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Research article

Ecological assessment and DNA barcoding of major pollinators of *Ziziphus mauritiana* in Bangladesh

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Abstract

Ziziphus mauritiana, a key fruit crop, depends on insect pollination, yet its pollinator diversity remains understudied in Bangladesh. This study combines ecological observation and DNA barcoding to assess the diversity and genetics of its pollinators. A total of 33 species from Hymenoptera, Diptera, Lepidoptera, and Coleoptera were identified, with bees being the most dominant (55.2%), followed by flies (29.1%). Peak pollinator activity occurred between 10:00 AM and 12:00 PM, with *Apis* species and *Chrysomya megacephala* showing sustained activity, highlighting their ecological significance. Molecular identification of the seven most frequent pollinator species was confirmed through cytochrome c oxidase subunit I (COI) gene sequencing. Nucleotide composition analysis revealed a pronounced AT bias (71.6%), consistent with typical insect mitochondrial DNA composition. Genetic distance and t-SNE analyses showed clear taxonomic clustering, with interspecific nucleotide differences ranging from 0.1064 to 0.3770, supported by haplotype network and phylogenetic tree. Haplotype analysis revealed 1 to 4 mutational steps within *Apis*, while distant species exhibited between 28 and 77 mutational steps. Additionally, the sequence similarity within *Apis* was 84%, in contrast to the 74% similarity in wasps. These findings enhance our understanding of *Z. mauritiana* pollination ecology and highlight the value of integrating ecological surveys with molecular tools for effective pollinator conservation.

Keywords: COI gene; DNA barcoding; Insect pollinators; Pollinator diversity; Sustainable fruit production

1 | Introduction

Ziziphus mauritiana Lam. (Indian jujube or Boroi) is a drought-tolerant fruit tree of considerable ecological and economic importance, widely cultivated in tropical and subtropical regions particularly in South Asia (Arndt et al. 2001; Shihan 2017; Ye & Qin 2019). The reproductive success of *Z. mauritiana* is highly dependent on insect-mediated pollination, as its protandrous flowers exhibit temporal separation of male and female phases, requiring effective pollinator activity for optimal fruit set and yield (Rama et al. 1989). Pollinators play a vital role in sustaining biodiversity and enhancing the yield of economically important fruit crops, with about 75% of major global crops benefiting from animal-mediated pollination (Klein et al. 2007; Potts et al. 2016).

A diverse array of insect visitors, including bees, wasps, flies, and ants, are among the most effective pollinators (Rama et al. 1989; Medel et al. 2018). In Eastern India, particularly Odisha, 13 insect pollinator species were recorded on *Z. mauritiana* flowers, indicating a complex pollination system (Padhy 2021). However, the productivity of jujube is increasingly threatened by the global decline in pollinator populations, driven by habitat loss, intensive farming, pesticide use, and climate change (Kluser & Peduzzi 2007; Goulson et al. 2015).

Studying the taxonomy and ecology of pollinator insects of a specific area is crucial for understanding their roles in improving fruit set, ecosystem functioning, biodiversity conservation, and sustainable agriculture (Ollerton et al. 2011; Potts et al. 2010). Accurate identification of pollinating insects is crucial for understanding plant-pollinator interactions, conserving biodiversity, and ensuring crop productivity, especially in economically important species like *Z. mauritiana* (Klein et al. 2007; Potts et al. 2010). Traditional morphological identification often fails to distinguish closely related or cryptic species, leading to incomplete biodiversity assessments (Zarei et al. 2021). To overcome these limitations, DNA barcoding particularly using mitochondrial cytochrome c oxidase I (COI) gene sequences has emerged as a powerful molecular tool for precise identification of insect pollinators (Hebert et al. 2003; Bell et al. 2016). Globally, it has uncovered hidden diversity and successfully identified cryptic pollinator species (Lowe et al. 2022).

Despite the ecological and economic significance of *Z. mauritiana*, there remains a notable lack of comprehensive research in Bangladesh that integrates both ecological observations and molecular identification of its pollinators. Although DNA barcoding has demonstrated strong potential for accurate species identification and biodiversity assessment, it has yet to be applied to *Z. mauritiana* pollinators in this region. To address this gap, the present study combines field-based ecological surveys with DNA barcoding to identify key pollinator species, evaluate their abundance, diversity, and foraging behavior, and investigate their ecological roles within selected agroecosystems. As the first DNA-based profiling and ecological assessment of *Z. mauritiana* pollinators in Bangladesh, this research will offer foundational insights critical for pollinator conservation, sustainable orchard management, and informed strategies for breeding and fruit production (Klein et al. 2007; Potts et al. 2010).

