphism in *D. modestum*, with larger, broad-winged females, underscores their role in oviposition and host infestation. Similar correlations between female size, fecundity, and host preference have been reported in *B. dorsalis* and *C. capitata* (Zhou et al. 2020). Therefore, control strategies should target female populations through baiting and field sanitation synchronized with fruit maturation and oviposition peaks.

4.3.2 Evolutionary stability and ecological adaptability: The moderate substitution rate and stabilizing selection detected in *D. modestum* suggest long-term genetic stability, implying that management strategies can rely on consistent diagnostic traits and molecular markers without rapid resistance evolution. The morphometric flexibility (particularly in head-thorax-wing complexes) likely aids ecological adaptability, explaining its spread across multiple hosts and environments. These findings echo observations in *Bactrocera* and *Anastrepha*, where evolutionary conservatism in genetic architecture coexists with ecological plasticity (He et al. 2024).

4.3.3 Integration into IPM frameworks: The integrated morphometric–molecular framework offers a strong basis for management of *D. modestum*. Reliable diagnosis via morphometric traits and COI barcoding enables more accurate surveillance. Recognising female morphometry (larger size, broader wings) as a key driver of dispersal and oviposition suggests that female-targeted interventions (protein baits, attract-and-kill and timed sanitation) could be especially effective, as seen in other fruit fly systems (Zhou et al. 2020). The evolutionary stability of the lineage means diagnostic markers and trait associations are likely to remain valid over time, supporting long-term IPM strategies. Morphometric quality control metrics may also guide mass-rearing for eventual SIT applications, given that thorax-wing profiles relate to male competitiveness (Rodriguero et al. 2002). Considering the pest's moderate evolutionary rate and phenotypic consistency, sustainable management should emphasise agroecological strategies**,** such as host-crop diversification, sanitation, and habitat manipulation, rather than assuming rapid genetic evolution or resistance.

5. Conclusion

The integrative morphometric and molecular characterization of *D. modestum* presented in this study provides a strong scientific basis for its accurate diagnosis, evolutionary understanding, and management in tropical fruit ecosystems. Morphometric differentiation, particularly the pronounced sexual dimorphism and the PCA-defined "size-breadth" axis, underscores the behavioral and functional variation that drives female dispersal and oviposition success. Molecular analyses through mt-COI barcoding and Bayesian phylogeny validated species identity with 97.8-99.2 % sequence similarity and established *D. modestum* as an evolutionarily stable lineage diverged from *Carpomya* around 9-11 Mya.

These findings collectively support the formulation of precision-based Integrated Pest Management (IPM) strategies. Female-biased morphology highlights the need for sex-targeted controls, including protein-bait traps and field sanitation during oviposition peaks. Stable molecular markers enable reliable molecular surveillance and quarantine diagnostics**,** ensuring early detection in mixed fruit-fly populations. Furthermore, morphometric traits such as thorax width and wing span may serve as fitness indicators in mass-rearing or Sterile Insect Technique (SIT) programs, facilitating quality control of released males. Integrating these morphometric and molecular insights strengthens eco-adaptive, sustainable pest management frameworks and enhances long-term resilience of fruit production systems against *D. modestum* infestations.

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Author's contribution

KP conceptualized and designed the study, curated and visualized the data, and prepared the initial draft of the manuscript. **BU** contributed to study design, field validation,

and data interpretation. **PDKJ** served as the corresponding author, developed the experimental methodology, validated the results, reviewed the final manuscript, and critically revised the manuscript for intellectual content. **SS** contributed study resources, provided technical inputs, overseeing the study's scientific and editorial integrity. **SM** and **AD** performed statistical and molecular data analyses. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Disclaimer

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Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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