2 | Materials and methods

2.1 | Collection of insect pollinators

Insect pollinators of *Z. mauritiana* were collected from four distinct habitats: commercially cultivated areas, wild environments, urban areas, and rooftop gardens, located at various geographical coordinates across Bangladesh (Fig. 1). Sampling was conducted two full days per week at each sampling site, from 6:00 AM to 6:00 PM, throughout the blooming period of *Z. mauritiana* (10 February to 30 April 2024), to ensure thorough coverage of pollinator activity across the entire daylight period. Flower-visiting insect pollinators were captured using standard entomological sweep nets with a circular hoop diameter of 30 cm and a net depth of approximately 60 cm. Simultaneously, high-resolution field photographs were taken to document pollinators in direct interaction with *Z. mauritiana* flowers, serving as visual confirmation of their foraging behavior and pollination activity. The collected samples were brought to the laboratory for detailed analysis, where a total of 316 specimens were examined.

2.2 | Morphological studies

Collected insect specimens were subjected to detailed morphological characterization using a Leica EZ4 Stereo Zoom Microscope or Canon EOS 50D camera. Morphological features were documented, and specimens were identified to the genus or species level based on established entomological keys and taxonomic literature (Zettler et al. 2016; Gullan & Cranston 2014; Borror & White 1970). Pollinators were distinguished from general flower visitors based on their behavior, frequency and duration of visits, and specialized morphological features. Specimens were examined for pollen loads and anatomical traits, such as corbiculae, to confirm their role in pollination.

2.3 | DNA extraction, COI amplification, and sequencing

Genomic DNA was extracted from seven insect specimens, chosen for their high abundance and frequent visits to *Z. mauritiana* flowers, using the Wizard® Genomic DNA Purification Kit (Promega, USA) following Aslam et al. (2019a, 2019b) with slight modifications. DNA concentration and purity were assessed using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, USA).

A 658 base pair fragment located at the 5′ end of the mitochondrial cytochrome c oxidase subunit I (COI) gene was targeted for amplification. The universal primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994) were used for PCR amplification. The thermal cycling conditions included an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 45.5°C for 1 minute, and extension at 72°C for 2 minutes, with a final elongation step at 72°C for 7 minutes. Amplified products were then purified using the Promega Wizard® SV Gel and PCR Clean-Up System, following the manufacturer's recommended protocol. DNA sequencing was performed using the BigDye® Terminator v3.1 cycle sequencing kit, with sequencing carried out using the LCO1490 primer to obtain the forward strand sequence.

2.4 | Sequence processing and analysis

Raw sequence data were initially visualized and refined using FinchTV and BioEdit v.7.0.5. Multiple sequence alignment was performed using the ClustalW algorithm implemented within MEGA XI software. The final alignment comprised of 625 base pairs. Phylogenetic analyses were conducted in MEGA XI using the Maximum Likelihood (ML) method based on the Tamura-Nei (TN93) substitution model, which was selected as the best-fit model. Tree robustness was assessed by performing 1000 bootstrap replications. Additionally, haplotype network analysis was conducted using the TCS method in PopART v1.7 to explore genetic relationships among the samples.

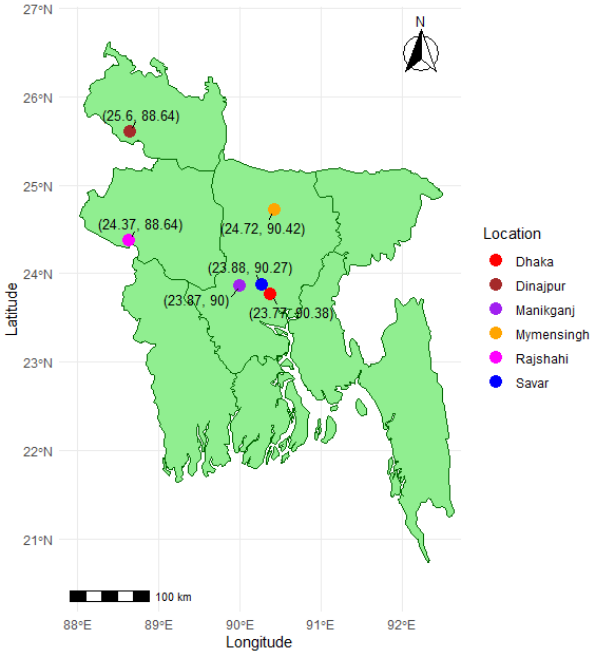

Figure 1. Sampling locations for pollinator collection of *Z. mauritiana* across Bangladesh.

2.5 | Ecological and diversity data analysis

The diversity data analysis was conducted using R v. 4.5.0 (R Core Team 2025) to visualize and interpret patterns in the diversity metrics. The dataset, structured in a CSV format, was imported and prepared for analysis. A heatmap was generated using the pheatmap (Gu 2022) to visualize the matrix of diversity values, with hierarchical clustering applied to rows and columns to identify patterns and relationships. Bar chart was constructed using ggplot2 (Murrell 2009) to compare diversity metrics across different categories, providing a clear representation of relative differences. Principal Component Analysis (PCA) was performed in R using the prcomp function from the stats package (Baldwin et al. 2012) to reduce dimensionality and visualize species differentiation. t-Distributed Stochastic Neighbor Embedding (t-SNE) for nonlinear dimensionality reduction, implemented in R using the Rtsne function from the Rtsne package (Van Der Maaten et al. 2014). All visualizations were tailored to highlight key insights into the diversity data, ensuring effective communication of results.

Table 1. Diversity and abundance of insect pollinators.

Order	Families	Species	Genera	Families Count
Hymenoptera	Vespidae, Apidae, Evaniidae, Sphecidae, Halictidae, Colletidae, Formicidae	18	11	7
Diptera	Syrphidae, Rhagionidae, Calliphoridae, Stratiomyidae, Muscidae	9	6	5
Lepidoptera	Nymphalidae, Hesperiidae, Pieridae, Erebidae	5	5	4
Coleoptera	Scarabaeidae	1	1	1
Total	—	33	23	17

Photo plate 1. Pollinators of *Ziziphus mauritiana*: 1. *Antodynerus flavescens* 2. *Delta Conidium* 3. *Vespa affinis* 4. *Delta arcuata* 5. *Apis florea* 6. *A. cerana* 7. *A. melifera* 8. *Evania appendigaste* 9. *Chalybion japonicum* 10. *Polistinae sp.* 11. *Lasioglossum sp.* 12. *Lasioglossum serenum* 13. *Hylaeus sp.1* 14. *Hylaeus sp.2* 15. *Protaetia fusca* 16. *Eumenes sp.* 17. *chalybion bengalense* 18. *Lasius niger* 19. *chalybion californicum* 20. *Eristalinus sp1* 21. *Eristalinus sp2* 22. *Rhagio scolopaceus* 23. *Asarkina ericetorm* 24. *Eristalinus arvorum* 25. *Chrysomya megacephala* 26. *Microchrysa flaviventris* 27. *Synthesiomyia nudiseta* 28. *Ypthima baldus* 29. *Iambrix salsala* 30. *Appias olferna* 31. *Pelopidas conjuncta* 32. *Amata cyssea* 33. *Hydrotaea ignava*

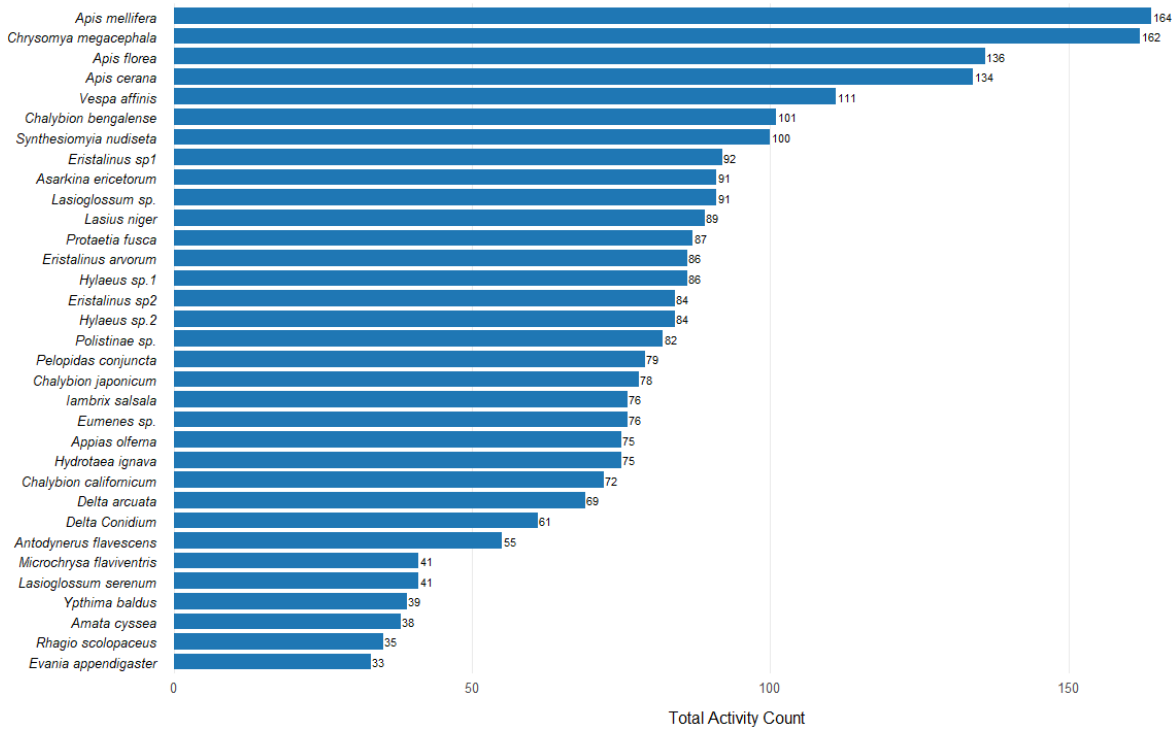

Figure 2. Abundance of 33 pollinating insect species associated with *Z. mauritiana* in the study area.

3 | Results

3.1 | Insect pollinator diversity and temporal foraging behavior

A total of 33 pollinating insects (Photo plate 1) were identified through morphological characterization using standard taxonomic keys. The 33 identified pollinator species belonged to four insect orders and seventeen families (Table 1). The recorded orders included Hymenoptera, Diptera, Lepidoptera, and Coleoptera. Bees were the most abundant group, represented by 18 species from 11 genera and 7 families. Flies followed with 9 species, then butterflies with 5 species, while beetles were the least represented, with only a single species.

Abundance analysis (Fig. 2) showed that *Apis* species, *Vespa affinis*, *Chrysomya megacephala*, and *Chalybion bengalense* were the most dominant pollinators in the study area. Their high abundance highlights their importance in local pollination networks. In contrast, species like *Evania appendigaster* and several flies were less abundant, indicating a limited role. These findings underscore the ecological significance of dominant pollinators for pollination services and conservation.

Activity patterns of 33 pollinator species were observed at different time intervals (Table 2), categorized into low, medium, and high abundance levels. Most species showed peak activity between 10:00 AM and 12:00 PM, with *Apis* species and *Chrysomya megacephala* maintaining high activity throughout the day. In contrast, species like *Evania appendigaster* and *Microchrysa flaviventris* exhibited lower and more restricted activity. These patterns suggest a

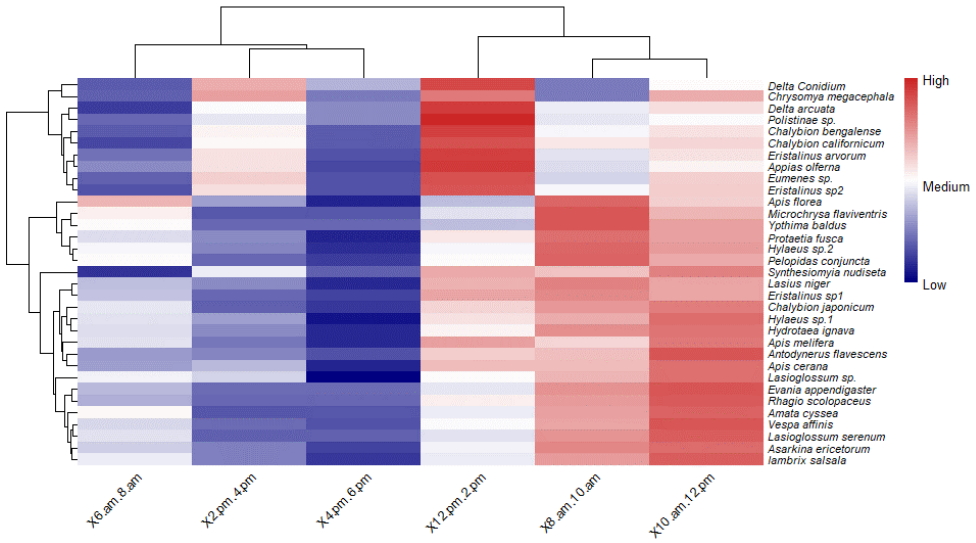

Figure 3. Heatmap analysis of diel activity patterns in insects: Temporal variations in abundance and foraging behavior. Activity levels: High (Red - increased activity), Medium (White- moderate activity), Low (Blue - reduced activity).

Table 2. Temporal activity patterns of pollinator species observed at different time intervals.

Species Name	6 am– 8 am	8 am–10 am	10 am– 12 pm	12 pm– 2 pm	2 pm– 4 pm	4 pm–6 pm
Antodynerus flavescens	+	++	+++	++	+	
Delta Conidium		+	++	+++	++	+
Vespa affinis	++	+++	+++	++	+	+
Delta arcuata	+	++	++	+++	++	+
Apis florea	+++	+++	+++	++	++	+
Apis cerana	++	+++	+++	+++	++	+
Apis mellifera	+++	+++	+++	+++	++	++
Evania appendigaster	+	++	++	+		
Chalybion japonicum	++	+++	+++	++	+	
Polistinae sp.	+	++	++	+++	++	+
Lasioglossum sp.	++	+++	+++	++	+	
Lasioglossum serenum	+	++	++	+		
Hylaeus sp.1	++	+++	+++	++	+	
Eumenes sp.	+	++	++	+++	++	+
Hylaeus sp.2	++	+++	+++	++	+	
Chalybion bengalense	+	++	++	+++	++	+
Lasius niger	++	+++	+++	++	+	
Chalybion californicum	+	++	++	+++	++	+
Eristalinus sp1	++	+++	+++	++	+	
Eristalinus sp2	+	++	++	+++	++	+
Rhagio scolopaceus	+	++	++	+		
Asarkina ericetorum	++	+++	+++	++	+	
Eristalinus arvorum	+	++	++	+++	++	+
Chrysomya megacephala	++	+++	+++	+++	+++	++
Microchrysa flaviventris	+	++	++	+		
Hydrotaea ignava	++	+++	+++	++	+	
Synthesiomyia nudiseta	+	+++	+++	+++	++	+
Ypthima baldus	+	++	++	+		
Iambrix salsala	++	+++	+++	++	+	
Appias olferna	+	++	++	+++	++	+
Pelopidas conjuncta	++	+++	+++	++	+	
Amata cyssea	+	++	++	+		
Protaetia fusca	++	+++	+++	++	+	

correlation with ecological factors such as temperature and resource availability.

Heatmap analysis (Fig. 3) showed distinct temporal variations in insect flower visitation, with peak activity occurring between 10:00 AM and 12:00 PM, followed by 8:00 AM–10:00 AM and 12:00 PM–2:00 PM, as indicated by red. Visitation was lowest between 4:00 PM–6:00 PM, 2:00 PM–4:00 PM, and 6:00 AM–8:00 AM, marked by blue. Hierarchical clustering revealed strong associations between mid-morning and noon, with distinct patterns in early morning and late afternoon. Species clustering further highlighted specific foraging behaviors.

3.2 | Molecular identification of pollinators and their genetic makeup

A total of seven pollinator specimens were successfully sequenced, and BLAST analysis revealed 99–100% similarity with existing sequences in the NCBI GenBank database, confirming their identification as seven distinct species (Table 3). All sequences were subsequently submitted to GenBank and assigned unique accession numbers (Table 3). These results demonstrate the effectiveness of COI barcoding in accurately distinguishing pollinator species.

The COI sequences were aligned (Fig. 4) to assess genetic similarity among pollinator species. *Apis* species showed 84% similarity, indicating close relatedness, while *Vespa affinis* and *Chalybion bengalense* shared 74%, suggesting greater divergence. The highest similarity (86%) was observed between *Synthesiomyia nudiseta* and *Chrysomya megacephala*. Variable regions (positions 50–60, 250–280, 410–440, 500–510) may reflect species-specific adaptations, while conserved regions indicate genetic stability across taxa.

Nucleotide composition of the COI gene sequences was analyzed, encompassing the 1st, 2nd, and 3rd codon positions (Table 4). The overall base composition of the COI fragment varied among specimens, showing a pronounced AT bias, which is consistent with expectations. The average AT content (T+A) was 71.6%, while the GC content (C+G) averaged 28.4%.

Table 3. GenBank accession number and the GPS location.

Species name	GPS Position	Accession No.
Apis florea	23.769570 N, 90.373105 E	MH378769.1
Apis cerana	23.821836 N, 90.412510 E	MG587944.1
Apis mellifera	23.864986 N, 90.003342 E	OP435365.1
Chalybion bengalense	23.524395 N, 90.154359 E	PQ796138.1
Vespa affinis	23.849410 N, 90.258229 E	MH036512.1
Chrysomya megacephala	23.880030 N, 90.265546 E	MG557664.1
Synthesiomyia nudiseta	23.876073 N, 90.267057 E	MG572240.1

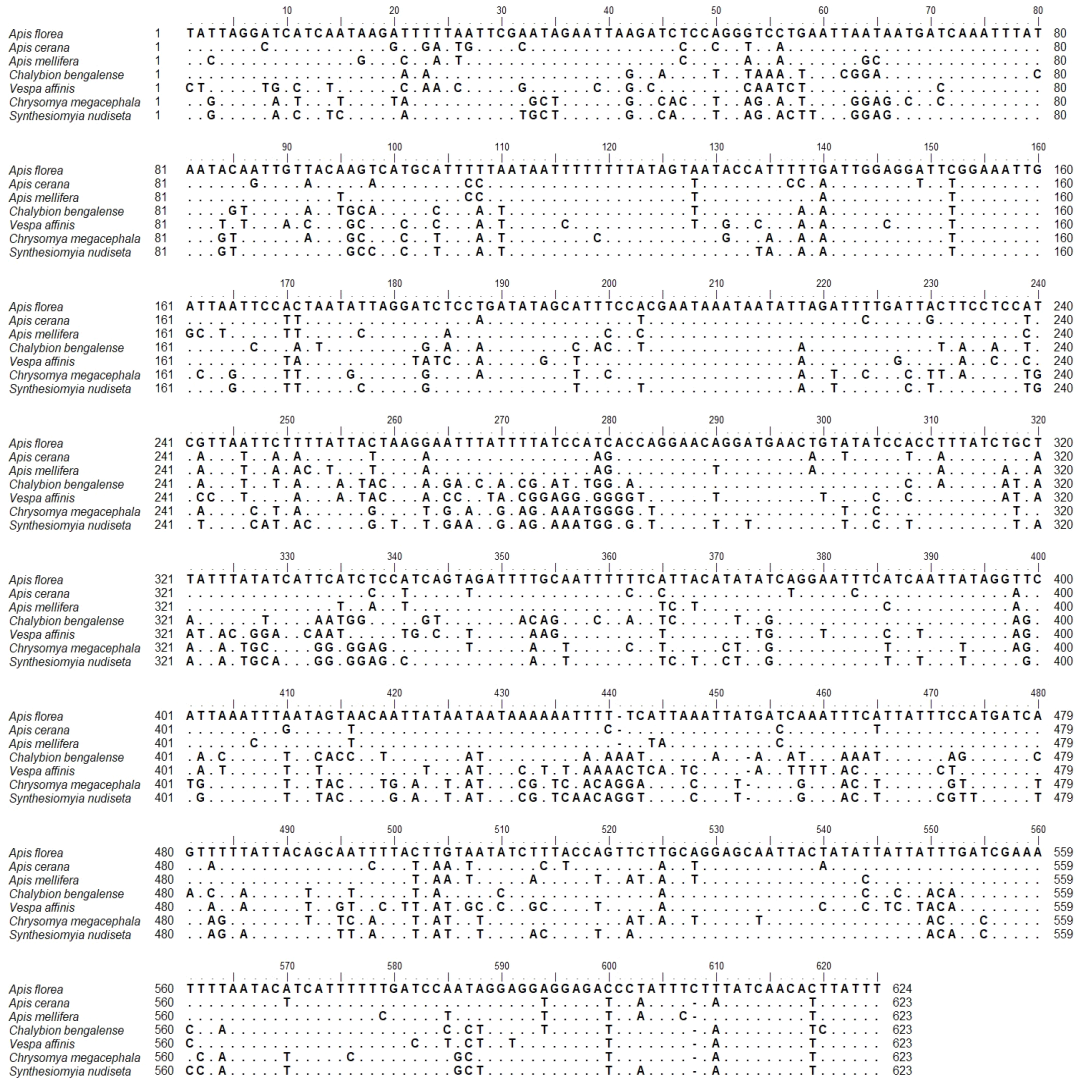

Figure 4. Multiple sequence alignment of sequenced COI genes from seven pollinators of *Z. mauritiana*. Letters represent the non-conserved regions, while dots indicate the conserved portions among the sequences.

Table 4. The nucleotide base composition of the sequenced pollinator species.

Species	T(U)%	C%	A%	G%	Total	A+T%	G+C%
Apis florea	41.6	14.0	33.4	11.0	652	**75**	25
Apis cerana	40.7	15.1	32.7	11.6	649	73.4	26.7
Apis mellifera	40.5	15.3	33.1	11.1	640	73.6	26.4
Chalybion bengalense	36.4	14.7	36.8	12.1	634	73.2	26.8
Vespa affinis	39.6	18.3	29.0	13.1	666	68.6	31.4
Chrysomya megacephala	38.5	15.3	30.4	15.8	639	68.9	31.1
Synthesiomyia nudiseta	38.8	15.5	29.9	15.8	659	68.7	**31.3**
Average	39.5	15.5	32.1	12.9	648.4	71.6	28.4

Pairwise genetic distance was analyzed to demonstrate partial sequence divergences between and within species (Table 5). The interspecific nucleotide difference among the species ranged from 0.1064 to 0.3770. The two fly species (*Chrysomya megacephala* and *Synthesiomyia nudiseta*) exhibit the lowest genetic distance (0.1064), confirming their strong genetic similarity. Additionally, the highest genetic distance is between *Vespa affinis* and *Chrysomya megacephala* (0.3770), indicating significant genetic divergence.

3.3 | Genetic relationship among the pollinators

The t-SNE plot analysis (Fig. 5) shows clear clustering patterns, reflecting both intra- and inter-group genetic relationships. *Apis* species (*A. florea*, *A. cerana*, *A. mellifera*) display minimal genetic distances, consistent with their close taxonomic ties. The wasps (*Chalybion bengalense* and *Vespa affinis*) and flies (*Chrysomya megacephala* and *Synthesiomyia nudiseta*) also cluster closely within

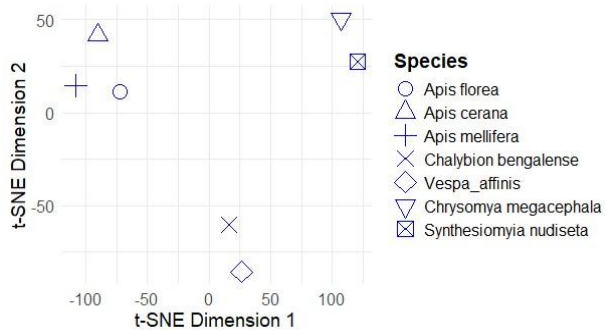

Figure 5. Revealing phylogenetic patterns in pollinators: A t-SNE and genetic distance approach.

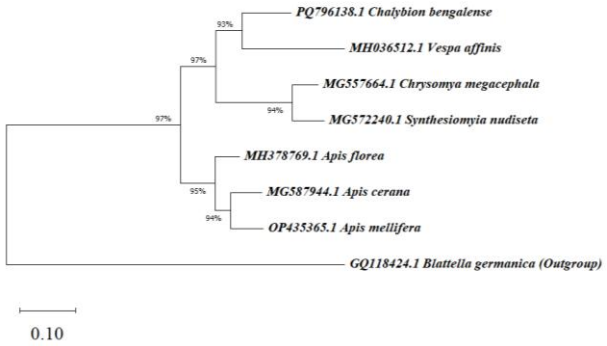

Figure 7. The maximum likelihood phylogenetic tree based on COI gene sequences. A total of 679 nucleotide positions from the COI gene sequences were considered in the final dataset for analysis.

Table 5. Interspecific genetic distances among 7 sequence species.

Species Name	1	2	3	4	5	6
Apis florea						
Apis cerana	0.1239					
Apis mellifera	0.1260	0.1153				
Chalybion bengalense	0.2748	0.2824	0.2838			
Vespa_affinis	0.3592	0.3723	0.3525	0.2680		
Chrysomya megacephala	0.3225	0.3242	0.3512	0.2884	**0.3770**	
Synthesiomyia nudiseta	0.3392	0.3620	0.3495	0.3051	0.3592	**0.1064**

their respective groups. Greater genetic distances between flies and wasps, and between *Apis* and wasp species, highlight deeper evolutionary divergence, aligning with taxonomic classifications and providing insights into their evolutionary relationships.

The TCS haplotype analysis (Fig. 6) revealed varying levels of genetic diversity across the studied species. Closely related species *Apis cerana* (yellow), *Apis mellifera* (purple), and *Apis florea* (red) exhibited minimal mutational differences (1–4 steps), suggesting a recent common ancestry. In contrast, *Vespa affinis* (light purple) and *Chrysomya megacephala* (blue) showed greater divergence, with 77 and 28 mutational steps respectively, indicating deeper evolutionary separation. The inferred haplotypes highlight potential ancestral connections among the observed sequences.

The phylogenetic analysis of pollinator species using the maximum likelihood method (Fig. 7) reveals distinct genetic clustering among the species studied. The *Apis* species (*A. florea*, *A. cerana*, and *A. mellifera*) form a close clade with high bootstrap support, indicating a shared evolutionary history. *Vespa affinis* and *Chalybion bengalense* also cluster together, suggesting a possible regional genetic variation. The two Dipteran species (*Chrysomya megacephala* and *Synthesiomyia nudiseta*) exhibit a clear separation from the Hymenoptera species, reflecting their greater evolutionary distance. The analysis includes *Blattella germanica* as an outgroup, which aids in rooting the tree. The bootstrap values (ranging from 93% to 97%) further support the robustness of the clades, providing strong evidence for the genetic relationships among the species.

4 | Discussion

This study presents a comprehensive ecological and molecular analysis of the insect pollinators of *Z. mauritiana* in Bangladesh, identifying 33 species across four insect orders. Among these, Hymenoptera, particularly *Apis* bees emerged as the most dominant group, accounting for 55.2% of the observed species. This finding aligns with earlier studies emphasizing the essential role of bees, especially honeybees, in pollinating fruit crops (Garratt et al. 2014; Roubik 1995; Klein et al. 2007). Their high abundance and widespread presence across the study sites confirm their role as

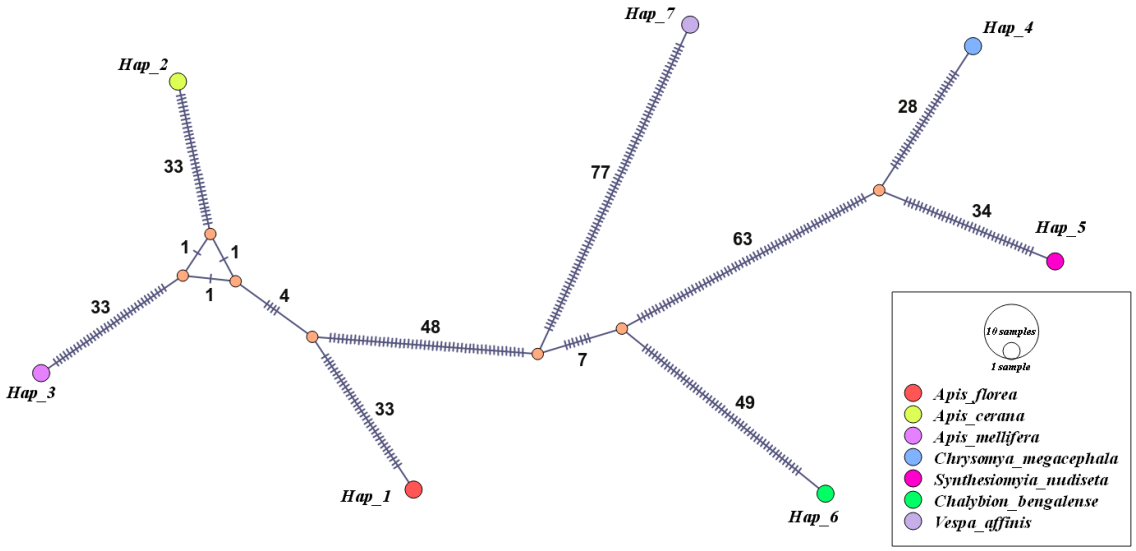

Figure 6. TCS haplotype network of mitochondrial COI gene showing the mutational relationships among pollinator species. Small circles represent immediate common ancestors, and mutational steps are indicated by hatch marks and numbers.

primary pollinators, contributing significantly to flower fertilization and optimal fruit set. These findings reinforce the conclusion that honeybees are essential to tropical and subtropical agricultural systems where *Z. mauritiana* is cultivated, as previously suggested by Free (1993).

Dipteran species, particularly from the families Syrphidae and Muscidae, comprised 29.1% of the pollinator community, making them the second most prevalent group. Although typically considered secondary pollinators, their role in this study underscores their complementary function to bees. As shown in other agricultural systems, Dipterans visit flowers that bees might overlook and remain active during specific conditions such as warmer, sunnier periods (Larson et al. 2001). This adaptive foraging behavior enables them to occupy ecological niches that enhance the overall pollination success of *Z. mauritiana*.

Beetles, though present, had a minor role in the pollination process. Their limited contribution is consistent with trends observed in other systems where beetles act more as incidental visitors rather than efficient pollinators (Gottsberger 1999). The reduced ecological impact of beetles in this context further emphasizes the dominance of bees and Dipterans in effective pollination.

Temporal activity patterns revealed a peak in pollinator visits between 10 AM and 12 PM, a period associated with optimal foraging conditions such as temperature and floral resource availability (Brittain et al. 2013). Notably, honeybees maintained foraging activity throughout the day, a trait also observed in other studies (Abrol 2015). In contrast, solitary bees and wasps showed reduced activity, likely due to thermoregulation needs or competition avoidance (Heinrich 1993; Carvalheiro et al. 2010). This variation reflects niche partitioning and behavioral adaptations among pollinator groups.

Insect pollinators that were highly abundant and frequently visited *Z. mauritiana* flowers were successfully identified using COI gene sequencing, demonstrating the effectiveness of DNA barcoding in resolving species-level identification across morphologically similar and taxonomically challenging insect groups. This molecular approach proved especially valuable for Hymenoptera, Diptera, and Coleoptera, where traditional morphological keys often fall short, by accurately distinguishing cryptic bee species, visually similar hoverflies, and poorly characterized floral-visiting beetles (Treanor et al. 2013; Jordaens et al. 2015).

Molecular data from COI sequences revealed a high AT content (71.6%), characteristic of insect mitochondrial genomes (Aslam et al. 2019; Rain et al. 2019; Simon et al. 1994). A high sequence similarity (84%) among *Apis* species indicates recent evolutionary divergence (Raffiudin & Crozier 2007), while greater variation in *V. affinis* suggests older divergence. The small genetic difference between *C. megacephala* and *S. nudiseta* indicates that they are closely related (Nelson et al. 2007). In contrast, the high bootstrap value of 93% for *C. bengalense* and *V. affinis* highlights their significance in evolutionary studies (Goulson et al. 2015). Conserving a diverse range of pollinators, especially bees and flies, is essential for effective pollination of *Z. mauritiana*. Temporal and genetic data can guide targeted strategies like staggered hive use (Brittain et al. 2013) and region-specific breeding (Rader et al. 2016).

5 | Conclusions

The integration of ecological observations with DNA barcoding in this study offers a powerful framework for the accurate assessment of pollinator communities, which is critical for biodiversity conservation and the management of pollination services in agroecosystems. This study emphasizes the vital role of diverse pollinator species, especially bees and flies, in the successful pollination of *Z. mauritiana*. Molecular insights into the genetic diversity of pollinators further support the importance of preserving this diversity for sustained crop productivity. Future research should focus on enhancing our understanding of pollinator behavior, their genetic relationships, and their movement patterns across landscapes, to refine conservation efforts and promote sustainable agricultural practices.

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Authors' contributions

M.T.R. collected samples, performed taxonomic identification, conducted data analysis, and prepared the manuscript. A.F.M.A. designed the research, supervised the project, conducted research, and revised the manuscript. They contributed critically to the drafts and gave final approval for publication.

Conflicts of interest

The authors declare no conflict of interest.

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